Effect of high levels of CO₂ on the electrochemical behaviour and the enzymatic and non-enzymatic antioxidant system in black and white table grapes stored at 0°C.

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ABSTRACT

BACKGROUND

In this study, we analysed the effect of the application of high levels of CO₂ (20 kPa CO₂ + 20 kPa O₂ + 60 kPa N₂) for 3 days at low temperature on a white table grape cultivar (Superior Seedless) in comparison to a black one (cv. Autumn Royal) in terms of quality parameters. Likewise, as a novelty, we used a solid state voltammetry methodology to analyse the effect of the 3-day gaseous treatment in berry tissues from both cultivars in the first stage of storage. We also determined the role of non-enzymatic (total phenols, total anthocyanins and antioxidant activity) and enzymatic antioxidant (catalase (*GCAT*), ascorbate peroxidase (*VcAPX*) gene expression) system in the behaviour of both cultivars.

RESULTS

Our results indicate that the application of a 3-day gaseous treatment was effective in maintaining the quality of black and white table grapes for up to 28 days of storage at 0°C. The electrochemical methodology applied was useful to differentiate between skin and pulp samples, and between black and white table grape cultivars. Some molecular mechanisms were modulated in the skin of both cultivars to overcome oxidative stress which was not manifested with the same intensity in grapes treated with CO₂ for 3 days. However, some differences in reference to the non-enzymatic system were observed.

CONCLUSION

The 3-day gaseous treatment was useful to maintain black and white table grapes quality at 0°C. Furthermore, the voltammetry analysis indicated that CO₂-treated samples from the skin and pulp showed more similarity to freshly-harvested ones.

KEYWORDS: Ascorbate peroxidase, Catalase, Solid state voltammetry, Total anthocyanins, Total phenolics, *Vitis vinifera*.

INTRODUCTION

The global changes which have occurred in the agri-food industry in recent decades have drastically affected postharvest technologies. Furthermore, given the increasing concern about issues related to the environment and sustainable development, consumers are demanding for a greater reduction in the use of chemical products. As a consequence, the incorporation of postharvest technologies that reduce the use of agrochemical compounds is vital to maintain the quality of fruit and vegetables during their storage. In this sense, the application of a gaseous treatment with 20 kPa CO₂ for 3 days at 0°C was effective in maintaining the quality of red table grapes during postharvest storage at low temperature by reducing total decay, weight loss and rachis browning.^{1,2,3} Although table grapes are classified as chilling tolerant, our previous works have shown that the 3-day gaseous treatment helped red table grapes to withstand temperature shifts at 0°C.^{1,3} However, it is important to mention that, to date, the effectiveness of the 3-day gaseous treatment has only been analysed in red table grapes.

Plants present the innate ability to synthesize, constitutively or in response to abiotic and biotic stress conditions, non-enzymatic antioxidants including secondary metabolites such as phenolic compounds, tannins, and anthocyanins. Likewise, some authors have found a significant correlation between antioxidant capacity, total phenols and anthocyanins in different types of fruit.^{4,5} However, there have been variations in this response depending on the cultivar and the maturity state of fruit.^{4,6} In previous works, we observed that the storage of Cardinal table grapes at 0°C under atmospheric conditions increased total anthocyanin content paralleled by an increase in antioxidant activity. This trend, nevertheless, was not denoted in CO₂-treated grapes since the total anthocyanin slightly increased while antioxidant activity sharply decreased.^{6,7}

The balance between production and detoxification of reactive oxygen species (ROS) is crucial for cell survival during exposure at low temperature.⁸ Different works show evidence that even during the stress situation, ROS production is not necessarily a symptom of cellular dysfunction but could represent a signal that would lead to the adjustment of the cellular machinery in adverse situations.⁸ It is known that fruit, when exposed to low temperature or different postharvest treatments, modulate enzymatic and non-enzymatic antioxidant system. In table grapes, Yuan et al.⁹ observed an induction of different antioxidant enzymes, including ascorbate peroxidase (APX) during the storage of Kyoho fruit at 2°C. On the other hand, in the case of Cardinal table grapes, the beneficial effect of high CO₂ levels to control oxidative stress through the induction of the enzymatic antioxidant system depends on the type of tissue and could be more closely related with the reduction of rachis browning rather than with a general response in table grapes.¹⁰ However, it is unknown whether this is a general response in table grapes or whether the cultivar analysed does have a part to play.

The aim of this study was to determine the role of the non-enzymatic and enzymatic antioxidant system in how table grapes respond to low temperature and high levels of CO₂ in the first stage of storage. Considering that the response could be modulated depending on the analysed cultivar, we quantified total anthocyanins and total phenols in the skin of white (Superior Seedless, SS) and black (Autumn Royal, AR) table grapes and evaluated its correlation with the antioxidant activity, together with the

expression of genes (*GCAT* and *VcAPX*) that encode antioxidant enzymes. In addition, as a novelty, lyophilized samples of the skin and pulp of black and white CO₂-treated and non-treated table grapes were directly analysed by solid-state voltammetry. This methodology has allowed the characterization of fresh tomatoes according to their phenols and ascorbic acid content¹¹ and for the reactivity of phenolic compounds with ROS to be monitored after their *in situ* electrochemical generation.¹²

MATERIAL AND METHODS

Plant material

Table grapes (*Vitis vinifera* L.) of AR (black) and SS (white) cultivars were collected from an orchard belonging to the company named Frutas Esther S.A located in Abarán (Murcia) in the months of November (AR) and July (SS), according to its optimum state of maturity for commercialization. The bunches were transferred to the ICTAN laboratory on the day of collection and those that did not present mechanical or pathological defects were randomly divided into two lots and stored at $0 \pm 0.5^{\circ}\text{C}$ with a relative humidity of 95% in 1 m³ neoprene cabins. Ten boxes (3 kg of table grapes/box) were stored in each cabin. One batch was stored under normal atmospheric conditions (non-treated) and the other batch was stored in a gas mixture that contained 20 kPa CO₂ + 20 kPa O₂ + 60 kPa N₂ (CO₂-treated) for 3 days. Thereafter, CO₂-treated bunches were transferred to air under the same conditions as non-treated ones until the end of the storage period (28 days). Five clusters (approximately 300 g each cluster) were sampled periodically, the skin and pulp of berries were collected, frozen in liquid nitrogen and stored at -80°C until analysis.

Quality assessments

Soluble solids content (SSC), titratable acidity (TA) and pH were determined at day 0 and at day 28 in CO₂-treated and non-treated table grapes as described by Sanchez-Ballesta et al.¹ Bunches weight was recorded on the day of harvest and after 28 days of storage at 0°C. Cumulative weight losses were expressed as a percentage loss of the original weight. Total decay and expressed as the percentage of decayed berries at day 28 with respect to the original cluster weight. Rachis browning at day 28 was determined by using the subjective scale indicated by Vazquez-Hernandez et al.³

Relative gene expression by quantitative real-time RT-PCR (RT-qPCR)

Total RNA extraction and cDNA synthesis were performed according to Vazquez-Hernandez et al.³ Relative gene expression of *VcAPX* (GeneBank accession no. DQ887095) and *GCAT* (GeneBank accession no. XM_003635412) was assayed at day 0, at the end of the gaseous treatment and after 3 days of storage at 0°C under normal atmosphere using RT-qPCR as described by Rosales et al.¹⁰ *Actin1* (XM_002282480) from *V. vinifera* was used as internal control. The specificity of products was validated according to Vazquez-Hernandez et al.³

Analysis of total phenols content

For the phenols extraction, 0.25 g of skin and pulp tissues from both cultivars at day 0, at the end of the gaseous treatment and after 3 days of storage under normal atmosphere were homogenized with 0.5 mL of 1% methanol HCl by ultrasonication for 10 min. The extracts were centrifuged at 10,000 g at 4°C for 10 min and the supernatant was collected. These steps were repeated twice obtaining a final volume of 1.5 mL. The supernatants were filtered with 0.45 µm nylon filters and stored at -80°C. The content of total phenols in the extracts of the two varieties of table grapes was determined by the Folin-Ciocalteu method¹³ and expressed as mg of gallic acid equivalents g⁻¹ fresh weight (FW).

Analysis of total anthocyanin content

The study of total anthocyanin content in the skin of AR and SS table grapes was carried out the same days as the total phenols content using the differential pH method¹⁴ as described by Sanchez-Ballesta et al.⁷ The results were expressed as mg of malvidin-3-glucoside g⁻¹ FW.

Antioxidant activities measured by ABTS and FRAP methods

For the determination of the antioxidant activity of AR and SS grapes it was used the same extract as for the determination of total phenols. Antioxidant activity was quantified by the ABTS⁺ method described by Re et al.¹⁵ and also following the FRAP method¹⁶. A calibration curve was obtained with a Trolox solution as standard reference compound (0, 0.1, 0.4, 1, 2 and 3 mM). The results were expressed in mM of Trolox equivalents g⁻¹ FW.

Electrochemical instrumentation and methods

Lyophilized samples from table grapes at day 0, at the end of the gaseous treatment and after 3 days of storage at 0°C were used. Electrochemical analyses were performed at 298 ± 1 K in a thermostated cell with CH I660 equipment (Cambria Scientific, Llwynhendy, LLaneli UK). A BAS MF2012 glassy carbon-working electrode (geometrical area 0.071 cm²), a platinum wire auxiliary electrode and an Ag/AgCl (3 M NaCl) reference electrode were used in a typical three-electrode arrangement. The glassy carbon-working electrode was previously polished with 1.00, 0.30 and 0.05 µm alumina slurries and then sonicated in redistilled water, ethanol and redistilled water, successively. The voltammetry was measured in films, generated from the lyophilized samples of the skin and pulp, on glassy carbon-working electrode. For that, ca, 1 mg of solid samples was placed in an agate mortar and pestle together with 1 mL of ethanol. After the mixture was homogenized, 50 µL of the suspension was dropped onto the surface of the glassy carbon-working electrode and evaporated in air.¹⁷ In addition, two anthocyanin standards, pelargonidin-3-glucoside and peonidin-3-glucoside, purchased by Extrasynthese (France), were analysed under the same conditions. Square wave voltammetry was used as detection mode.

Statistical analyses

Data from at least three replicates per sample were subjected to analysis of variance (one-way ANOVA) using the Tukey-b test ($P < 0.05$) (SPSS v. 23.0, IBM). The relationship between *VcAPX*, *GCAT*, total anthocyanins, total phenols and antioxidant activity was described as Pearson product moment correlation coefficient (r), $P < 0.05$ or $P < 0.01$. Principal components analysis (PCA) was applied to voltammetric data using the correlation matrix and linear regression.

RESULTS AND DISCUSSION

Effect of a 3 day- CO_2 treatment on the quality of black and white table grapes stored at 0°C

The application of high CO_2 levels for 3 days did not modified the SSC, TA and pH values in both AR and SS table grapes after 28 days at 0°C , in comparison to non-treated grapes (Table 1). The CO_2 treatment was effective in controlling the weight loss of AR and SS bunches after 28 days, as well as controlling the rachis browning in comparison to non-treated table grapes. Although the effect of high levels of CO_2 on weight loss has been previously observed in table grapes⁷ and other fruit,^{18,19} in the case of rachis browning it is not a common response. Thus, storage of Red Globe table grapes at 0°C for up to 12 weeks under controlled atmospheres (above 10 kPa CO_2) accelerated rachis browning.²⁰ In the present work, this is the first time that a 3-day CO_2 -treatment at 0°C was applied to a white cultivar, obtaining similar results to black table grapes regarding weight loss and rachis browning.

It is important to note that total decay was not recorded in SS grapes, even after 28 days at 0°C under the conditions assayed. However, the CO_2 treatment significantly decreased the percentage of total decay detected in non-treated AR grapes. At this point, it is important to note that table grape from both cultivars were cultivated in the same commercial orchard. Artés-Hernández et al.²¹ showed that a short cold storage (7 days at 0°C + 4 days at 8°C) plus 2 days at 20°C under modified atmosphere packaging was effective in controlling weight loss, rachis browning and fungal attack in Superior Seedless bunches during the shelf-life period. Similarly, continuous and intermittent ozone treatments applied to Superior Seedless grapes were effective in controlling total decay after 72 days at 5°C .²² Thus, the fact that in our storage conditions no total decay was recorded could be related to the time of storage (28 days) in comparison to the 72 days assayed by Cayuela et al.²² and due to the fact that we did not analyse a shelf-life period as Artés-Hernández et al.²¹ So, further work would be needed to analyse in deep the storage time at 0°C at which total decay would be manifested in Superior Seedless grapes.

General voltammetric pattern of black and white tables grapes treated with high levels of CO_2 for 3 days and stored at 0°C

The square wave voltammetry responses obtained for the lyophilized pulp and skin samples corresponding to the black AR cultivar were shown in Fig. 1. The voltammogram is dominated by a main oxidation process at +0.75 (A₂) and +0.50 V (A₁) vs. Ag/AgCl in the case of the pulp and skin, respectively, denoting a clear difference in the composition of the antioxidant constituents between both tissues. The voltammograms associated with the pulp and skin samples of 3-day CO_2 -treated and non-

245 treated white table grapes stored at 0°C exhibited a higher similarity showing, in both
246 cases, oxidation processes at ca. +0.80 (A2), +0.60 (A1) and +0.30 (A1) (Fig. S1). Given
247 that the electrochemical response for each cultivar was significantly different, this
248 methodology could be successfully applied for cultivar characterization and screening as
249 was previously done for tomatoes and tea samples.^{11,17}

250 According to previous electrochemical studies,^{11,12} signals in the A₁ potential
251 region (ca. +0.60-0.20 V) (Fig. 1) can be assigned to compounds such as ascorbic acid,
252 caffeic acid derivatives and phenolic compounds with oxidizable *o*-catechol-like motifs,
253 namely catechin, quercetin, myricitin and other flavonoids previously found in
254 grapes.²³ However, phenolic compounds, which do not have an *o*-catechol unit, display a
255 signal ca. at +0.75 V, as can be the case of hydroxybenzoic and hydroxycinnamic acids.
256 Because of the electrochemical profile of flavonoids in solution,²⁴ the process A₁ can be
257 attributed to the two-electron, two proton oxidation of the 3',4'-dihydroxybenzoic moiety
258 (ring B) yielding to the corresponding *o*-quinone. This process can often be accompanied
259 by the oxidation of the 5,7-dihydroxyl groups, occurring at higher potentials (A₂) (Fig.
260 S2).

261 On the other hand, the interaction of the phenolic compounds with ROS has been
262 monitored after the *in situ* generation of these species by using negative potentials in the
263 presence of dissolved oxygen (Fig. S3).¹² In this case, the voltammograms obtained for
264 the pulp and skin of freshly-harvested AR grapes can be also observed in Fig. 1. In
265 contrast to the results obtained for fresh tomatoes,¹² non-significant differences were
266 detected in the potential range between +1.0 and -0.2 V due to the presence of ROS, most
267 likely caused by the loss of the electroactive compounds during the sample manipulation
268 including the liophilization process. However, as can be seen in Fig. 1D, highlights the
269 appearance of a new signal at ca. -0.60 V in the voltammogram corresponding to the skin
270 samples. Interestingly, the apparition of this new signal only occurred in the skin samples
271 from the black grape cultivar, and thus might be related to the presence of anthocyanin
272 compounds that can be found in this tissue.²⁵ This assumption was confirmed by
273 analysing commercial standards for pelargonidin-3-glucoside and peonidine-3-glucoside
274 whose voltammograms exhibited, in addition to an anodic peak in the 0.25 to 0.55 V
275 region, an intense signal at negative potentials (-0.70 and -0.60 V, respectively) (Fig. S4).
276 Curiously, the signal obtained for the black grape cultivar appeared at the same potential
277 as for peonidine-3-glucoside (-0.60 V), which is known as the main anthocyanin in red
278 table grapes.^{6,26} Thus, our results seem to indicate that the signal observed at ca. -0.60 V
279 in the skin of the black table grapes, and not in the white ones, could be properly related
280 to peonidin-3-glucoside, confirming that the electrochemical analyses employed are
281 useful to satisfactorily differentiate between black and white grape cultivars. Moreover,
282 the differences in the oxidation potential values and/or peaks intensities could make it
283 possible to discriminate among grape cultivars, and, as we will see below, even
284 postharvest storage conditions could affect the content of electroactive compounds.

285 Consequently, in order to evaluate the influence of the cultivar, tissue and
286 postharvest treatment on the electroactive compounds, multivariate analysis of the
287 voltammetric data has been performed. Values of the ratio between the current at a given
288 potential and the maximum peak current ($i(E)/i_{\max}$) in square wave voltammetry have

been used for the PCA, taking potentials between +0.20 and +1.00 V at intervals of 50 mV. An optimal sample classification according to tissue (component 1) and cultivar (component 2) (Fig. S5) was achieved from the electrochemical results. On the other hand, the two-dimensional PCA related to the CO₂-treated and non-treated samples can be observed in Fig. 2. The PCA extracted two components which explain 100% of variation in all cases. For pulp samples, the first component which explains 71 and 82% of the variance for black and white table grape cultivars, distinguishes between samples stored for 3 days at 0°C in air (non-treated) and those treated with high levels of CO₂ and freshly-harvested (t0), most likely due to a decrease in the electroactive components presented in the tissue (Fig. 2A and 2C). The skin samples showed different behaviour according to the cultivar. Indeed, the PCA obtained for the white grape cultivar identifies similarities between samples at time 0 and those stored in air (Fig. 2D). While for AR table grapes, freshly-harvested samples are separated in the first component from those stored for 3 days, even if samples treated with CO₂ are in the limit of both components, which could be attributed to the lower reduction of electroactive components than in the case of samples stored in air (Fig. 2B). In previous works, we have pointed out that the 3-day high CO₂ treatment help red/black table grapes (Cardinal or Autumn Royal) to withstand temperature shifts at 0°C^{1,3} and thus CO₂-treated berries reached low ion leakage, water loss and rachis browning in comparison to non-treated ones. Likewise, a microarray analysis revealed substantial changes in the transcriptome of Cardinal table grapes as a response to storage at 0°C, whereas the number of genes which changed expression because of the 3-day CO₂ was much lower.² Consequently, the behaviour of CO₂-treated samples is closer to that of freshly-harvested ones and the electrochemical results obtained in the present work reinforce this idea, with the exception of the skin of SS grapes where non-treated samples showed more similarities with samples at time 0.

Effect of a 3-day high CO₂ treatment on non-enzymatic antioxidant compounds in the skin of black and white table grapes stored at 0°C. Determination of total phenolic content, total anthocyanin levels and antioxidant activity

Taking into account that the electrochemical studies showed differences in the antioxidant constituents between black and white table grapes, we have analysed non-enzymatic antioxidant compounds in the skin and pulp of both cultivars. However, we only show results obtained from the skin because total phenolic content and antioxidant activity from the pulp were very low and did not present significant differences among the samples analysed (data not shown).

The results showed that the modulation of total phenolic and anthocyanin content, as well as the antioxidant activity in CO₂-treated and non-treated skin samples were different in the first stage of storage at 0°C depending on the cultivar (Fig. 3). At this point, it is important to note that although grapes were harvested at different times (July and November), fruit were picked on its optimum state of maturity for commercialization. In the black cultivar, storage at low temperature reduced the total phenolic content in the skin of both CO₂-treated and non-treated table grapes, although the decrease was significantly higher in those exposed to the gaseous treatment (Fig. 3A). By contrast, in

the white cultivar, although the content was lower in comparison to the black one, the application of high levels of CO₂ significantly increased the total phenolic content in comparison to freshly-harvested fruit (Fig. 3B). Šamec and Piljac-Žegarac²⁷ observed that the total phenolic content of red and white table grapes decreased or did not vary after 2 days of storage at 4°C, respectively, increasing from the fourth day onwards in both cultivars. Artés-Hernández et al.²¹ recorded that Superior Seedless table grapes stored for 7 days at 0°C followed by 4 days at 8°C under modified atmosphere packaging did not change the total phenolic content, showing a slight decrease during the subsequent shelf-life. Likewise, the application of a pretreatment with high CO₂ levels (30 kPa) during the postharvest dehydration of white wine grapes at 20°C maintained the total polyphenol concentration.²⁸

Mazza et al.²⁹ indicated that varietal factors determine the concentration, distribution and accumulation of polyphenols in grapes. Furthermore, it is known that the synthesis of polyphenols in grapes is also determined by many factors such as temperature, water availability and sun exposure of the clusters. In this sense, we have observed in red Cardinal table grapes that the total phenolic content and their modulation in response to high CO₂ levels and low temperature varied depending on the maturity stage.⁶ Thus, the differences observed in the pattern of accumulation of the total phenolic content between Cardinal⁶ and Autumn Royal (this work) could be related to varietal factors, and also because unlike Cardinal cultivar (which matures from late spring to mid-summer), Autumn Royal is a late-maturing table grape cultivar (from autumn to early winter).

The total anthocyanin content decreased similarly in the skin of both CO₂-treated and non-treated AR table grapes after 3 days at 0°C (Fig. 3A). In the white cultivar, the anthocyanin content was 128 times lower than in the black cultivar, and showed no changes after storage at 0°C in treated or non-treated grapes (Fig. 3B). In both cultivars, a significant moderate correlation was noticed between total phenolic and anthocyanin content ($r = 0.761$ (AR), $r = 0.757$ (SS), $P < 0.05$). It is already known that anthocyanin synthesis continues after harvest as well as during long-term cold storage, and is inhibited in fruit treated with high CO₂ concentrations.³⁰ In reference to the anthocyanin content, our results are not in concordance with previous ones obtained in Cardinal table grapes stored at low temperature^{6,7} and 3 days of storage at 0°C were not enough to induce its content in AR. This fact could be linked to the differences in the harvest time between Cardinal and AR, where there is a temperature shift from the field to 0°C which was higher in Cardinal table grapes harvested in July, in comparison to AR picked in November. Although future studies are needed to address this assumption, the anthocyanin concentration was higher in aster flowers grown under temperature shift 17/9°C (day/night) and it declined when the temperature shift was lower (23/15°C and 29/21°C (day/night)).³¹ Stiles et al.³² suggested that increased anthocyanin production at low temperature in flowers is adaptive because of the warming effect of anthocyanins.

The antioxidant capacity determined by the ABTS and FRAP assays decreased in CO₂-treated AR grapes, whereas it did not change in non-treated ones. In this sense, a significant strong positive correlation ($r = 0.917$, $P < 0.01$) was observed between the results obtained by both assays. However, in the white table grapes, the ABTS and FRAP

assays showed a decrease in the antioxidant activity in non-treated fruit. By contrast, in this cultivar the CO₂ treatment increased the antioxidant activity, although it was only significant when it was determined by the FRAP method. Likewise, as in the black cultivar, a significant strong positive correlation ($r = 0.905$, $P < 0.01$) was noticed between ABTS and FRAP determinations. The statistical analysis indicated that in both cultivars the total phenolic content correlated with the antioxidant activity, but with differences depending on the assay used and on the cultivar. Thus, in the black cultivar a strong positive correlation was observed between the total phenolic content and antioxidant capacity using the ABTS assay ($r = 0.933$, $P < 0.01$), and showed a lower correlation in the case of FRAP determination ($r = 0.751$, $P < 0.05$). However, in the white cultivar a fairly good correlation was found between the FRAP values and total phenolic content ($r = 0.909$, $P < 0.01$), being lower with the ABTS ($r = 0.695$, $P < 0.05$). The antioxidant capacity of grape-derived products is affected by their total phenol content, which is influenced mainly by the grape variety³³. Conversely, correlation values between antioxidant capacity and anthocyanins were moderate and non-significant (ABTS, $r = 0.48$; FRAP, $r = 0.15$, $P < 0.05$). In a similar vein, different works evidenced that anthocyanins seem to be less significantly correlated with the antioxidant properties in grapes,³⁴ which is consistent with our results. These findings indicated that other phenolics compounds which are different from anthocyanins seem to participate in the antioxidant activity recorded.

Effect of high levels of CO₂ on gene expression of antioxidant enzymes in the skin and pulp of black and white table grapes stored at 0°C

To determine the role of antioxidant enzymes in the effect of CO₂ in black and white table grapes stored at 0°C, we have analysed changes in the expression of *GCAT* and *VcAPX* (Fig. 4). Results showed that *GCAT* gene expression was significantly induced by low temperature in the skin of non-treated AR and SS berries (Fig. 4) and that the application of high levels of CO₂ restrained this accumulation. In the pulp, the pattern of expression was different in both cultivars. Thus, while *GCAT* increased in the black cultivar regardless of the atmosphere, in the white cultivar the application of high levels of CO₂ induced a sharp increase in the *GCAT* transcript accumulation (Fig. 4). The pattern of *VcAPX* accumulation was quite similar in both tissues of each cultivar (Fig. 4). In the skin and pulp of AR fruit, the storage at 0°C significantly decreased the gene expression in both treated and non-treated fruit. However, this decrease was noticed in CO₂-treated SS samples, whereas the gene expression did not change in fruit stored in air. A previous study indicated that the beneficial effect of the pretreatment with high CO₂ levels to control oxidative stress by the induction of the enzymatic antioxidant system depends on the type of tissue.¹⁰ In this sense, although we had previously suggested that *APX* participate in removing the putative high levels of H₂O₂ in the skin of non-treated Cardinal grapes,³⁵ in the case of AR and SS this fact seems to be due to *CAT*. But it is important to note that, in the case of the skin, the accumulation of *APX* or *CAT* transcripts in CO₂-treated samples was always significantly lower than the levels achieved in non-treated ones. Conversely, Ni et al.³⁶ reported that a gaseous treatment with hydrogen

sulfide alleviated postharvest senescence and rotting of Kyoho grape by enhancing the activities of APX and CAT in both grape skin and pulp during storage. Similarly, chitosan coating positively influenced the physical-chemical and nutraceutical traits of three strawberry cultivars, reducing postharvest oxidative stress through the increase of APX activity but not CAT.³⁷ However, we have observed that the beneficial effect of the CO₂ treatment to control oxidative stress by modulating genes implicated in the enzymatic antioxidant system was closely related with the reduction of rachis browning rather than with a general response in table grapes.¹⁰

CONCLUSIONS

Our results indicate that the application of a 3-day gaseous treatment was effective in maintaining the quality of black and white table grapes. Moreover, the present work showed that the solid state voltammetry methodology can be used for characterising table grape cultivars and monitoring postharvest treatments. Furthermore, the electrochemical approach was useful to confirm that the behaviour of CO₂-treated samples, independently of the cultivar, was closer to that of freshly-harvested ones reinforcing previous results using a transcriptomic approach.² In this sense, the activation of *GCAT* gene expression in the skin of non-treated grapes from both cultivars could be activated to overcome oxidative stress due to cold storage which was less noticeable in CO₂-treated samples.

ACKNOWLEDGMENTS

This work was supported by CICYT projects AGL2014-53081-R and AGL2017-85291-R (MINECO/AEI/FEDER, UE). The authors are grateful to Frutas Esther S.A for providing tables grapes.

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FIGURE CAPTIONS

Figure 1. Solid state square wave voltammetry at glassy carbon-working electrode of pulp (A, B) and skin (C, D) from AR cultivar. Potential scan initiated at -0.05 V (A, C) and -1.05 V (B, D) in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

Figure 2. Two-dimensional PCA loading plots of the pulp (A, C) and skin (B, D) of the two principal components explaining 100% of the variance for AR (A, B) and SS (C, D) cultivars. Data were obtained from values of the ratio between the current at a given potential and the maximum peak current ($i(E)/i_{max}$) in square wave voltammetry, taking potentials between -0.8 and $+0.2$ V at intervals of 50 mV.

Figure 3. Changes in total phenolic content, total anthocyanin levels and antioxidant activity measured by ABTS and FRAP in the skin of non-treated and CO₂-treated AR (A) and SS (B) table grapes stored at 0°C. Different letters on the bars indicate that the values are statistically different using the Tukey-b test ($P<0.05$).

Figure 4. Expression levels of *GCAT* and *VcAPX* in the skin and pulp of non-treated and CO₂-treated AR (A) and SS (B) table grapes stored at 0°C. Values are the means of three replicates $\pm$ S.E. Different letters on the bars indicate that the values are statistically different using the Tukey-b test ($P<0.05$).

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609 **Table 1.** Soluble solids content (SSC), titratable acidity (TA), pH, weight loss, total decay and rachis browning index of AR and SS table grapes treated with CO₂ and
 610 stored during 28 days at 0°C.

	Autumn Royal			Superior Seedless		
	Freshly-harvested	0°C		Freshly-harvested	0°C	
		28 d Air	3 d CO ₂ + 25 d Air		28 d Air	3 d CO ₂ + 25 d Air
SSC (%)	12.87 ± 0.18 a	13.00 ± 0.26 a	13.40 ± 0.26 a	14.77 ± 0.75 a	14.25 ± 0.58a	15.53 ± 0.42 a
TA (% Tartaric Acid)	0.46 ± 0.04 a	0.41 ± 0.01 a	0.43 ± 0.02 a	0.47 ± 0.02 a	0.48 ± 0.01 a	0.49 ± 0.01 a
Maturity Index (SSC/TA)	27.97	31.70	31.16	31.42	29.68	31.69
pH	3.88 ± 0.04 a	3.77 ± 0.06 a	3.81 ± 0.03 a	3.72 ± 0.06 a	3.65 ± 0.04 a	3.68 ± 0.01 a
Weight Loss (%)	-	5.10 ± 0.17 b	4.30 ± 0.18 a	-	3.62 ± 0.40 b	2.09 ± 0.02 a
Total Decay (%)	-	4.40 ± 0.60 b	2.30 ± 0.50 a	-	-	-
Rachis Browning Index	-	3.70 ± 0.20 b	2.70 ± 0.20 a	-	3.00 ± 0.40 b	2.00 ± 0.35 a

611 Values are the mean of three replicate samples ±SE.

612 Different letters within each row indicate that means are statistically different according to Tukey-b test ($P \leq 0.05$)

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614

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Figure 1

Figure 2

Figure 4