COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Postharvest treatment with 1-MCP in apple 'Gala' mutants: physicochemical characterization, bioactive compounds and antioxidant activity

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The aim of this study was to evaluate the application of 1-MCP on physicochemical characterization and antioxidant activity of 'Gala' apples mutants, harvested at two different times and maintained in refrigerated atmosphere (0 °C) during 90 days. 'Mondial Gala', 'Imperial Gala' and 'Galaxy' were obtained from commercial orchards. After harvesting, half of the fruits were treated with 1-MCP, and control fruits were maintained in the same condition, but without the treatment. Skin color, pulp firmness, titratable acidity, total soluble solids, total phenolic compounds, anthocyanin and antioxidant activity were evaluated in peel and pulp. The results show that using 1-MCP, pulp firmness was higher than the control. Titratable acidity analysis showed statistical differences for apple clones, harvest point and treatment with 1-MCP. Total soluble solids content was not influenced by the treatments. Epidermis color was statistically influenced by clone (a*, L* and C*), and by harvest point (L*), but 1-MCP did not affect this parameter. The content of total polyphenols and antioxidant activity was higher in the peel when compared to the pulp. 1-MCP proved to be effective in maintaining postharvest quality in all clones and at two harvest points tested.

Highlighted Conclusions
1) Refrigerated atmosphere during 90 days maintained the fruit quality.
2) Total phenolic compounds are higher in apple peel.
3) 1-MCP proved to be effective in maintaining postharvest quality.

Apples are globally appreciated for their taste, aroma and texture and also have been strongly associated with reduced risks of diseases such as cancer and cardiac disorders (McCann et al. 2007). Phytochemicals have received much attention because of their antioxidant potential (Tsuda et al. 1994, Leyva-Corral et al. 2016), and apples have one of the highest levels of antioxidant activity among vegetables (Chinnici et al. 2004).

Apple is the most important temperate fruit marketed as fresh fruit in the national and international context (Mello 2004). According to Jesionkowska (2006) is known the consumers preference for reddish fruit, for that reason, orchards with standard 'Gala' have been replaced by strains with intense red color. 'Gala' cultivars have a high respiratory activity and ethylene production, facts that reduce their longevity in storage, making it difficult to supply the market in the off season (Brackmann 1992).

Low temperatures may not be enough to maintain the fruit quality, being necessary the use of other techniques, such as, ethylene inhibitors (Pinheiro et al. 2005). 1-MCP (1-methylcyclopropene) is a water-soluble powder that has been tested as an ethylene inhibitor. 1-MCP binds to ethylene receptors in fruit tissue and prevents the action of ethylene (Corrent et al. 2004). So postharvest use of 1-MCP may be an alternative to maintaining apples quality (Fante et al. 2013).

Previous studies were carried out to optimize the extraction of bioactive compounds and quantify the antioxidant activity using different extractors in apples from 'Gala' cultivar (Ferrareze et el. 2014). Moreover, physical-chemical, bioactive compounds and antioxidant activity were evaluated in three 'Gala' mutants (Galaxy, Imperial Gala and Mondial Gala) collected at two moments of harvest (Ferrareze et al. 2017).

The aim of this study was to evaluate the application of 1-MCP on physicochemical characterization and antioxidant activity of 'Galaxy', 'Imperial Gala' and 'Mondial Gala' apples harvested at two different times and maintained in refrigerated atmosphere for 90 days.

MATERIAL AND METHODS

Plant Material. This study was conducted at Embrapa Grape and Wine laboratories (Bento Gonçalves, RS, Brazil). Apple fruits used (*Malus domestica* Borkh) of 'Mondial Gala', 'Imperial Gala' and 'Galaxy' was from commercial orchards in the City of Vacaria, RS, Brazil. Fruits were harvest in 2007. These orchards were close to each other, presenting plants with the same characteristics with respect to age (7 years), rootstock (M-7), row spacing (3.5 m) and between plants (0.7 m). The fruits were harvested at two maturation stages: Harvest 1 (physiological maturity) and Harvest 2 (10 days after harvest 1).

Application of 1-MCP. After harvesting, fruits were treated with 625 ppb of 1-MCP (Agro Fresh – 0.14 %). 1-MCP was placed in a sealed glass vial where it was dissolved with 10 mL of aqueous sodium dodecyl sulfate solution (0.1 % and 50 °C). After dissolution, the vial was opened and 1-MCP reached its gaseous form. Chambers were kept closed for 48 hours, at 20 ºC. Control fruits were maintained in the same condition, but without 1-MCP treatment. After the treatment, samples were stored for 90 days in refrigerated atmosphere (0 °C and 95 % relative humidity).

Physicochemical characterization. Skin color, pulp firmness, titratable acidity, total soluble solids, antioxidant activity, total phenolic compounds and anthocyanins were evaluated in fruits harvested at two maturation points.

For the skin color determination, it was used a colorimeter (Minolta, Chroma Meter CM-508D), and L*, a* and C* color parameters were evaluated. Two measurements were taken in the equatorial region of each fruit. Pulp Firmness (Lb pol^{-2}) was measured with a manual penetrometer (Bishop FT 327), with an 11 mm tip. The analysis was made on two opposite sides of the fruit in equatorial region, the epidermis was removed prior the evaluation. Titratable acidity (C mol L^{-1}) was performed for each repetition using 10 mL of juice diluted in 90 mL of distilled water and titrated using a digital burette with 0.1 mol L^{-1} of sodium hydroxide solution until pH 8.1, determined with a digital pHmeter. Total soluble solids were determined using a refractometer (Atago, model PR 101, 0-45 %) with temperature correction, and were expressed in °Brix.

Total phenolic compounds, anthocyanin and antioxidant activity. Folin-Ciocalteau reagent was used for determining the content of phenolic compounds as described by Swain and Hillis (1959). Extraction was performed according to Ferrareze et al. (2014).

Peel anthocyanin were evaluated according to Takos et al. (2006) with modifications. 5 g of liquid nitrogen grinded peel were macerated with 10 mL of HCl: methanol 1% (v/v) and let for 24 h in the dark at 4 °C. Subsequently the samples were centrifuged for 15 minutes at 13,000 xg. The supernatant was collected and read at 520 nm in a spectrophotometer. The results were expressed as mg kg^{-1}.

Antioxidant activity was measured through the removal of the radical DPPH (1.1-difenil-2-picrilhidrazil), it was determined according to Brand-Williams et al. (1995). Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) was used as standard to calibration curve. The results were expressed as mg TEAC (Trolox Equivalent Antioxidant Capacity) 100 g^{-1} fruits.

Statistical analysis. A factorial experiment was performed using completely randomized design with three replications of 10 fruits. The factors studied consisted of 3 cultivars, 2 harvest times (harvest 1 and harvest 2) and 2 treatment (control and 1-MCP). Statistical analysis was performed using the SAS statistical software. The results were submitted to analysis of variance. Tukey test ($p<0.05$ and $p<0.01$) was adopted to compare averages.

RESULTS AND DISCUSSION

Table 1 presents the results of physicochemical characteristics in 'Gala' apple clones at two maturation points (1 and 2) treated or not with 1-MCP and maintained in refrigerated atmosphere (RA) during 90 days.

Pulp firmness did not present difference between 'Galaxy', 'Imperial Gala' and 'Mondial Gala' clones. Using 1-MCP, it was obtained a statistical effect on pulp firmness in the harvest point, confirming the beneficial effect of 1-MCP on the control of ethylene, similar to the data obtained by Brackmann (2005).

In the Figure 1 it can be observed that pulp firmness in the fruits treated with 1-MCP presented an average value of 15.51 Lbs pol^{-2}, higher than the control (13.42 Lbs pol^{-2}). This difference after 3 months of storage is beneficial to conservation and commercialization. Moreover, beneficial effects of 1-MCP in maintaining pulp firmness are observed in avocado (Feng et al. 2000, Jeong et al. 2002), damascus (Fan et al. 2000) and apple (Rupasinghe et al. 2000, Deell et al. 2002).

Table 1. Physicochemical characteristics in apple clone at two maturation points (1 and 2) treated or not with 1-MCP and maintained in refrigerated atmosphere (RA) during 90 days.

Parameters	Harvest point	Treatment	'Galaxy'	'Imperial Gala'	'Mondial Gala'	CV %
Pulp Firmness (Lbs pol^{-2})	1	Control	14.41 a	13.56 a	13.42 a	11.24
		1-MCP	16.03 a	15.37 a	15.86 a	13.20
	2	Control	12.96 a	13.09 a	12.42 a	10.90
		1-MCP	15.07 a	15.7 a	15.05 a	12.55
Titratable Acidity (C mol L^{-1})	1	Control	3.74 a	3.6 a	4.67 a	10.97
		1-MCP	4.5 B	4.57 B	5.18 A	3.86
	2	Control	3.55 a	3.48 a	4.21 a	7.12
		1-MCP	3.27 a	4.14 a	4.50 a	20.71
Total Soluble Solids (°Brix)	1	Control	12.67 a	12.53 a	13.37 a	4.77
		1-MCP	12.97 a	13.03 a	13.47 a	5.72
	2	Control	12.57 a	12.33 a	12.43 a	5.77
		1-MCP	13.03 a	12.63 a	13.02 a	9.10
Color (a*)	1	Control	34.15 a	33.01 a	35.26 a	8.41
		1-MCP	33.80 a	35.26 a	35.43 a	8.08
	2	Control	35.57 a	34.50 a	20.28 a	6.81
		1-MCP	34.68 a	34.49 a	36.18 a	6.34
Color (C*)	1	Control	40.12 a	40.37 a	40.93 a	5.40
		1-MCP	40.11 a	40.10 a	41.77 a	6.40
	2	Control	39.61 a	39.13 a	29.63 a	7.12
		1-MCP	39.47 B	38.24 B	42.17 A	5.94
Color (L*)	1	Control	41.98 a	42.50 a	45.28 a	11.38
		1-MCP	43.93 a	41.50 a	43.56 a	10.06
	2	Control	37.92 B	41.00 A	42.51 A	8.92
		1-MCP	40.17 AB	37.56 B	43.27 A	10.73

The values in the row that have the same lowercase letters do not differ with 5% probability level and those with the same uppercase letters do not differ at the 1% probability level.

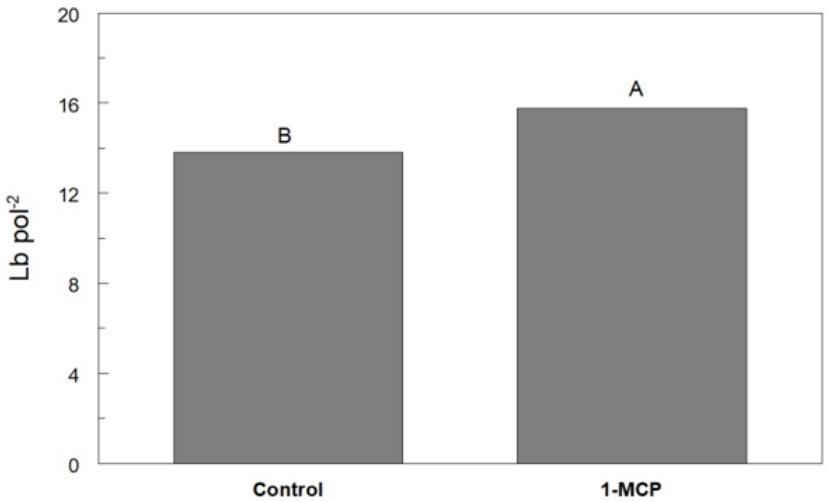

Figure 1. Pulp firmness in apple 'Gala' mutants treated or not with 1-MCP and maintained in refrigerated atmosphere (RA) during 90 days.

Differently from that observed for pulp firmness, it was noted statistical differences in titratable acidity between apple clones, harvest point and treatment with 1-MCP. 'Mondial Gala' presented higher acidity values compared

with other clones ($p<0.05$) for titrated acidity (Figure 2). This fact reflects the importance of the appropriate harvest point, because acidity is an indication of the fruit quality, due its decrease along the refrigerated storage (Girardi 2004). According to Serek et al, (1995), the increase of ethylene production accelerates the respiratory intensity, which increases the consumption of organic acids, which explains the higher acidity contents in fruits treated with 1-MCP, since it is known that inhibits the ethylene action (Figure 3).

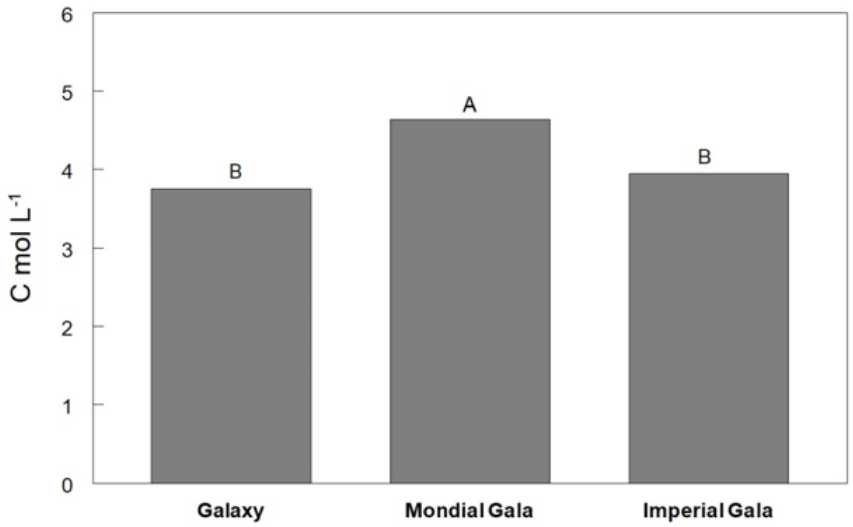

Figure 2. Titratable acidity values in 'Mondial Gala', 'Galaxy' and 'Imperial Gala' obtained at 2 harvest time and maintained in refrigerated atmosphere (RA) during 90 days.

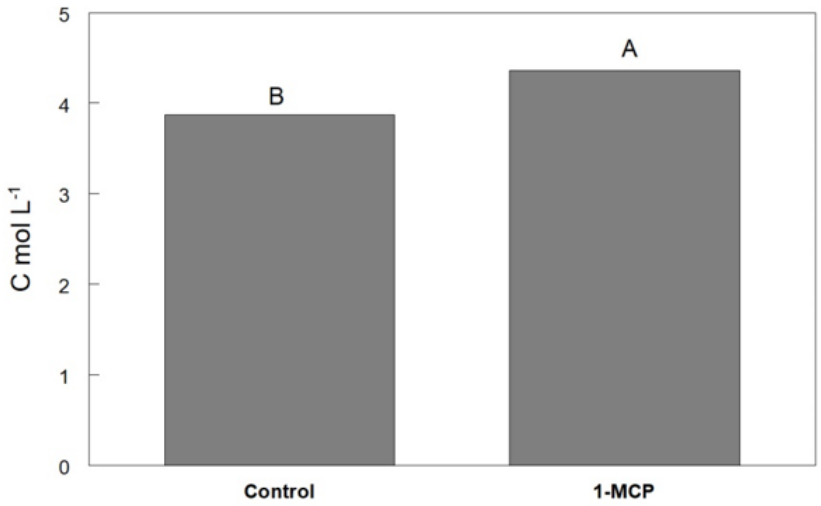

Figure 3. Titratable acidity values in apple 'Gala' mutants treated or not with 1-MCP and maintained in refrigerated atmosphere (RA) during 90 days.

Total soluble solids content was not influenced by the treatments evaluated. These results are in agreement with the expected, being very similar to the values obtained at the harvest time (Ferrareze et al. 2017), and do not suffer major variations during storage. Sugar content is a main factor of fruits consumption quality and can be influenced by some factors such as sun exposure, irrigation, rootstock and fertilization. The use of 1-MCP did not present statistical difference and this result is in accordance to Corrent et al. (2004), Brackmann (2005), and Pinheiro et al. (2006), because ethylene does not influence this physiological parameter (Ayub et al. 1996).

Epidermis color was statistically influenced by clone (a*, L* and C*), and by harvest point (L*). 1-MCP did not affect this parameter because color fruits are not influenced by ethylene (Ayub et al. 1996). According to the Figure 4, color parameters of 'Mondial Gala' clone presented lower values (a* and C*) than 'Galaxy' and 'Imperial Gala' apples, which did not present statistical difference.

Table 2 presents the results of total phenolic compounds, anthocyanin and antioxidant activity in apple clones at two maturation points (1 and 2) treated or not with 1-MCP and maintained in refrigerated atmosphere (RA) during 90 days.

Pulp and peel phenolic compounds presented statistical difference in apple clone and harvest point, and the application of 1-MPC was significant just for the pulp. Peel polyphenol was statistically higher than the pulp (Figure 5). 'Galaxy' (520.53 mg GAE 100 g^{-1}) clone on peel presented more compounds compared with 'Mondial Gala' (425.61 mg GAE 100 g^{-1}) and 'Imperial Gala' (445.06 mg GAE 100 g^{-1}), and this fact occurred also for the pulp. According to Figure 5, the average values of polyphenols in apple are in accordance by other authors (Ju et al. 1996, Leja et al. 2003), which are higher than found in strawberry, pineapple, blackberry, guava, grape and uvaia (Kuskoski et al. 2006, Abe et al. 2007, Sganzerla et al. 2018). Figure 6 shows that polyphenol contents were lower in fruits harvested with more advanced maturation (harvest 2), for both peel and pulp.

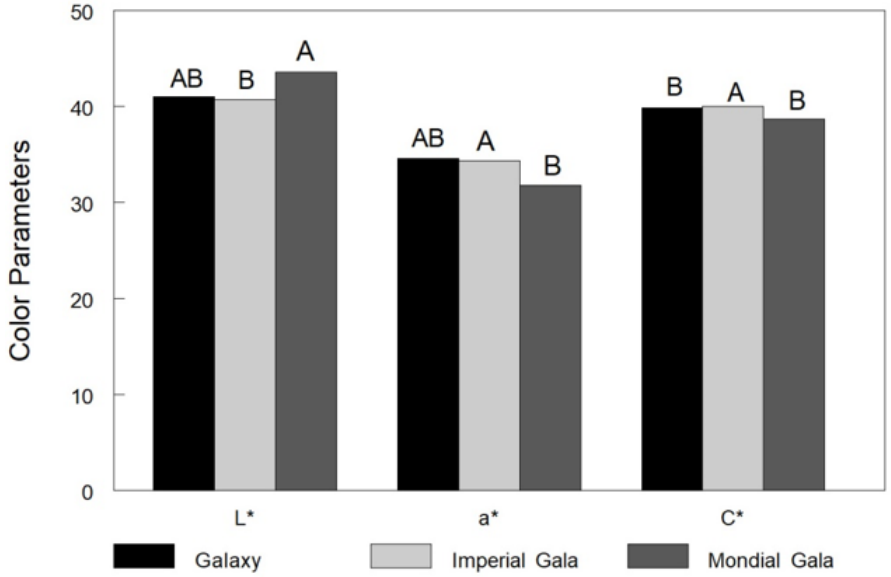

Figure 4. Color parameters (L*, a* and C*) in 'Galaxy', 'Imperial Gala' and 'Mondial Gala' obtained at 2 harvest time and maintained in refrigerated atmosphere (RA) during 90 days.

Table 2. Total phenolic compounds, anthocyanin and antioxidant activity in apple clones at two maturation points (1 and 2) treated or not with 1-MCP and maintained in RA during 90 days.

Parameters	Harvest point	Treatment	'Galaxy'	'Imperial Gala'	'Mondial Gala'	CV %
Peel Phenolic Compounds (mg GAE 100g^{-1})	1	Control	566.07 a	438.47 b	470.84 ab	8.5
		1-MCP	513.37 a	493.03 a	460.66 a	9.38
	2	Control	526.77 A	466.4 A	340.3 B	8.79
		1-MCP	475.91 a	382.34 a	430.64 a	20.71
Pulp Phenolic Compounds (mg GAE 100g^{-1})	1	Control	407.1 A	334.46 A	92.05 B	16.02
		1-MCP	153.75 a	148.2 a	152.62 a	14.81
	2	Control	226.99 a	158 a	128.89 a	36.26
		1-MCP	214.75 A	73.15 B	152.48 A	3.63
Anthocyanin (mg kg^{-1})	1	Control	57.76 A	51.10 B	58.10 A	3.13
		1-MCP	59.60 B	67.19 A	52.32 C	1.25
	2	Control	109.73 A	57.19 B	54.91 B	1.93
		1-MCP	74.59 a	71.57 a	53.16 a	29.32
Peel Antioxidant Activity (mg TEAC 100g^{-1})	1	Control	4288.02 ab	3795.37 b	4741.24 a	6.14
		1-MCP	4784.32 a	4267.39 a	4591.65 a	5.37
	2	Control	4639.42 a	4471.03 a	4813.04 a	7.08
		1-MCP	4783.27 a	4779.62 a	4564.75 a	3.63
Pulp Antioxidant Activity (mg TEAC 100g^{-1})	1	Control	162.93 B	220.92 A	83.37 C	4.6
		1-MCP	89.06 B	115.68 A	127.12 A	5.06
	2	Control	126.82 a	137.37 a	141.29 a	4.14
		1-MCP	133.54 A	84.4 C	118.84 B	4.44

The values in the row that have the same lowercase letters do not differ with 5% probability level and those with the same uppercase letters do not differ at the 1% probability level. GAE: Gallic Acid Equivalent; TEAC: Trolox Equivalent Antioxidant Capacity.

Anthocyanin content in apple peel showed a similar behavior of phenolic compounds. Most levels of polyphenols found in apple peel are directly related to the content of anthocyanin present in this fruit. Thus, harvest 2 (74.19 mg kg^{-1}) presented higher values of anthocyanin in relation to harvest 1 (62.67 mg kg^{-1}) being consistent with the values observed at the harvest moment (Ferrareze et al. 2017).

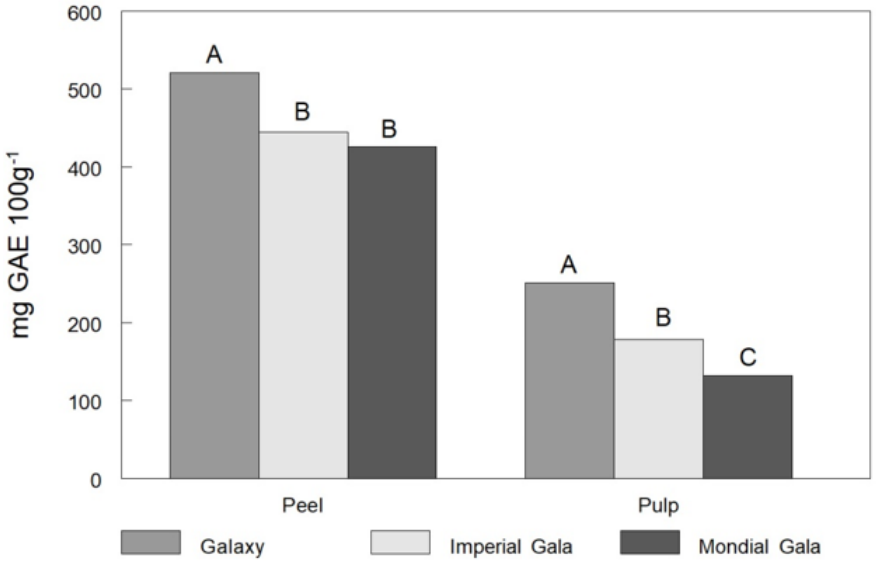

Figure 5. Total phenolic compounds in peel and pulp of 'Galaxy', 'Imperial Gala' and 'Mondial Gala' mutants maintained in refrigerated atmosphere (RA) during 90 days.

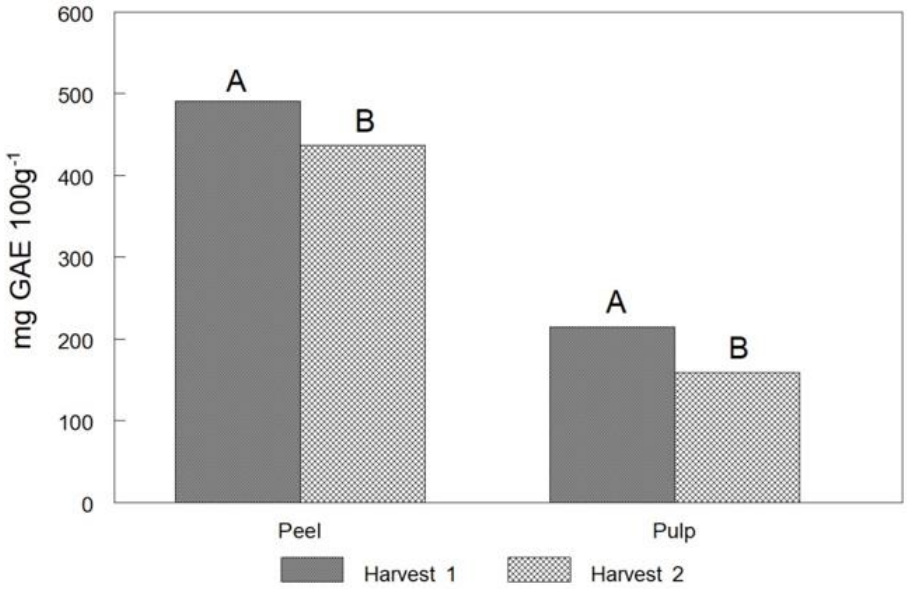

Figure 6. Total phenolic compounds in peel and pulp of 'Gala' mutants obtained at 2 harvest time and maintained in refrigerated atmosphere (RA) during 90 days.

Antioxidant activity in apple 'Gala' mutants presented statistical behavior similar to phenolic compounds (Table 2). The antioxidant activity in peel and pulp of 'Gala' clones studied in this work demonstrate the functional potential of this fruit in human diet (Figure 7). Total antioxidant activity in peel and pulp of 'Gala' obtained at 2 harvest times are presented in Figure 8, the advance of maturation increased the antioxidants peel content, being this in accordance to Ferrareze et al. (2017). However, in the pulp, the opposite occurred, where at harvest 2 there was a significant reduction in the values, that may be related to a higher metabolic activity of these fruits, since these compounds are sources of cellular defense against oxidative stress (Yang et al. 2001).

In conclusion, the storage system tested (refrigerated atmosphere) during a period of three months (90 days) maintained fruit quality in the different clones and treatments tested. The content of total polyphenols and

antioxidant activity wore higher in the peel when compared to the pulp. In addition, 1-MCP proved to be effective in maintaining postharvest quality in all clones and at two harvest points tested.

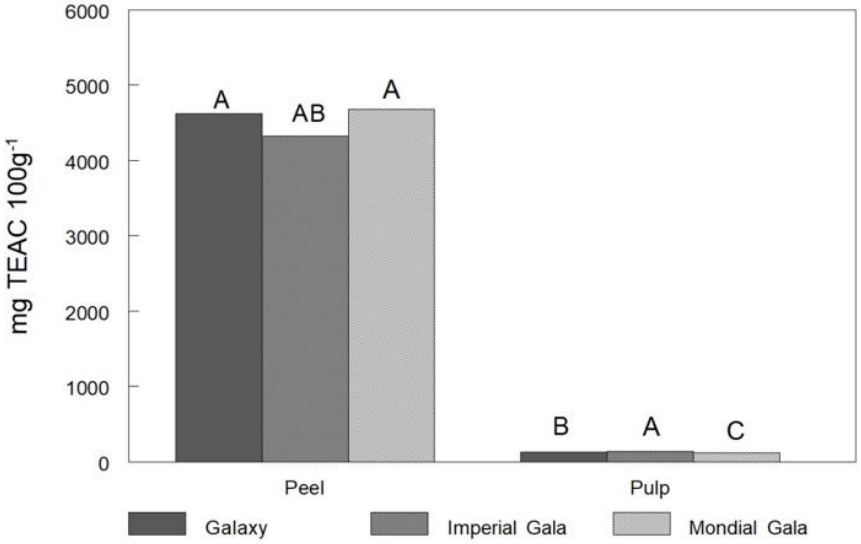

Figure 7. Total antioxidant activity in peel and pulp of 'Gala' mutants maintained in refrigerated atmosphere (RA) during 90 days.

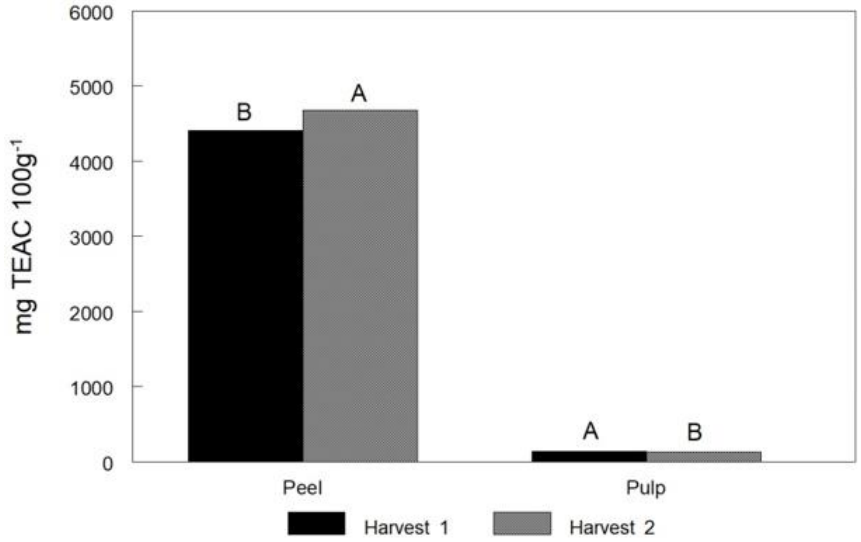

Figure 8. Total antioxidant activity in peel and pulp of 'Gala' obtained at 2 harvest time and maintained in refrigerated atmosphere (RA) during 90 days.

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