COMMUNICATIONS IN PLANT SCIENCES

Physico-chemical and bioactive compounds characterization of apple 'Gala' mutants harvested at two different time points

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In the past years, new apple clones have been propagated on a large scale for their flavor characteristics and greater red color presence in the epidermis, which provide commercial advantages in national and international markets. The objective of this work was to evaluate the physicochemical characteristics and functional properties of 'Galaxy', 'Imperial Gala' and 'Mondial Gala' apples harvested in the city of Vacaria, RS, Brazil in two diferent harvesting time. The analyzed variables were pulp firmness (PF), total soluble solids (TSS), titratable acidity (TA), iodine-starch (IA), skin color (L*a*b*), total polyphenols, anthocyanins and antioxidant capacity of pulp and peel. The results obtained at the time of harvest show that the parameters of pulp firmness, titratable acidity, total soluble solids, anthocyanins and antioxidant activity of the peel presented a highly significant difference between the clones studied. The cultivar 'Mondial Gala' showed a different behavior from the other clones, in the values of firmness of pulp, acidity and total soluble solids, showing that these fruits were at a stage of maturation less advanced, ideal for long-term storage. The levels of total polyphenols and antioxidant capacity were significantly higher in the peel when compared to the pulp for the three clones and for the two harvest times. However, anthocyanin levels were higher for the Mondial Gala clone in both harvests, although anthocyanins and antioxidant capacity were better at harvest 2 for all clones, results that collaborate with more intense red color and thus better commercial quality.

Highlighted Conclusions
Fruit quality varies on the clone studied.
'Mondial Gala' fruits are ideal for long-term storage and have more intense red color and better commercial quality.
The peel shows a higher total polyphenols and a better antioxidant capacity.

Fruiticulture is present in all Brazilian states and as an economic activity, involves around five million people, directly and indirectly. Brazil is the third largest fruit producer, with a production of around 40 million tons a year. Temperate fruit production, such as apples have grown in Brazil mainly due to modernization and better management of land ownership and all production factors, the adoption of low environmental impact technologies, aiming quality, product safety and environmental protection (Fachinello et al. 2011).

In south Brazil, 'Gala' orchards have been replaced by 'Gala' strains with more redish skin, like 'Galaxy' ('Galaxy ®'), Imperial Gala and 'Mondial Gala' ('Mondial Gala®' Mitchgla) (Denardi and Camilo 1992), to fulfill an internal and external market where consumers demand red color skin apples (Crassweller and Hollender 1989, Denardi and Camilo 1992, Gurnsey and Lawes 1999, Iglesias and Alegre 2006, Iglesias et al., 2008).

The standard 'Gala' shows good correlation between fruit color and ripeness. This is essential for fruit postharvest preservation. Before becoming yellow, Gala background color changes to a creamy-white tone, which is a good indicator of physiological state, from which the conservation potential decreases. For Gala strains showing early and intense reddish color, background could not be used as a maturation index, so it is necessary to use other methods to estimate the optimal harvest date (Trillot et al. 1995).

The apple cultivar Gala is well explored and accepted by the market. However, it is a cultivar that has 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, especially after September (Mitcham et al. 2002).

The apple besides being appreciated worldwide for her unique organoleptic characteristics, is associated with reduced risk of diseases like cancer and heart disease (Castro Monte 2006). Apples have one of the highest levels of antioxidant activity among vegetables (Chinnici et al. 2004). In the United States, for example 23% of the phenolic compounds consumed by the population are derived from apples, being the main source of these phytochemicals (Speisky 2006). Among the phytochemicals phenolic compounds have received much attention because of their antioxidant activity (Tsuda et al. 1994, Leyva-Corral et al. 2016). Many studies show that apples are a rich source of phenolic compounds, whose activity and concentration vary with the cultivar, maturity stage, environmental conditions, biotic and abiotic stress and the part of the fruit (Kjersti et al. 2004, Van Der Sluice et al. 2001, Dossett et al. 2011, Lee 2012).

According to Gurnsey and Lawes (1999) is known the consumers preference for reddish fruit, because it orchards with standard 'Gala' has been replaced by strains with intense red color. The red color is due to the presence of the pigment cyanidin-3-galactoside, an anthocyanin which may also scavenge free radicals (Yamasaki et al., 1996). Besides contributing to the color of flowers and fruits, anthocyanins act as a filter of ultraviolet rays on the leaves. Also, in certain plant species are associated with resistance to pathogens and act improving and regulating photosynthesis (Mazza and Miniati 1993). Anthocyanins have anticarcinogenic activity (Hagiwara et al. 2001, Kapadia et al. 1997), antioxidant (Wang et al. 2000, Youdim et al. 2000) and antiviral (Kapadia et al. 1997).

'Gala' mutants differ with respect to physico-chemical and functional properties. It is also known that fruits that remain on the plant longer can accumulate more phytochemicals compounds as they receive photosynthates for a longer period of time, however if the maturity stage is too advanced at the time of harvest, post-harvest conservation will be compromised. In this study physical-chemical and functional properties of three 'Gala' mutants: Galaxy, Imperial Gala and Mondial Gala, collected in two moments of harvest were evaluated.

MATERIAL AND METHODS

Plant material. This study was conducted at Embrapa Grape and Wine laboratories in Bento Gonçalves / RS, Brazil. Apple fruits used (*Malus domestica* Borkh) of 'Mondial Gala', 'Imperial Gala' and 'Galaxy' were from commercial orchards in the City of Vacaria / RS (2007 harvest). These orchards were close to each other, apresenting 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).

Physico-chemical analysis (quality). The following evaluations were performed: skin color, firmness, titratable acidity, iodine-starch, total soluble solids, antioxidant activity, total polyphenols and anthocyanins for the fruits harvested at two stages. For the skin color determination, we used a colorimeter MINOLTA Chroma Meter CM-508D. Two measurements were taken in the equatorial region of each fruit. These values correspond to the values of L*a*b* three-dimensional CIELAB scale system. The firmness was measured with a manual penetrometer (Bishop FT 327), with a 11 mm in diameter tip, the results were expressed in pounds (Lb Pol^{-2}). The analysis was made on two opposite sides of the fruit equatorial region, the epidermis was removed prior the evaluation and the analyzys was held by the same person. The determination of titratable acidity (TA) was made in a 10 mL sample of juice for each repetition, diluted in 90 mL of distilled water and titrated using a digital burette with 0.1N sodium hydroxide solution to pH 8.1 determined with a digital pH meter. The values were expressed in $Cmol^{-1}$. The starch degradation was determined by the iodine-starch content. The fruits were cut in the equatorial zone and half of the stem was immersed in a solution of 12 g of metallic iodine and 24g potassium iodide for 40 seconds. When removed from the immersion, the presence of starch was evaluated by comparing it with the Werner (1989) scale, where index 1 represents a maximum level of starch and 5 the absence of the same index. For the measurement of soluble solids, an Atago refractometer model PR 101 (0-45%) with temperature correction was used. The amounts were expressed in °Brix.

Analyses of the bioactive compounds. Total polyphenols concentration and antioxidant capacity were evaluated according to Ferrareze et al. (2007). Peel anthocyanins 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 min. at 13,000 x g. The supernatant

was collected and read at 520 nm in a spectrophotometer (Perkin Elmer UV / VIS Spectrometer Lambda Bio), the results were expressed in mg kg^{-1}.

Statistical procedures. For the 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). Statistical analysis was performed using the SAS statistical software. The results were submitted to analysis of variance. To compare averages, we adopted the Tukey test at 5% and 1% error probability. Figure 1 shows a diagram of the procedure performed.

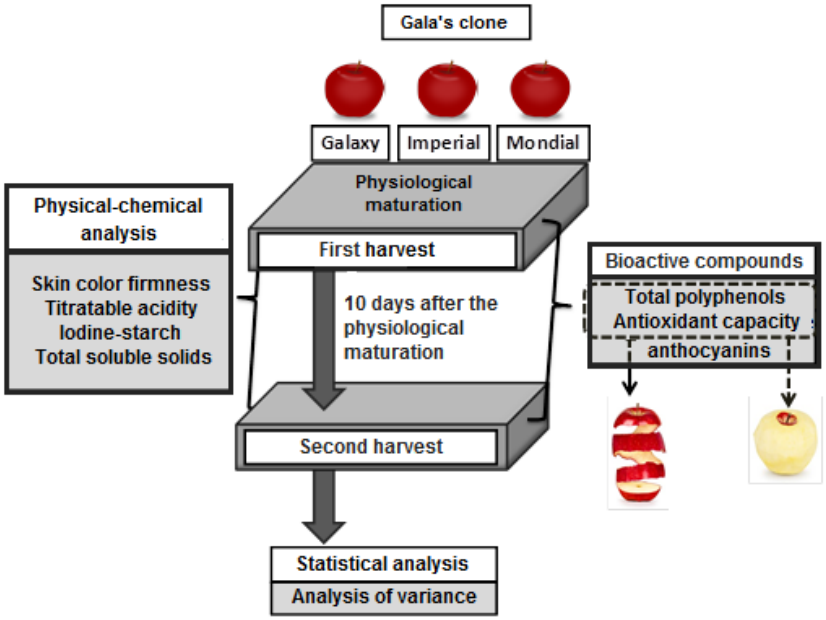

Figure 1. Diagram of the experimental procedure.

RESULTS AND DISCUSSION

The results were evaluated according to variance analysis for the clones: 'Galaxy', 'Imperial Gala' and 'Mondial Gala' obtained at two harvest times. Statistical significance levels show that harvesting parameters for pulp firmness, titratable acidity, total soluble solids, antioxidant activity of the peel and anthocyanins presented a significant difference between clones. This difference is shown in Figures 1 to 5, where by the Tukey test ($p < 0.05$) the mean differences observed for these variables were evaluated. The effect of interaction (Clone X Harvest Time) was significant only for bioactive compounds: antioxidant activity (peel) and polyphenols (peel and pulp). Mean anthocyanin data for each clone and harvesting periods are shown in Figures 4 and 5 respectively.

Table 1 presents results of the harvest time 1, which were within the average parameters for prolonged storage of the Gala cultivar (Girardi et al. 2002). Thus, it can be ensured that the maturation rates established by research were respected at the beginning of harvesting and subsequent storage and/or commercialization, this allow maximum efficiency in the conservation and maintenance of the fruits internal and external quality. For 'Gala' apple, the values recommended for harvest, are 17 to 19 lbs of pulp firmness, SS more than 11 ºBrix and AT between 5.2 to 6.0 Cmol L^{-1} (Girardi 2004).

The starch degradation index was 3.7 for 'Galaxy' and 'Imperial Gala' and 4.0 for 'Mondial Gala' on a scale of 1 to 5 (Werner 1989). These values are considered satisfactory for long periods of storage. The fruits of the studied clones show early and intense reddish coloration, the background color cannot be used as a maturation index, and it is necessary to use other methods (SS, AT, Color) to estimate the ideal harvest time.

At the harvest time 2 (harvested 10 days after the harvest 1), apples showed a significant increase in maturation stage, a loss of firmness, especially in 'Galaxy' and 'Imperial Gala'. However, according to variance analysis, only antioxidant activity (peel), polyphenols (peel and pulp) and anthocyanins presented significant difference related to harvest and clone interaction. Antolovich et al. (2000) says that a fruit phenolic profile is characteristic of the cultivar, while climatic and seasonal differences are of secondary importance. According to

Connor et al. (2005) the antioxidant and polyphenol contents can change significantly according to the cultivar and environmental conditions.

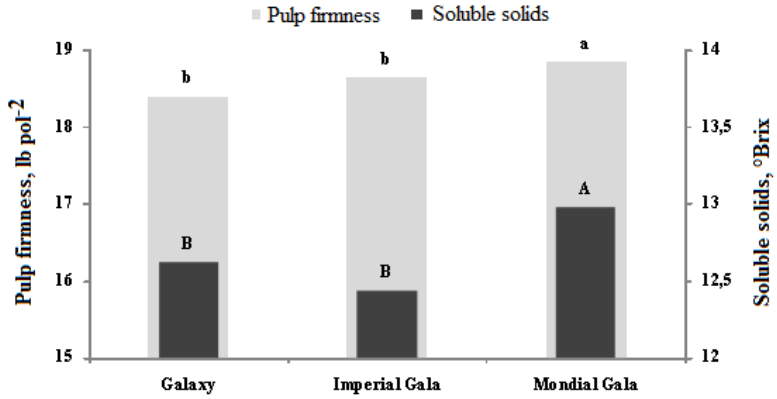

Figure 1. Pulp firmness and soluble solids of 'Galaxy', 'Imperial Gala' and 'Mondial Gala'.

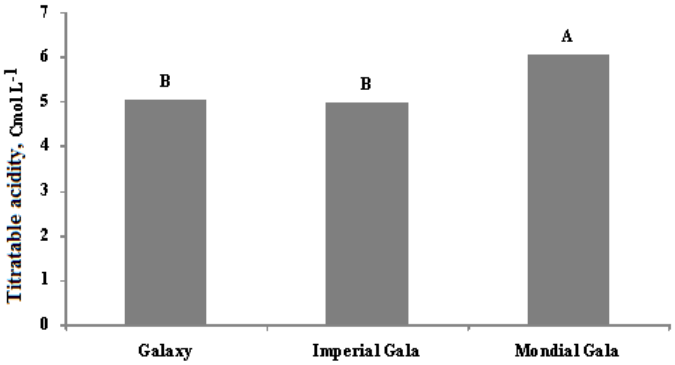

Figure 2. Total titratable acidity values in 'Mondial Gala', 'Galaxy' and 'Imperial Gala'.

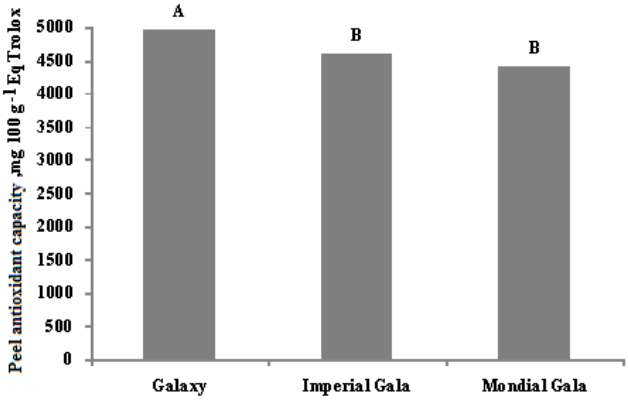

Figure 3. Total mean values of 'Mondial Gala', 'Galaxy' and 'Imperial Gala' peel antioxidant activity.

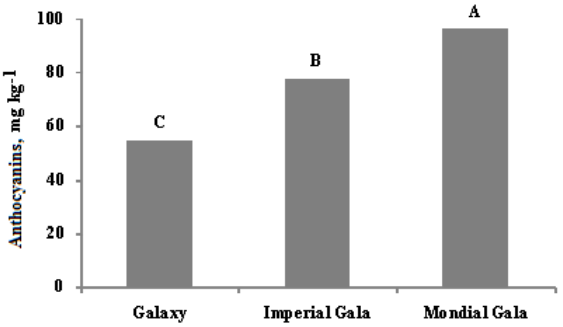

Figure 4. Total mean values of 'Mondial Gala', 'Galaxy' and 'Imperial Gala' anthocyanins.

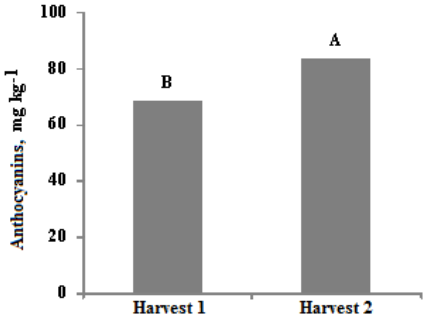

Figure 5. Anthocyanin content at harvest 1 and 2.

'Mondial Gala' had a different behavior from the other clones, especially at harvest 2, the values of pulp firmness, acidity and total soluble solids were higher than the other clones. Also for 'Mondial Gala', the peel antioxidant activity at harvest point 1 were lower than the other clones, with values of 4005.3 mg 100 g^{-1} Trolox Eq, while 'Galaxy' obtained 4779.6 mg 100 g^{-1} Trolox Eq and 'Imperial Gala' 4456.9 mg 100 g^{-1} Trolox Eq. This was probably the cause of the statistical variation among clones, observing that these fruits were in a less advanced stage of maturation, as can be observed in Figures (1 to 4). The anthocyanin values of 'Mondial Gala' (Table 1), were higher in both harvests. In this analysis the mean values for the two harvest points were 95.97 mg kg^{-1} for 'Mondial Gala', 77.48 mg kg^{-1} for 'Imperial Gala' and 54.37 mg kg^{-1} for 'Galaxy' (Figure 4). It is important to note that for each harvest time, all fruits were harvested on the same day and place, based mainly on the size and presence of red color. It can be verified that the color parameters (value a, b and L) did not present statistical differences among the clones, confirming that all the fruits had the same red visual appearance of the epidermis in both harvests.

Table 1. Physico-chemical characteristics and functional properties of 'Galaxy', 'Imperial Gala' and 'Mondial Gala' fruits from two harvest times. Means of 6 replicates.

Variable	Harvest	*'Galaxy'*	*'Imperial Gala'*	*'Mondial Gala'*	CV %
Pulp firmness (PF)	*1*	19.06 a	19.54 a	18.94 a	12.78
(Lbs)	*2*	17.73 a	17.73 a	18.75 a	11.04
Total soluble solids (TSS)	*1*	12.57 a	12.67 a	12.93 a	1.57
(°Brix)	*2*	12.67 AB	12.20 B	13.03 A	0.79
Titratable acidity (TA)	*1*	5.11 a	5.03 a	5.88 a	7.06
(Cmol L^{-1})	*2*	4.98 B	4.92 B	6.21 A	4.05
Iodine-starch (IA)	*1*	3.70 a	3.72 a	4.03 a	7.35
	2	4.62 a	4.40 a	4.00 a	6.25
Color	*1*	36.75 a	36.33 a	37.24 a	7.60
(Valor a)	*2*	38.43 a	36.73 a	38.47 a	7.88
Color	*1*	19.98 a	18.27a	19.88a	6.43
(Valor b)	*2*	18.76 a	18.70 a	19.73 a	6.52
Color	*1*	41.78 a	41.06 a	42.96 a	9.92
(Valor L)	*2*	41.09 a	42.67 a	43.12 a	12.26
Peel polyphenols	*1*	772.99 b	736.87 ab	1019.70 a	14.78
(mg 100 g^{-1} Eq de Trolox)	*2*	664.21 a	746.88 a	611.12 a	11.15
Pulp polyphenols	*1*	37.40 a	36.73 a	32.70 a	8.83
(mg 100 g^{-1} Eq de Trolox)	*2*	31.52 B	51.72 B	40.18 B	8.98
Peel antioxidant capacity	*1*	4779.62 a	4456.93 ab	4005.27 b	4.93
(mg 100 g^{-1} Galic acid Eq)	*2*	5140.69 a	4734.98 b	4798.68 ab	2.87
Pulp antioxidant capacity	*1*	444.47 a	450.53 a	479.94 a	13.71
(mg 100 g^{-1} Galic acid Eq)	*2*	473.02 A	430.32 B	453.33 A	1.37
Anthocyanins	*1*	51.59 C	74.23 B	79.67 A	1.30
(mg kg^{-1})	*2*	57.16 C	80.73 B	112.29 A	1.72

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.

Peel and pulp polyphenols content were influenced by the time of harvest and their interaction with the clone. The average values obtained in the peel among the three clones studied were 818 mg 100 g^{-1} gallic acid Eq at harvest 1, going to 674 mg 100g-1 gallic acid Eq at harvest 2. In the pulp, the values found were significantly lower, ranging from 36 to 41 mg 100g-1 gallic acid Eq at harvest 2 and 1 respectively, the results are in agreement with Nicolas et al. (1994), which obtained for different cultivars a peel/pulp ratio of 3 to 10. Ju et al. (1996) working with 'Delicious' and 'Ralls' apples also obtained markedly superior results for the peel when compared to the pulp.

In apples, the phenolic compounds are located in the vacuoles (97%), and in the cells of the epidermis and sub-epidermis their concentrations are higher than the amounts found in the internal tissues of the fruit. In different cultivars, this ratio, peel / pulp, of phenolic concentration may be 3 to 10 times higher (Nicolas et al. 1994).

The average peel antioxidant capacity at harvest time 2 (4891.45 mg 100 g^{-1} Trolox Eq) was highly superior ($p < .01$) than harvest time 1 (4413.94 mg 100 g^{-1} Trolox Eq). These results agree with the anthocyanins, where the values reached at harvest 2 (83.39 mg kg^{-1}) differed significantly from those obtained at harvest time 1 (68.50 mg kg^{-1}). These data is consistent because these fruits remained 10 days longer than the fruits of harvest 1 attached to the plant receiving photosynthates.

CONCLUSION

Pulp firmness, titratable acidity, total soluble solids, anthocyanins and antioxidant activity of the peel presented a highly significant difference between the clones studied. 'Mondial Gala' fruits were at a less advanced maturation stage, ideal for long-term storage. Total polyphenols and antioxidant capacity were significantly higher in the peel when compared to the pulp for the three clones and for the two harvest times. 'Mondial Gala' anthocyanin levels were higher at both harvests. Anthocyanins and antioxidant capacity were higher at harvest 2 for all clones, results implying in more intense red color and better commercial quality at this harvest point.

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