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Research Article

Antioxidant activity and bio compounds induced by salicylic acid and potassium from 'Flame' grapes

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Abstract

The objective of this study was to determine the antioxidant activity and bio compounds induced by salicylic acid (AS) and potassium (K) in 'Flame' grape peel and pulp. The applications were made in table grape of the 'Flame' variety, with 9 treatments and 3 repetitions, T1 control 0.0 (T2 AS 0.0, K 5 mM) (T3 AS 2.0, K 5.0 mM) (T4 AS 2.0, K 0 mM) (T5 AS 0.100, K 0.250 mM) (T6 AS 0.100, K 2. 50 mM) (T7 AS 1, K 2. 50 mM) (T8 AS 1, K .250 mM) (T9 AS 0. 25, K .625 mM) with 6 applications in the veraison stage throughout the cycle. The parameters of antioxidant capacity were determined by the DPPH and FRAP method, phenols, anthocyanins, flavonoids, pH, °Brix, titratable acidity and physical parameters in grape peel and pulp. Doses of AS 2.0, K 0 mM; AS 1, K 2.50 mM; and AS 0.25, K .625 mM influenced the increase in quality, as well as the bioactive and antioxidant activity. Anthocyanins were the main phenols in peel with 406.08 mg (C3G)/g^{-1}. 'Flame' table grape peels have a high content of compounds, favouring the antioxidant activity. A serving of unpeeled table grapes could provide up to 110 mg of phenols. The use of salicylic acid and potassium can be an alternative to enrich the nutritional quality of the grape and benefit the health of the population.

Keywords: anthocyanins; physicochemical parameters; *Vitis vinifera*

Introduction

Nowadays it has been proven that eating habits characterized by nutrition disorders and stress have had a negative effect on human health, bringing with it a significant increase in the number of chronic diseases such as obesity, diabetes, cancer, cardiovascular diseases and neurological disorders (Lee and Scagel, 2010). A study

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in Mexico determined that the main causes of death are related to heart diseases, accounting for 31.3% of deaths throughout the country (INEGI, 2013).

The *Vitis vinifera* grape is one of the products that is most widely consumed worldwide, due to its importance and commercial value which depends on the size of the berry, bunch, colour, juiciness and aroma (Cosme *et al.*, 2018). Veraison is an essential point in the development of the berry, because it is when the pigmentation of the peel, the synthesis of sugars and aromatic compounds begins (García *et al.*, 2019). In addition, grapes contain phenolic compounds, flavonoids and anthocyanins generating beneficial antioxidant effects for health, by preventing various damages related to tissue oxidation and that can reduce risks of cardiovascular diseases, diabetes, inflammations and cancers (Fortes *et al.*, 2011). Other authors mention that the peel of grapes, especially in epidermal cells, have a higher content of phenolic compounds of very different chemical structures unlike the pulp, such as anthocyanins which provide the colour and antioxidant activity trapping free radicals and exerting lipid control, with a protective effect against cardiovascular diseases (Sandoval *et al.*, 2008). Also, it is included as a functional food in its nutritional role and plays a role in shelf life (Molina-Quijada *et al.*, 2010).

On the other hand, growth regulators have become a considerable tool in viticulture, for their positive effect on the quality, appearance, size and development of the fruit (Kook, 2018). In the same way, it has been proven that AS is a hormonal compound with very broad functions in the physiological processes of plants, is part of a large group of phenolic compounds, and participates in many metabolic functions, such as coloration, absorption of nutrients, protein synthesis, resistance to diseases of the fruit, which reduces its deterioration, discoloration and prolongs its life (Glowaez and Rees, 2016). Likewise, applications of AS have been made, favouring the content of polyphenols, delayed the ripening, quality improvement and increased antioxidant potential in pomegranate arils and apricot fruits (Wang *et al.*, 2015; Fernández *et al.*, 2019). On the other hand, some researchers mention that the potassium activated the antioxidant system and reduced the production of free radicals (Devi *et al.*, 2012). Cakmak (2005) mentions that K reduces the activity of NADPH oxidases and thereby decreases the production of oxygen reactive species and an increase in NADPH oxidation can be up to 8 times greater in plants with low K supply than in plants with enough K. In addition, potassium deficiency causes a severe reduction in photosynthetic CO_2 fixation and deterioration in the partitioning and use of photosynthates.

Due to this, alternatives have been sought which could lead us to produce safer foods with a greater nutritional contribution, and that promote health by reducing the risk of suffering from chronic diseases. Without a doubt at a global level, it is of great importance (Tilman and Clark, 2014). Therefore, the objective of this study is to analyse the antioxidant activity and bio compounds induced by salicylic acid and potassium in table grapes from 'Flame' variety, in both pulp and peel.

Materials and Methods

Location

The work was carried out in field 3B in the city of Cuauhtémoc, Chihuahua. In a vineyard located at 28.481468, -106.971815. m.s.n.m. The vineyard is equipped with a structure for the driving system. The material used was 12-year-old 'Flame' table grapes in a 3 x 2 spread.

Treatments and experimental design

Applications of salicylic acid (AS) and potassium (K) were made by foliar spraying at different concentrations, with 9 treatments: (T1 control AS 0.0 K 0.0) (T2 AS 0.0, K 5 mM) (T3 AS 2.0, K 5.0 mM) (T4 AS 2.0, K 0 mM) (T5 AS 0.100, K 0.250 mM) (T6 AS 0.100, K 2.50 mM) (T7 AS 1, K 2.50 mM) (T8 AS 1, K .250 mM) (T9 AS 0.25, K .625 mM) with 16 plants per row in 3 rows and 3 repetitions, in a completely random factorial design. The applications were made in the veraison stage, and 6 applications were made. The harvest was obtained in September 2019.

Plant material

Once the grapes were collected, they were transferred to the laboratory of the Faculty of Agrotechnological Sciences UACH where they were washed with distilled water and then proceeded to remove the peel from the pulp. The pulp was saved for further analysis and the peel was taken to a drying stove at 50 °C for 24 h. After the process was finished, they were ground in a coffee mill and once the powder was obtained, they were sifted in a mesh No. 20 to obtain a particle size of 0.84 mm. Then, they were weighed to determine the yield in grams. The samples were refrigerated until use.

Sample preparation

1 g of sample was taken and homogenized with 20 ml of methanol at 80%, from there they were taken to the sonicator for an hour and then placed in the centrifuge at 4000 x g for 20 minutes at 4 °C. Finally, the supernatant was separated, and the residue was extracted once again for the realization of the analyses.

Variables to measure

Quality parameters

Weight. The total weight of the bunch was determined, then, 50 randomly taken grapes were weighed and the weight was recorded as g/ 100 in an analytical balance, doing this in triplicate AOAC, 2000. The titratable acidity as tartaric acid was determined by titration with NaOH 0.1N, with the methodology of the AOAC, 2000. The total Soluble Solids (°Bx) were determined in the pulp of the fruit with a digital refractometer brand ATAGO, with a scale of 0 to 53% and were expressed as °Bx. The grape ripeness index SSC/TA ratio was calculated as a defined maturity index, the determination of pH was measured with a pH meter and the humidity was determined by the 925.40 method of drying in open capsule.

Extraction of bioactive compounds phenols

They were determined according to the methodology described by Singleton and Rossi (1965). In a test tube, 1.5 ml of Na_2CO_3 at 2% was placed and 0.5 ml of Folin-Ciocalteau reactive at 50%, 2.75 ml of deionized H2O and 0.5 ml of the extract were added. The mixture was incubated at room temperature and in darkness for 30 minutes. Absorbance was measured at 765 nm on a Thermo Scientific spectrophotometer, G 10S UV Vis, (USA). The results were expressed as milligrams of gallic acid.

Flavonoids

They were performed according to the methodology of Zhishen *et al.* (1999). 500 µL of extract was placed and mixed with 150 µL of 5% sodium nitrite ($NaNO_2$), the reaction was left to stand for 5 min, then 150 µL of aluminium chloride ($AlCl_3$) at 10% and 500 µL of NaOH 1 M were added. The mixture was diluted to a final volume of 5 ml with distilled water. Absorbance was measured at 415 nm and quantified on a standard catechin curve (0.2-0.12 mg/mL). The resulting values are expressed in mg catechin equivalents per gram of sample (mg EC/g).

Anthocyanins

They were determined according to the differential pH method described by Wrolstad (1976). The extract was diluted 1:5 with potassium 0.025 M chloride, pH 1.0 and 0.4 M sodium acetate, pH 4.5, as buffers. The absorption spectrum of 460 nm to 710 nm. It was calculated using the formula $AM = (A \times MW \times DF \times 1000) / (\varepsilon \times l)$, where the absorbance of the sample $A = (A\lambda \text{ vis-max} - A700)\ pH1.0 - (A\lambda \text{ vis-max} - A700)\ pH4.5$, MW is the molecular weight (449.2), ε is the cyanidin-3-glycoside Molar absorption (26,900), DF is the used dilution factor, and 1 is the path length (1 cm). Results are reported as cyanidin-3-glucoside mg (C3G)/g sample.

Antioxidant capacity DPPH method

It was carried out according to the methodology of Meir *et al.* (1995). 100 μL from the extract were taken and mixed with 2.9 mL of a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) freshly prepared, and the mixture will be incubated for 60 min. in darkness. Absorbance shall be measured by spectrophotometry at A515 nm. For the white sample, the extract will be replaced by 0,5 mL of methanol.

Antioxidant capacity FRAP method

The antioxidant power by reduction of the ferric to ferrous ion was determined using the methodology of Rubio *et al.* (2016) with slight modifications. First, a stock solution was prepared, which was made under acidic conditions (pH 3.6), that includes a buffer solution of sodium acetate (300 mmol/L at a pH of 3.6). Subsequently, the iron-TPTZ complex was prepared with 20 mmol of $FeCl_3{\cdot}6H_2O$ in a solution of TPTZ (2,4,6-tripiridil-s-triazin) in 40 mmol of HCl. Once the Stock solutions were prepared, the working solution (FRAP solution) was prepared. The solutions were added at a ratio of 10:1:1 (Buffer: $FeCl_3{\cdot}6H_2O$: TPTZ·HCl). The readings were taken at 593 nm on a Fisher brand UV spectrophotometer. Ferric ion reduction occurs visually in a colour change, from transparent to blue. A standard curve was performed with trolox, with concentrations ranging from 0 to 200 M. Results were expressed as μM ET/g of sample.

Statistical analysis

The statistical analysis was performed using an analysis of variance and means of Tukey comparison ($p \geq 0.05$). For which the SAS statistical package was used. Version 9.0.

Results and Discussion

Physical parameters

The results of the physical parameters of the Flame grape are presented in Table 1 in which significant differences are observed $p \leq 0.05$ in the weight of the bunch, weight of the pulp, yield and humidity, being the dose of 0. 250 AS and 0.625 K mM which affected all parameters with an increase, this because cell division by the application of AS promotes the production of indoleacetic acid and naphthaleneacetic acid, which according to Salisbury and Ross (1994), are the main regulators of plant growth. Franco-Bañuelos *et al.* (2019), present humidity values of 78.7 to 80.9%. Similar to those obtained with 79.56%, who also mention that humidity is an important factor due to the high level of sugars required during ripening period to improve the quality of the berry. Agreeing with Hernández *et al.* (2011) who mention that physical characteristics are a very important factor, in relation to the size of the berry, since it is a determining characteristic in the quality of the grape, because it is directly or inversely related to the proportion of the pulp, peels and seeds and with the concentration of phenolic compounds, which have an effect in the prevention of chronic-degenerative diseases. This is corroborated by Khandaker *et al.* (2011), by mentioning that salicylic acid (SA), o-hydroxybenzoic acid, is one of the phenolic compounds produced in the plant.

Table 1. Physical parameters determined in the flame grape, treated with salicylic acid and potassium

Treat ment	Weigh of the bunch	Weigh of 50 grapes (g)	Pulp weight (g)	Peel weight (g)	Dry peel weight	Yield (%)	Humidity (%)
11	708.87b*	132.90 a	113.50 a	11.46 a	2.53 a	22.09 b	77.91 a
22	752.90 b	133.83 a	109.87 b	12. 46 a	2.56 a	20.13 b	79.81 a
33	807.80 b	138.20 a	118.90 a	12.06 a	2.46 a	20.53 b	75.47 a
44	900.63 a	125.47 b	109.80 b	11.16 a	2.43 a	21.81 b	78.19 a
55	683.63 b	122.80 a	106.73 a	10.40 a	2.56 a	24.05 a	75.35 b
66	779.63 b	135.83 a	118.93 a	12.36 a	2.50 a	20.27 b	79.73 a
77	632.60 b	131.93 a	114.13 a	11.83 a	2.63 a	22.60 b	77.40 a
88	637.80 b	120.83 b	102.17 b	12.10 a	2.86 a	23.74 a	76.26 a
99	907.13 a	139.43 a	120.43 a	12.83 a	3.13 a	24.43 a	79.57 a

*Means with different letters are different according to Tukey's test with p≤ 0.05. Each value represents the average of three replicas.

Quality parameters

Table 2 shows significant differences p ≤0.05 in the titratable acidity parameters, soluble solids and acidity/solids ratio. The dose of AS 0.25, K 0.625 presented the maximum value of 1.80% titratable acidity and 19.06 °Bx of soluble solids. The values of pH 3.22 and soluble solids 18.0 °Bx obtained by Hernández *et al*. (2011) are similar to those of this work. Lo'ay (2017) reports SS values of 17 °Bx and AT % of 0.78 and a AT/SS ratio of 41.88 in grapes treated with AS, obtaining favorable results, however, their values are lower than those obtained in this work. Titratable acidity indicates the content of tartaric and malic acids that constitute free organic acids, and it is the result of physiological processes of respiration, which are a component of the flavor and they decrease as the fruit ripens due to the dilution of acids with the increase of the size of the berries and increased pH, as a result of the migration of the acid compounds Franco-Bañuelos *et al*. (2019). Also, the SS/AT ratio trends to increase and this ratio is used in critics as an indicator of maturity (Dorey *et al*., 2016). Therefore, according to the results obtained, these were affected by the AS and K and meet the satisfactory degree of maturity. On the other hand, Puerto *et al*. (2014) made applications of potassium sulfate and mention that the effect influenced the quality variables, evidencing results at a lower dose, as is the case of this study.

Table 2. Physical parameters determined in the pulp of flame grapes, treated with salicylic acid and potassium

Treatment	Volume (ml)	pH	Titratable acidity (%)	SS (°Bx)	AT/SS Ratio
1	48.00 a*	3.36 a	1.33 a	14.93b	11.22 b
2	47.00 a	3.22 a	0.60 b	15.03b	25.05 a
3	46.66 a	3.20 a	0.73 b	16.13 a	22.09 a
4	49.33 a	3.22 a	0.56 b	16.06 a	28.67 a
5	46.66 a	3.27 a	0.60 b	17.26 a	28.76 a
6	47.33 a	3.22 a	0.50 b	15.66b	10.44 b
7	46.33 a	3.30 a	1.13 a	18.00 a	15.92 b
8	48.33 a	3.24 a	0.83 b	18.10 a	21.80 b
9	48.33 a	3.42 a	1.80 a	19.06 a	38.10 a

*Means with different letters are different according to the Tukey test with p≤ 0.05. Each value represents the average of three replicas.

Biocomposites in pulp and peel of Flame grape

The content of phenolic compounds, flavonoids and anthocyanins evaluated in the pulp and peel of Flame grape are shown in Table 3. The results indicate significant differences p ≤0.05 in the evaluated treatments. The dose of AS 0.25, K 0.625 had a higher content of phenols in pulp and peel. The increase in flavonoids in pulp and peel was observed in T7 and T4 respectively. The highest presence of anthocyanins in pulp and peel was detected in T4 and T9. Values that were influenced by the different applications of AS and K. It is not yet fully elucidated how AS increases phenolic compounds. However, AS is a molecule that induces the biosynthesis of compounds through the activity of Phenylalanine ammonium lyase (PAL) responsible for the de novo synthesis of phenols (Fernández *et al.*, 2019). In a study conducted by Molina-quijada *et al.* (2010), in grape peels, is shown that they have a phenol content of 10.60 mg g^{-1} and flavonoids of 8.81 mg g^{-1} lower than those obtained in this study. However, they mention that the phenol content can vary from 0.55 to 37.94 and even reach up to 50.00 mg g^{-1} as it was in this case. The increase in the concentration of phenols and flavonoids is due to the fact that AS generates biochemical stress in the cell suspensions and tissues of plants, leading to an increase in secondary metabolites in accordance with the established by Diaz *et al.* (2016). According to Hernández *et al.* (2011) the phenolic compounds of the grape are mostly concentrated in the peels, coinciding with the results obtained in this work since the peels have a higher content of compounds. In the same way, the presence of anthocyanins in the peels is quite high unlike the pulp. In a study carried out by Cantos *et al.* (2002) in the pulp of the Flame variety reports an anthocyanin content of 34.3 mg/Kg, Castillo and Gutiérrez (2014) obtained an anthocyanin content of 45.93 mg of malvidin 3 glucose/100 g. Anthocyanins contribute in a certain way to the contribution of the antioxidant capacity of the fruits and depending on their structure, it will be the way to act on the radicals. Kuskoski *et al.* (2005) point out that there is a greater correlation in antioxidant capacity with the content of phenols in fruits that are rich in anthocyanins, an assertion that coincides with what was obtained in this work. All these values vary depending on the region, varieties and crop management, as well as the methodologies and processes carried out.

Table 3. Content of phenols, flavonoids and anthocyanins in flame grape pulp, treated with salicylic acid and potassium

Treatment	Phenols (mg GAE/g-1)		Flavonoids mg/(EC)g-1		Anthocyanins (mg C3 G/g-1)	
	Pulp	Peel	Pulp	Peel	Pulp	Peel
1	29.33 cbd	134.00 e	1.27 e	101.31 e	0.38 d	113.35 d c
2	35.06 cb	143.26 de	5.04 dc	348.25 b	2.40 b	77.88 d
3	22.06 ed	194.20 b	2.85 de	212.63 d	1.64 cb	271.65 bac
4	21.86 ed	165.60 dc	11.47 b	395.29 a	3.79 a	182.99 bdc
5	26.46 ced	206.66 ba	6.30 c	209.34 d	0.76 cd	405.54 a
6	19.20 e	185.33 bc	11.62 b	297.61 c	0.88 cd	32.55 d
7	37.60 b	189.26 b	15.55 a	240.54 d	1.64 cb	294.72 ba
8	31.20 cb	146.06 de	6.14 c	302.16 c	1.39 c	178.57 bdc
9	54.26 a	217.93 a	11.47 b	233.80 d	3.41 a	406.08 a

*Means with different letters are different according to the Tukey test with p≤ 0.05. Each value represents the average of three replicas.

DPPH antioxidant activity in pulp and peel

Figure 1 shows the results obtained of antioxidant activity by the DPPH method in the 'Flame' grape, in which significant differences are observed p ≤0.05 in the evaluated treatments. The greatest antioxidant activity in the pulp occurs at doses of AS 2.0 and K 0.00 mN and in the peel at doses of AS 0.100 and K 2.50 mM. Franco-Bañuelos *et al.* (2019) obtained values of 6.7 to 45.3 µmol ET 100 g^{-1} in grape pulp applying auxins and they also mention that growth regulators play an important role in the production of secondary metabolites, and increase the synthesis of total antioxidants, very similar results to the ones obtained in this work. However, larger amounts were obtained in the peel, which coincide with other authors such as Chaves (2016) who obtained values of 661.17 (µm Trolox/g) higher than those obtained in this work. The results obtained confirm that the peel of red and green table grapes presented an important antioxidant capacity, attributed mainly to the high content of phenolic compounds, which have the ability to donate hydrogens to sequester radicals (Martínez-Valverde *et al.*, 2000).

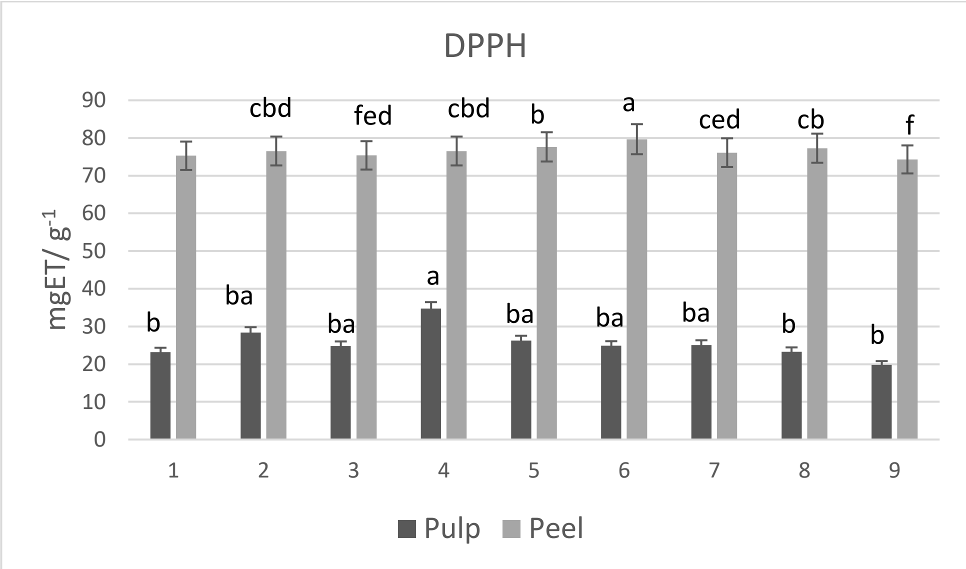

Figure 1. DPPH antioxidant activity in pulp and peel of 'Flame' grape.
Means with different letters are different according to Tukey's test with $p \leq 0.05$

FRAP antioxidant activity in pulp and peel of 'Flame' grape

The antioxidant activity determined in the pulp and peel of 'Flame' grape is observed in Figure 2, with significant differences $p \leq 0.05$ in the evaluated treatments. T6 had greater antioxidant activity in the peel and T9 in the pulp. Authors such as Vicente (2019) obtained values of 0.36 to 0.75 µMol AG/g in grape pulp and values of 1.64 to 3.80 µMol AG/g in grape peel in different varieties evaluated, which confirms that the peels present the highest values, results that are highly dependent on the concentrations of AS and K applied. Evaluating the antioxidant capacity by FRAP is very important because metals such as Fe^{2+} are able to initiate lipid peroxidation reactions of unsaturated fatty acids and some polyphenols have the ability to chelate different metal ions with which they avoid the deterioration of lipids (González, 2016). This allows us to better understand the physiological activity of these compounds according to their solubility (Martínez-Valverde *et al.*, 2000).

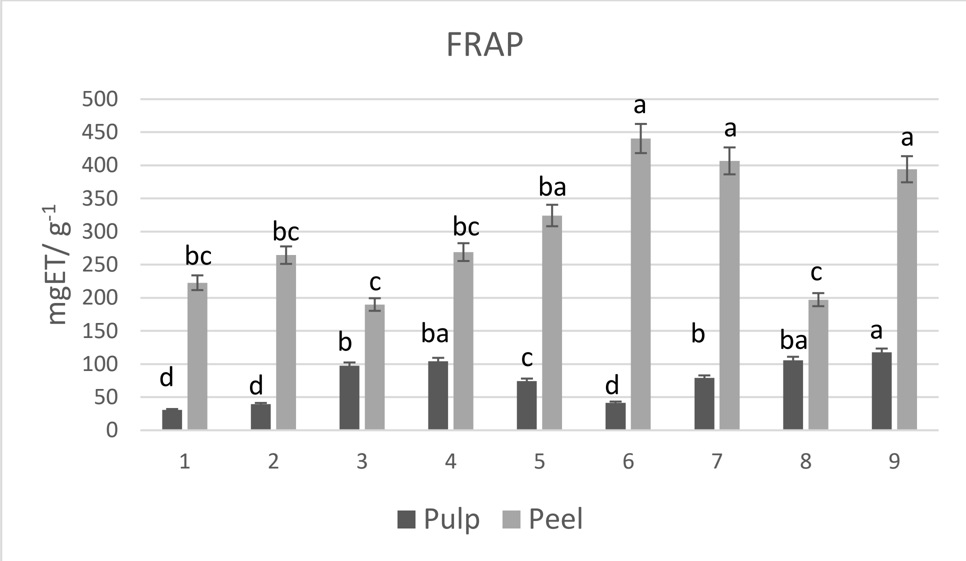

Figure 2. FRAP antioxidant activity in pulp and peel of 'Flame' grape
Means with different letters are different according to Tukey's test with p≤ 0.05

Correlations

An analysis of correlations of the antioxidant capacity against the phenols, flavonoids and anthocyanins of the 'Flame' grape was carried out, in which a high correlation of DPPH and phenols of 0.94153, DPPH and flavonoids of 0.9043 and FRAP and phenols of 0.88852 were observed. In addition, it is observed that flavonoids are the main components of phenols, which give it its antioxidant capacity. This correlation shows that most grape samples can be well represented by DPPH and FRAP, since there is an affinity with the DPPH radical and confers a greater reducing power in FRAP, which is supported by the obtained result. These results are the same as those obtained by Franco-Bañuelos *et al.* (2017).

Table 4. Correlations (R^2) of the antioxidant capacity against the phenols, flavonoids and anthocyanins of the 'Flame' grape

Correlations (R^2)			
	Phenols	Flavonoids	Anthocyanins
DPPH	0.9415	0.9043	0.7269
FRAP	0.8885	0.7847	0.6781
PHENOLS	-	0.8577	0.8325

Likewise, these results suggest that the consumption of grapes could have the same health benefits that certain fruits and vegetables have presented, in addition to their possible use of the peel as a raw material to obtain natural antioxidants that can be useful in the food, pharmaceutical and cosmetic industry.

Conclusions

It is concluded that the applications of salicylic acid and potassium favored the increase of the evaluated parameters and with it the quality. The doses of AS 2.0, K 0 mM; AS 1, K 2.50 mM; and AS 0.25, K 0.625 mM influenced the increase in quality, bioactive and antioxidant activity. Flame table grape peels show a high content of compounds, favoring antioxidant activity. The use of salicylic acid and potassium can be an alternative to enrich the nutritional quality of the grape and benefit the health of the population.

Authors' Contributions

J.M.S.P and E.S.CH. designed the study. M.C.S.C. and M.J.R.R. analyzed the data. M.A.F.C. and K.F.V.F prepared the manuscript, while K.F.V.F. and J.M.S.P. conducted the experiments. M.A.F.C.; N. A. S.S and S.P.A. organized the data and performed the statistical analysis. All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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