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ORIGINAL RESEARCH ARTICLE

Changes of anthocyanins and residual ethylene of table grapes with color promotants treated

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ABSTRACT

Different color-promoting treatments were tested on table grape cv. "Flame Seedlees" to evaluate changes on flavonoids such as anthocyanins and the residual ethylene produced. Treatments were spray-applied at the onset of veraison. The control was Ethrel at 250 ppm (ETH), Salicylic Acid at 100 ppm (AS), Melatonin at 25 ppm (MEL) and 1:1 mixtures of ETH+AS, ETH+MEL and AS+MEL. The trials were conducted in triplicate after harvest, measuring Total Soluble Solids (% TSS), total acidity (% tartaric acid), pH, residual ethylene (ppm) and anthocyanin content ($mg \cdot cm^{-2}$). It was found that treatments ETH, AS, MEL and ETH+AS reached 16% TSS, standing out with lower values ETH +MEL (14.27%) and AS+MEL (15.17%) ($p \leq 0.05$). ETH reached 0.83 ppm of residual ethylene, while a sum effect was appreciated in ETH+AS (0.5 ppm) and ETH+MEL (0.35 ppm), but not beneficial as it did not reflect quality characteristics. Only differences ($p \leq 0.05$) in anthocyanin content were recorded between ETH (0.019 $mg \cdot cm^{-2}$) and AS+MEL (0.003 $mg \cdot cm^{-2}$). The subjective color of the grape bunches in the field made it possible to relate it to the objective results of the analyses performed. This research provides commercially important information on the substitution of Ethrel by natural compounds such as AS and MEL, as they show similar effects on the quality of "Flame Seedless" table grapes. In addition, these compounds do not have an ethylene residual greater than 0.2 mg/kg.

Keywords: Color; Residual; Melatonin; Salicylic Acid

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1. Introduction

In table grapes, especially in red grapes such as cv. "Flame Seedless", several studies have been conducted to obtain uniformity in berry color[1,2], since this is one of the quality characteristics of relevance both commercially and for the consumer. It is also known that berry color can be affected during berry development and ripening. Applications of compounds that help grape coloration have been applied during veraison, a stage where color change begins, there is an increase in anthocyanins, total soluble solids (TSS) and aromatic compounds, and the content of organic acids decreases. On the one hand, there is a strong trend towards the use of natural growth regulators, biostimulants, among others, that do not generate residuals and are more compatible with the environment. However, compounds such as Ethrel are still used which, although some favorable results have been obtained in increasing the color of grapes[3,4]; it is also being restricted by international markets such as Europe. On the other hand, salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature, it is easy and safe to use in pre- or post-harvest applications. This has been employed, alone or in combination, in preharvest to increase color and maintain quality in

grapes[5–7]. In a previous study, Gámez *et al.*[8] treated "Flame Seedless" grapes during veraison with 50 ppm AS at pH 7.5. They observed an increase in color values from 1.10 to 4.26 on the CIRG scale, where 3.66 is the estimated value for red grapes. Likewise, substances such as abscisic acid and SA, have been evaluated to induce endogenous ethylene synthesis with effects similar to Ethrel[9–11]. As for Melatonin (N-acetyl-5-methoxytryptamine) (MEL), it is a secondary metabolite in plants and its role is as a phytohormone[12]). Vitallini *et al.*[13] related high antiradical activity with a synergistic effect between MEL and grape polyphenols. Melatonin has the ability to cross cell membranes and enter different subcellular compartments due to its amphipathic nature[14], so that an exogenous application of melatonin is easily absorbed. During pre-veraison, exogenous application of melatonin in grapes causes changes in fruit size and weight, favoring its synchronization in the ripening process, as well as a greater accumulation of sugars[15]. In addition to its antioxidant capacity[16], applied MEL (100 µM) in strawberries[17] favored the accumulation of anthocyanins, activating enzymes for ATP supply for the phenylpropanoid pathway. Therefore, the main objective of the present research was to apply exogenously AS, MEL and their combination, as an alternative to obtain the effects produced by Ethrel on the quality characteristics of table grape "Flame Seedless".

2. Materials and methods

2.1 Raw materials and treatments

The study was conducted on 40 table grape (*Vitis vinifera* L.) cv. "Flame Seedless" plants within the vineyard "Las Mercedes", located in the region of Pesqueira, San Miguel de Horcasitas, Sonora, Mexico. Treatments were single spray application： the control consisted of Ethrel solution at 250 ppm (ETH), Salicylic Acid at 100 ppm (AS), Melatonin at 25 ppm (MEL) and 1:1 mixtures of ETH+AS, ETH+MEL and AS+MEL. The mixtures were prepared with double distilled water, 21.7% ethrel (ETHREL^{mr} 240, BAYER), salicylic acid (FAGALAB, MEX), melatonin 5 mg (tablets) and 95% ethyl alcohol (FAGALAB, MEX). Applications were made at midday, during the veraison stage, approximately 60 days after full flowering.

2.1.1 Analyses performed

After harvest (14 days after applications), 15 bunches from each of 5 plants were taken at random from each treatment and immediately transferred to refrigerated coolers to reduce field heat for the following analyses. For firmness, from each treatment, 100 berries were randomly cut from the top, middle and bottom of the clusters. To evaluate the total titratable acidity (AOAC 942.15, 1990), from the previous group, 40 berries were crushed and the filtrate was neutralized with NaOH 0.1 N (SIGMA, Missouri, USA) in an automatic titrator Mettler (DL67, Switzerland) reporting the result as percentage of tartaric acid (% TA). From the previous filtrate, the sample to measure Total Soluble Solids (% TSS) was obtained using an ATAGO digital refractometer (PR-101, Tokyo, Japan), using HPLC water as blank.

2.1.2 Residual ethylene

The evaluation of this gas was performed by GC-HS, using the method proposed by Tseng *et al.*[18], modified by Gámez *et al.*[19]. This test was performed in triplicate, incubating 250 µL of the sample at 60 ℃ for 1 h in an Isotemp water bath (128, Pennsylvania, USA). 1 mL of the headspace was injected into a VARIAN gas chromatograph (Star 3400, California, USA) with a flame ionization detector (FID) and a Hayesep N 80/100 column. The C.H. residual was reported in ppm.

2.1.3 Anthocyanins

These were analyzed according to the method described by Peppi[19], Neff and Chory[20] and Xu *et al.*[21], with certain modifications. 1.0 g of grape skins obtained from 7 berries were ground in a cold mortar in triplicate. These were mixed with 10 mL of methanol with 1% (v/v) HCl. The filtrate of the mixture was taken to a Varian spectrophotometer (Cary50, Mulgrave, Australia) taking readings at 530 and 657 nm. The anthocyanin content was expressed as mg·cm^{-2}.

2.1.4 Analysis of results

The experiment was carried out on the basis of a completely randomized design with three replications. The data were subjected to a one-way analysis of variance and the means with significant differences were compared with the Tukey-Kramer test. Statistical analysis was performed using the NCSS program, version 12, with a confidence level of $p \leq 0.05$.

3. Results

3.1 Quality characteristics

In general, similar results were obtained between the ETH control and the treatments with their combinations. According to NMX-FF-026-SCFI-2006, "Flame Seedless" table grapes must contain a minimum of 16% TSS in any of its categories. As shown in **Table 1**, this value was achieved in ETH, AS, MEL and ETH+AS. Despite the differences among treatments ($p \leq 0.05$), those that stood out with the lowest TSS value were ETH+MEL (14.27%) and AS+MEL (15.17%). In this last variable, about 0.1% less acidity was obtained with respect to the other treatments. In another study[22], applying 100 ppm AS in "Fame Seedless" a slightly lower value of TA of 0.76% was obtained, although a higher percentage implies a lower degree of maturity[23]. The pH resulted slightly lower in the treatments combined with MEL. The above results suggest that MEL, in combination with ETH or AS, reduced grape ripening. Tijero-Esteve[24], the results of the study, after 19 days, found a lower accumulation of anthocyanins in cherries treated with 10^{-5} M MEL compared to the control. The delay in the accumulation of this pigment directly affected fruit ripening, suggesting that the exogenous application of melatonin inhibits the ripening process of cherries.

Table 1. Changes in TSS, TA and pH quality characteristics of "Flame Seedless" table grape by effect of exogenous applications of Ethrel (ETH), salicylic acid (AS), melatonin (MEL) and their combinations

Treatments	TSS (%)	TA (%)	pH
ETH	16.33 ± 0.03^{b}*	$0.87 \pm 0.01\ l^{b}$	3.28 ± 0.003^{bc}
AS	16.13 ± 0.03^{bc}	0.88 ± 0.007^{b}	3.32 ± 0.018^{b}
MEL	16.70 ± 0.06^{a}	0.89 ± 0.006^{b}	3.42 ± 0.015^{a}
ETH-AS	16.10 ± 0.00^{c}	0.86 ± 0.002^{b}	3.38 ± 0.012^{a}
ETH+MEL	14.27 ± 0.07^{e}	0.97 ± 0.005^{a}	3.24 ± 0.007^{cd}
AS+MEL	15.17 ± 0.03^{d}	0.95 ± 0.008^{a}	3.20 ± 0.015^{d}

* means in column with different superscript are different ($p \leq 0.05$). Standard error (±).

3.2 Residual ethylene

It was expected that the highest ethylene values would be found in the application of ETH. This reached 0.83 ppm ethylene, showing significant differences with respect to the other treatments (**Figure 1**). Residual ethylene can give an idea of how much of this compound has been used metabolically to produce some effect, in this case for grapes. Thus, it could be assumed that part of the amounts recorded for AS (0.16 ppm) and MEL (0.09 ppm) were used to produce the changes in the quality characteristics mentioned above in this study. In addition, a sum effect of ethylene content in ETH+AS (0.5 ppm) and ETH+MEL (0.35 ppm) was appreciated, but not beneficial as it was not reflected in the quality characteristics. As for the AS+MEL mixture (0.23 ppm), the sum effect of ethylene values is probably due to AS, since MEL has been associated with the reduction of one of the promoters of ethylene synthesis, such as ABA[25].

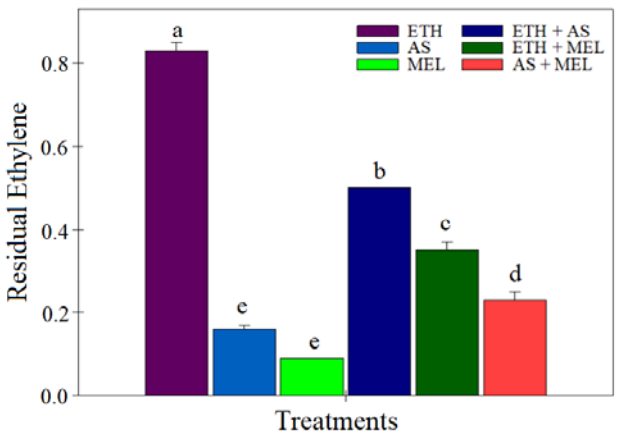

Figure 1. Response of residual ethylene (ppm) in "Flame Seedless" table grapes to exogenous applications of Ethrel (ETH), salicylic acid (AS), melatonin (MEL) and their combinations.

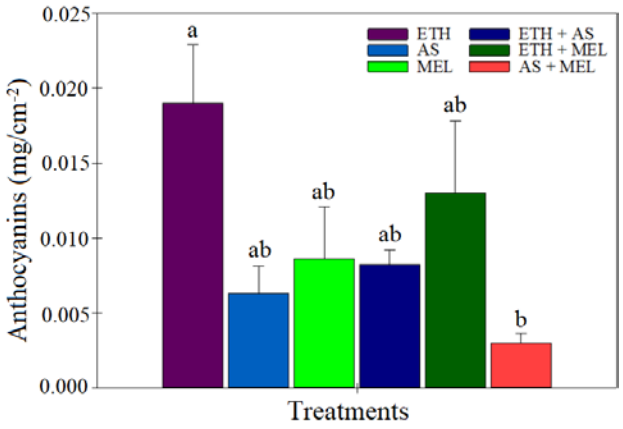

Figure 2. Anthocyanin content (mg·cm^{-2}) for "Flame Seedless" table grapes by effect of exogenous applications of Ethrel (ETH), salicylic acid (AS), melatonin (MEL) and their combinations.

3.3 Anthocyanins

No significant differences in total anthocyanin values were found for ETH (0.019 mg·cm^{-2}), AS (0.0063 mg·cm^{-2}), MEL (0.0086 mg·cm^{-2}), ETH+AS (0.0082 mg·cm^{-2}) and ETH+MEL (0.013 mg·cm^{-2}). While the difference ($p \leq 0.05$) occurred between ETH and AS+MEL (0.003 mg·cm^{-2}) (**Figure 2**). It is likely that the combination with AS potentiated the MEL response. It has been reported that exogenous application of AS in pre-harvest "Niagara Rosada" grapes induces increased serotonin and MEL content with beneficial effects on increasing shelf life[26]. Similarly, pre-harvest spraying of 1.5 mM with AS proved to be an effective means to improve quality and prolong postharvest life of grape cv. "Flame Seedless"[6]. It was also successful in maintaining anthocyanin content and grape skin color. On the other hand, Rodriguez-Naranjo *et al.*[27], did not find MEL in any of the analyzed parts of the grape (pulp, seed or peel). However, in a previous study with ELISA tests it was found present in the peel of eight different grape varieties. In any case, whether or not MEL is present in grape skins, it has been shown that the response to exogenous application of melatonin depends on the concentration applied, considering that it could be present endogenously. At low concentrations (10^{-5} M), MEL delays anthocyanin accumulation, while at high concentrations (10^{-4} M), an increase in acidity is observed[24]. In our case MEL alone did not retard anthocyanin accumulation. Rather, as mentioned, there were no significant changes in their accumulation that could be related to changes in peel color. Regarding the above, Peppi *et al.*[28] mention that anthocyanin concentrations between 0.01 and 0.04 mg·cm^{-2} had little effect on berry brightness and hue, so they recommend measuring color, not only anthocyanins, when evaluating the quality of red table grapes. These are values very close to those found in our experiment.

3.4 Color of grapes in the field

Lancaster *et al.*[3], referring to anthocyanins, mentioned that the relationships between pigment concentrations of a fruit and its color may not be linear. This applies to what was found in our study. It was interesting to see that objective measurements, such as residual ethylene, were related to observations of the fruit in the field (**Figure 3**). The photograph shows how ETH showed a greater number of colored berries. However, in many clusters the color was not completely covered in all the grapes, and colored berries of different sizes were observed, even in the small ones. In the latter, the flavor was acidic and tasteless. The color observations with the AS treatment were very similar to those of the ETH applications. As can be seen in the image, with AS there was a more complete color coverage in the berry, not by zones as was seen with ETH, and even the size of the grape was more uniform. The flavor of the grapes with AS was similar when tasting berries from the other treatments that had similar characteristics in terms of size and color. Except for the grapes with ETH+MEL and AS+MEL treatment, which coincided with the data obtained in SST. Another aspect that we were able to observe in these combinations was that the berry apparently presented a larger and more uniform size in the clusters. This effect was reported by Liu *et al.*[29], when applying a dose of 100 µmol·L^{-1} of MEL in pear variety Zaosu, where fruit size and weight increased compared to the control. Likewise, the combinations of ETH+AS and ETH+MEL, visually were the least able to cover the berries with color compared to the other treatments. Interestingly, these two combinations showed higher residual ethylene content than berries treated individually

with AS or MEL. In the treatment with MEL alone, the clusters were more compact and with more green berries. Finally, grapes with AS+MEL showed more red color than MEL alone.

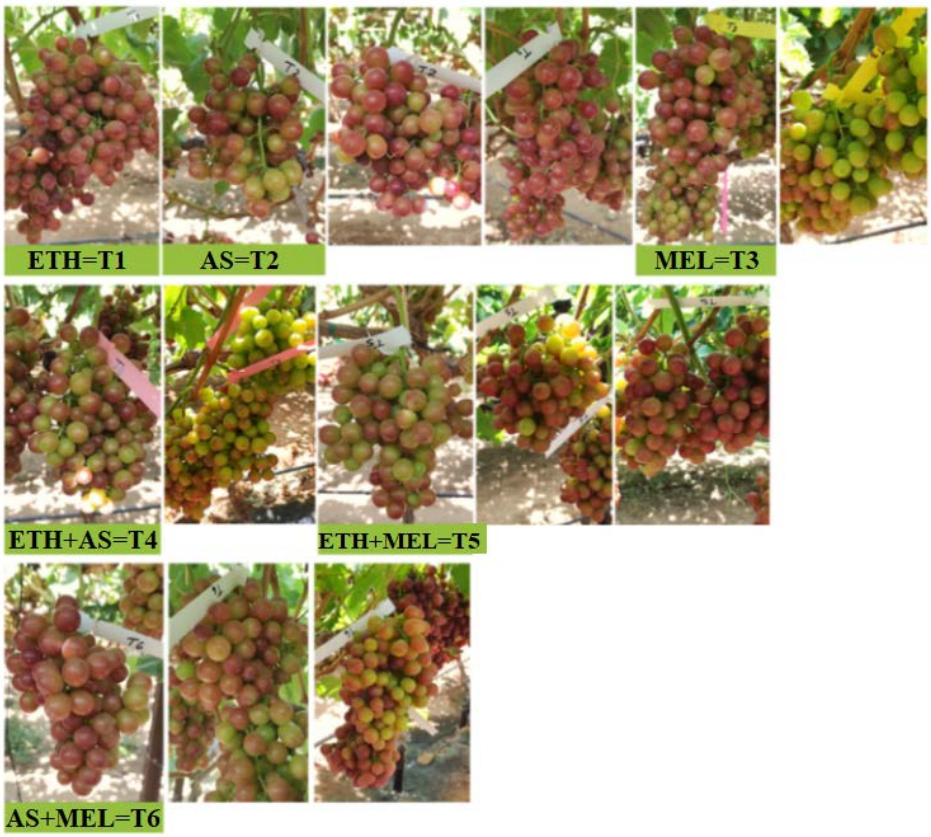

Figure 3. Images of "Flame Seedless" table grape clusters in the field, with treatments and just before harvest.

Even with these observations, we considered that a date beyond 14 days was required to harvest and further evaluate the effects of AS and MEL. The above is supported by previous observations, especially with AS where berry color was even better covered than with Ethrel in several applications[8,30]. On the other hand, the agronomic practice in vineyards has been to make multiple applications of ethylene and other chemicals in their eagerness to achieve in less time the coloration of the grapes. Therefore, this research provides commercially important information regarding the substitution of Ethrel by natural compounds such as AS and MEL, as they show similar effects on the quality of "Flame Seedless" grapes. In addition, these compounds do not have an ethylene residual greater than 0.2 mg/kg.

5. Conclusions

Salicylic acid could be a potential substitute for Ethrel by allowing a similar response on quality variables, anthocyanin content and skin color in "Flame Seedlees" table grapes. The application of melatonin and its combination with salicylic acid could be very useful during pre- and postharvest handling of this variety.

Conflict of interest

The authors declared no conflict of interest.

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