



## Bioactive compounds and antioxidant activity of jaboticaba var. *Pingo de mel* during its physiological development

Lismaíra Gonçalves Caixeta GARCIA<sup>1</sup>, Flávio Alves da SILVA<sup>2,3\*</sup>, Eduardo Ramirez ASQUIERI<sup>2,3</sup>,  
Eduardo Valério de Barros Vilas BOAS<sup>3,4</sup>, Clarissa DAMIANI<sup>3,5</sup> 

### ABSTRACT

The bioactive compounds and *in vitro* antioxidant activity of jaboticaba fruits var. *Pingo de mel* were evaluated during their physiological development. The fruits were harvested ten days after anthesis until maturity, at intervals of four days between collections. The period between anthesis and maturity was 34 days. Higher phenolics levels, hydrolysed tannins, and antioxidant activity were observed in 10 days after anthesis, while vitamin C contents were higher throughout the ripening process. At the end of development (34 days), higher levels of vitamin C and lower hydrolysed tannin were observed. It is known that the lower the hydrolysed tannins, the better the sensory characteristics of the fruit, and considering the benefits of vitamin C, it is necessary to harvest the jaboticaba fruits var. *Pingo de mel* in 34 days after anthesis for better use of these compounds.

**Keywords:** anthesis; *Myrciaria* sp.; vitamin C; hydrolysed tannins.

**Practical Application:** Monitoring the development of jaboticaba to determine the maximum levels of bioactive compounds.

### 1 Introduction

Jaboticaba is a non-climacteric, highly perishable fruit native to Brazil, and commonly named as brazilian grape tree, sabará, among others. The fruit is a black spherical berry with a thin and fragile peel, and whitish pulp that is slightly acidic and sweet, with an astringent taste. The composition and nutritional value of jaboticaba fruits and products are characterized by their high content of carbohydrates (mainly glucose and fructose), dietary fiber, minerals, vitamins, and also other bioactive compounds (Alejandro et al., 2013a, b; Reynertson et al., 2008a; Wu et al., 2013a, b). The sweet taste and fruit acidity is probably due to the presence of sugars, organic acids and terpenes (Plagemann et al., 2012). In addition, several studies have shown significant antioxidant activity, anthocyanins (Einbond et al., 2004; Ascheri et al., 2006; Reynertson et al., 2008b), and high content of phenolic compounds in jaboticaba fruits.

Despite its great potential, there are no studies on the physiological development of jaboticaba var. *Pingo de mel*, which is widely cultivated in the Goiás region. During fruit maturation, the bioactive compounds undergo a series of complex biosynthesis process, leading to the changes in their composition and content in plant and plant-derived foods (Prasanna et al., 2007).

Given the above, this study aimed to characterize the jaboticaba fruits var. *Pingo de mel* for their bioactive compounds and *in vitro* antioxidant activity along fruit development, targeting their health benefits.

### 2 Materials and methods

The experiment was performed from September to October 2014, at the Fazenda & Vinícola Jaboticabal in New Fatima, district of Hidrolândia-GO (16° 55' 32.35 "S, and 49° 21' 39.76" W), 35.6 Km from Goiânia-GO. The area provided for the experiment contained about 700 trees, and seventy trees of similar size and age (7 years) were selected at random, and the branches were marked on the occasion of the anthesis (flower opening) with wool yarn of different colors. The experiment was performed from September to October 2014, at the Fazenda & Vinícola Jaboticabal in New Fatima, district of Hidrolândia-GO (16° 55' 32.35 "S, and 49° 21' 39.76" W), 35.6 Km from Goiânia-GO.

The fruits were collected in the morning and transported to the laboratory in polyvinyl chloride (PVC) plastic containers, in polystyrene boxes containing ice. For that, 800 fruits were harvested, representing the point 1 of analysis (10 DAA), 400 for the point 2 and 3 (14 and 18 DAA), and 200 fruits for the other points of analysis, divided into four equal lots, representing the replicates. Immediately after arriving at the laboratory, the fruits were selected for the presence of defects or plagues and washed in running water. On the day of harvest, extracts were extracted from the fruits for analysis of phenolic compounds, antioxidant activity *in vitro*, hydrolyzed tannins and vitamin C. The extracts were then frozen at -18 °C until analysis.

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<sup>1</sup>Programa de Pós-graduação em Administração – PPGA, Escola de Agronomia, Universidade Federal de Goiás – UFG, Goiânia, GO, Brasil

<sup>2</sup>Departamento de Ciência e Tecnologia de Alimentos, Universidade Estadual de Campinas – UNICAMP, Campinas, SP, Brasil

<sup>3</sup>Universidade Federal de Goiás – UFG, Goiânia, GO, Brasil

<sup>4</sup>Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil

<sup>5</sup>Departamento de Ciência dos Alimentos, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil

\*Corresponding author: [flaviocamp@gmail.com](mailto:flaviocamp@gmail.com)

## 2.1 Determinations

### Physicochemical determinations

To obtain the extracts, the samples (pulp and peel) were homogenized with ethyl ether in the ratio 1:20 (w/v) under stirring and protected from light for one hour. Subsequently, the extracts were filtered through Whatman filter paper #1, and the volumes were adjusted. The extract was dried on filter paper for two hours at 35 °C to evaporate the ethyl ether residue. Then, the residue was weighed into a beaker and ethanol was added in the ratio of 1:20, and the extraction process (stirring + filtration) was repeated. After filtration, the residue was kept on filter paper for 30 minutes at 50 °C to evaporate the residual solvent. Finally, water in the ratio of 1:20 was added to the residue from the previous extracts to obtain the aqueous extract, which was kept under stirring and protected from light for one hour. The extracts were stored in amber glass bottles at -18 °C until completion of the analysis.

### Phenolic compounds

Phenolic compounds were determined in the ether, ethanol and aqueous extracts in a spectrophotometer (Biospectro SP-220) at 750 nm, using the Folin-Ciocalteu reagent (Waterhouse, 2002). The quantification was done using a standard curve of gallic acid, and the results were expressed as mg gallic acid equivalents (GAE) per 100 g sample.

### Antioxidante activity

#### FRAP – Ferric Reducing Antioxidant Power

The antioxidant activity was determined in all extracts (ether, ethanol and aqueous) by the iron reduction method (FRAP - Ferric Reducing Antioxidant Power), according to Benzie & Strain (1996) with modifications by Rufino et al. (2010). Readings (tripyrindyl triazine ferrous complex) were measured in a spectrophotometer at 595 nm (Biospectro SP-220), and the results were expressed as  $\mu\text{mol FeSO}_4 \text{ g}^{-1}$  of fresh weight.

#### DPPH - 2,2-diphenyl-1-picrylhydrazyl

The antioxidant activity was determined in the ether, ethanolic, and aqueous extracts by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) according to Brand-Williams et al. (1995), with modifications by Borguini & Torres (2009). The degree of discoloration of the DPPH radical at 517 nm was measured in spectrophotometer (Biospectro SP-220) with a concentration of 0.2 mg mL<sup>-1</sup>, and the results were expressed as % discoloration. The absorbance readings were carried out after 20 minutes of reaction. The calculations were performed using the Equation 1:

$$\% \text{ discoloration by DPPH} = \left[ -1 \left( \frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs control}} \right) \right] * 100 \quad (1)$$

where *Abs sample* is the absorbance of the sample; *Abs blank* is the absorbance of the blank; and *Abs control* is the absorbance of control (750  $\mu\text{L}$  methanol + 1.5 mL DPPH).

#### ABTS<sup>+</sup> - 2,2 AZINO BIS (3-ethylbenzo thiazoline 6 sulfonic acid)

The antioxidant activity by the ABTS<sup>+</sup> radicals method was determined according to Miller et al. (1993) with modifications by Rufino et al. (2010) The absorbance was measured in a spectrophotometer (BiospectroSP-220) at 734 nm after 6 minutes of the addition of ABTS<sup>+</sup>. The results were expressed as  $\mu\text{mol Trolox g}^{-1}$  fresh weight.

#### Hydrolysed tannins

The content of hydrolysed tannins was measured in a spectrophotometer, according to Brune et al. (1991), with adaptations by Barcia et al. (2012). For tannins extraction, one g sample was mixed with 50 mL methanol and stirred for 10 min, followed by standing for one h. After filtration, two mL extract were removed and eight mL FAS reagent (89% of urea:ethyl acetate 1:1; 10% of 1% gum arabic in deionized water; and 1% ferric ammonium sulfate of 5% in HCl 1 M) was added. After 15 min of reaction, readings were performed in a spectrophotometer (Biospectro SP-220) at 680 nm (blue), using methanol to reset the equipment (blank). The results were expressed as mg gallic acid equivalent per 100 g sample.

#### Vitamin C

The vitamin C content was extracted using of the procedure described by Abushita et al. (1997). Reading were performed in spectrophotometer (Biospectro SP-220), and the results were expressed as mg of ascorbic acid per 100 g sample.

## 2.2 Statistical analysis

The experiment was carried out using a completely randomized design (CRD) composed of seven maturation stages and three replicates, each replicate consisting of 60 fruits. The variables evaluated were subjected to a polynomial regression analysis as a function of the harvesting dates. The computer software SISVAR was used to fit the regression models by way of an F test at the 5% probability level, to measure the significance of the proposed model.

## 3 Results and discussion

The stage of development of jaboticaba fruits var. *Pingo de mel* was 34 days, from anthesis to harvest, which represented the stages of growth, maturity and ripening. According to Donadio (2000), jaboticaba fruits ripen in about three weeks after flowering. However, this period can vary according to several factors, including species, climate, soil, temperature, and others. A significant difference ( $p < 0.05$ ) in the contents of bioactive compounds of jaboticaba fruits var. *Pingo de mel* was observed throughout the stage of development. The extraction process using solvents with different polarities allowed the extraction of phenolic compounds only in the ethanolic and aqueous extracts (Figure 1), once it was not possible to perform absorbance readings in the ether extract (data not shown). The content of phenolic compounds of the ethanolic extract of jaboticaba fruit decreased from the first harvest to

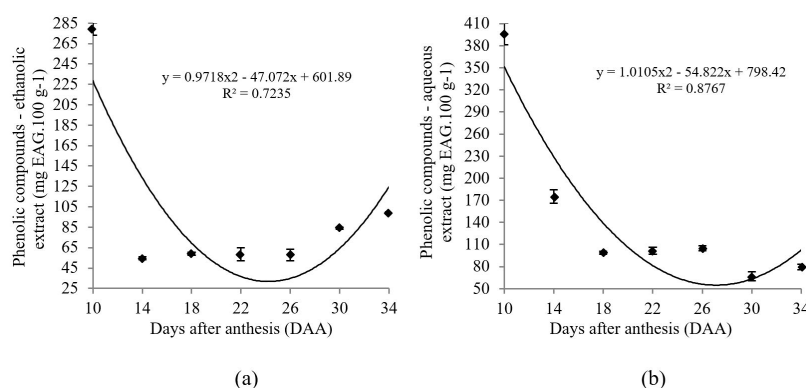
14 DAA, from 279.15 mg GAE 100g<sup>-1</sup> to 54.60 mg of GAE 100g<sup>-1</sup>. However, from 14 DAA to full ripening of fruits, an increase was observed, with values of 98.87 mg GAE 100g<sup>-1</sup> at 34 DAA. Alezandro et al. (2013a) evaluated the content of phenolic compounds of Sabará jaboticabas, and observed that the complete ripening led to a 67% decrease in the Folin-Ciocalteu reduction capacity in unripe berries.

Chitarra & Chitarra (2005) have reported that young fruits contain a higher concentration of phenolics in tissues, and these compounds play important roles in plant, acting as a defense mechanism, reducing during the stages of development, which explains the higher content of phenolic compounds at 10 DAA. Taiz & Zeiger (2006) have also pointed out an increase in the content of phenolic compounds during the fruit development, probably due to biotic and abiotic stresses that induces the secondary metabolism of the fruit.

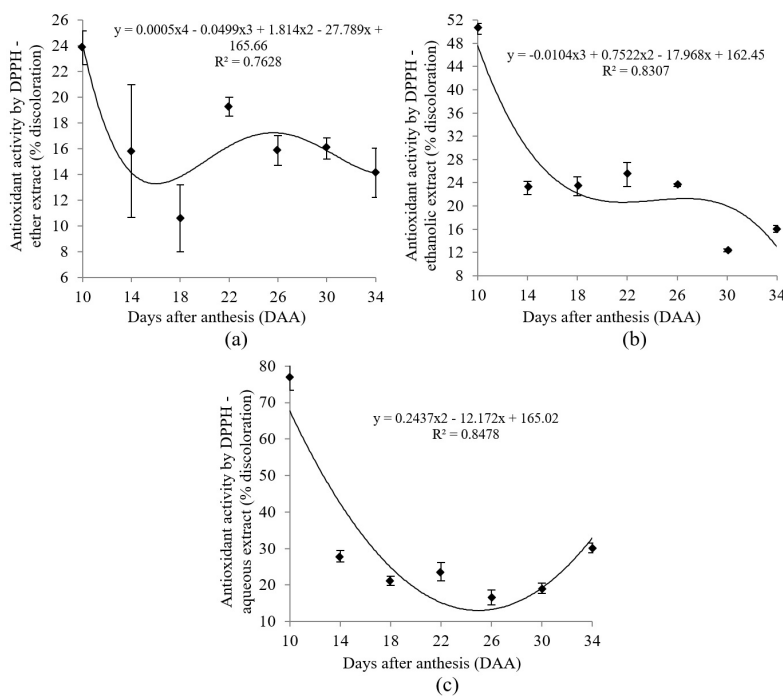
The content of phenolic compounds in the aqueous extract was higher (395.88 mg GAE 100g<sup>-1</sup>) when compared to the ethanolic extract. Moreover, different behavior was observed for the phenolic compounds in the ethanolic extract, decreasing during development and ripening of the fruits, with a final value of 79.35 mg GAE 100g<sup>-1</sup> sample.

The differences observed for various solvents were also found by Pellegrini et al. (2007) and Melo et al. (2008), who reported that the solubility in a given solvent is a peculiar characteristic of the phytochemical, which explains the absence of a universal extraction due to the structural diversity and sensitivity of the phenolic compounds to extraction conditions.

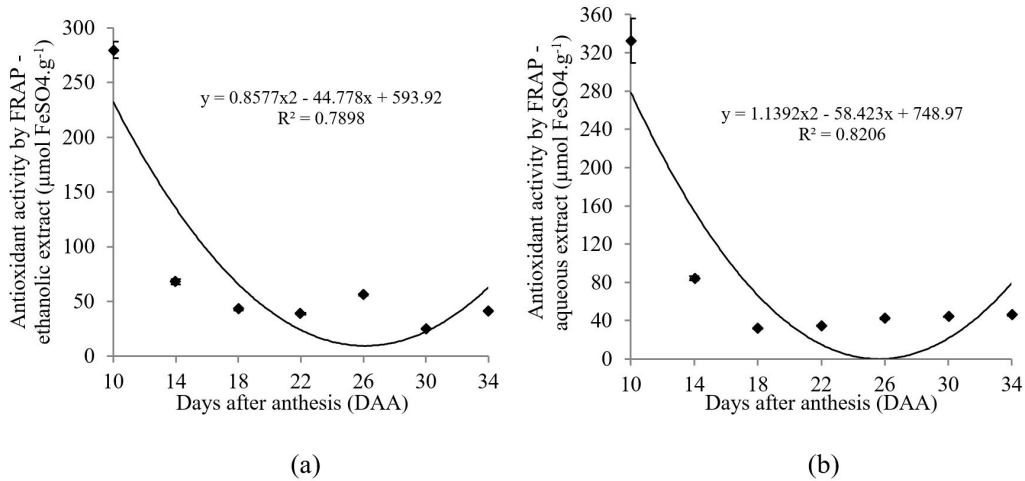
Similar tendency was observed for the *in vitro* antioxidant activity in the different extracts determined by various methods (Figures 2, 3 and 4), once the highest contents of phenolic compounds were observed at 10 DAA.



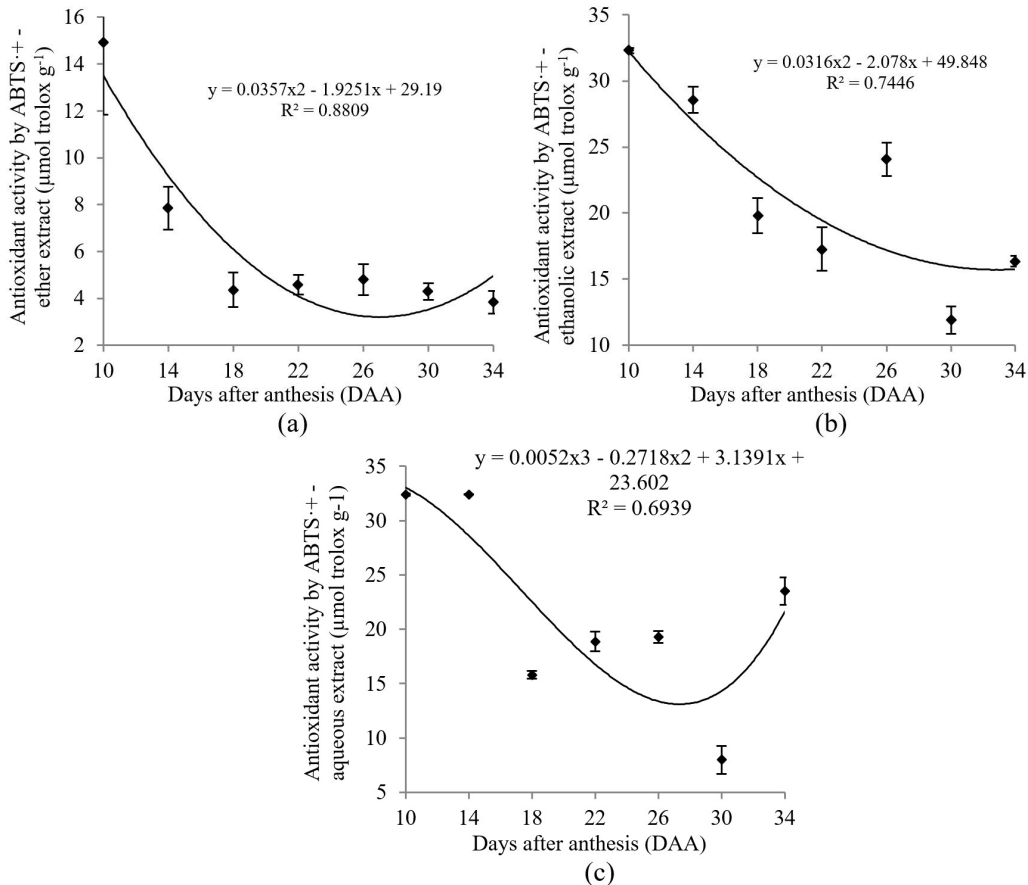
**Figure 1.** Mean values and standard deviation of phenolics content in ethanolic (a) and aqueous (b) extracts during the stages of development of jaboticaba fruits var. *Pingo de mel*.



**Figure 2.** Mean values and standard deviation of the *in vitro* antioxidant activity by DPPH method in the ether (a) ethanolic (b), and aqueous (c) extracts during the stages of development of jaboticaba fruits var. *Pingo de mel*.



**Figure 3.** Mean values and standard deviation of *in vitro* antioxidant activity by FRAP method in ethanolic (a), and aqueous (b) extracts during the stages of development of jabuticaba fruits var. *Pingo de mel*.

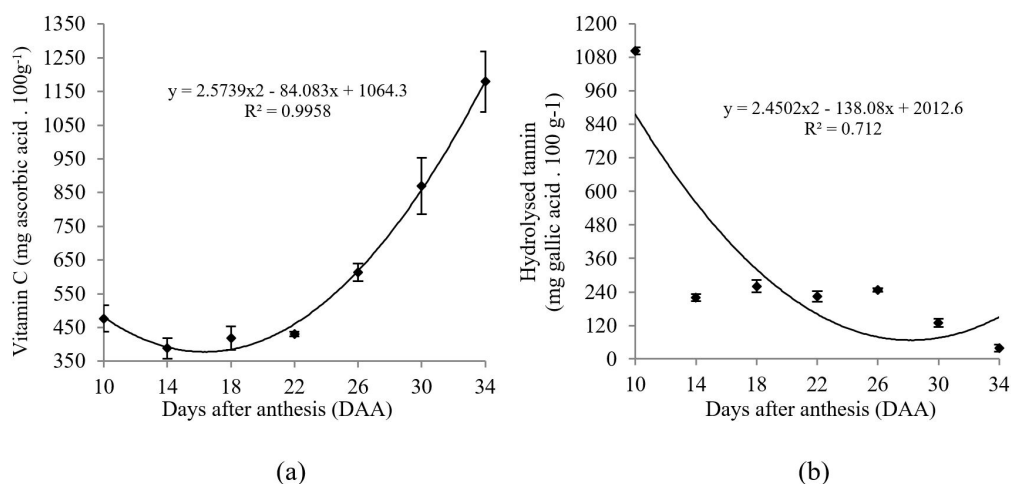


**Figure 4.** Mean values and standard deviation of the *in vitro* antioxidant activity by ABTS<sup>+</sup> method in the ether (a) ethanolic (b), and aqueous (c) extracts during the stages of development of jabuticaba fruits var. *Pingo de mel*.

A reduction in the antioxidant activity measured by the DPPH method (Figure 2) was observed until 18 DAA in the ether extract, increasing at 22 DAA, followed by a further reduction until ripening of the fruit, with 14% DPPH discoloration. In contrast, a decrease was observed in the ethanolic extract at 14 DAA, followed by stability up to 26 DAA, with a reduction

at 30 DAA, and slight increase at 34 DAA, with 16% DPPH discoloration.

The aqueous extract stood out when compared to the ether and ethanolic extracts, with higher values both in the initial stage of development as at the end of ripening, with almost double the discoloration percentage (30% discoloration). Zozio et al. (2014) and Wang et al. (2016) used the DPPH method, and also



**Figure 5.** Mean values and standard deviation of the vitamin C content (a) and hydrolysed tannin (b) during the stages of development of jaboticaba fruits var. *Pingo de mel*.

observed a higher antioxidant activity in *Ziziphus jujuba* in the initial ripening periods.

As previously reported, it was not possible to quantify the antioxidant activity by FRAP method in the ether extract (data not shown), while the ethanolic and aqueous extracts showed similar behavior (Figure 3), with a peak at 10 DAA, reduction at 14 DAA, followed by a stability until fruit ripening, with final concentrations of  $41.46 \mu\text{mol FeSO}_4 \text{ g}^{-1}$  and  $45.84 \mu\text{mol Fe}_2\text{SO}_4 \text{ g}^{-1}$ , in the ethanolic and aqueous extracts, respectively. Rufino et al. (2010) analyzed the antioxidant activity of the aqueous extract of ripe jaboticaba fruit by FRAP method, and found higher values ( $635 \pm 11.9 \mu\text{mol FeSO}_4 \text{ g}^{-1}$ ) when compared with the results of the present study.

When evaluating the antioxidant activity by ABTS<sup>+</sup> method, the ether extract showed the lowest antioxidant activity when compared to the other extracts, while ethanolic and aqueous extracts showed similar results (Figure 4).

The ether extract showed a decrease of the antioxidant activity throughout the stages of development of jaboticaba fruits, with initial and final values of  $14.91 \mu\text{mol trolox g}^{-1}$  and  $3.83 \mu\text{mol trolox g}^{-1}$ , respectively. Lower values were observed for the ethanolic extract up to 22 DAA, with subsequent increases and reductions until ripening, while the aqueous extract presented a low antioxidant activity at 14 DAA, with a tendency to stability up to 26 DAA, followed by a drop at 30 DAA, with new peak at 34 DAA.

Rufino et al. (2010) also investigated the antioxidant activity of the aqueous extract of ripe jaboticaba by the ABTS<sup>+</sup> method, and found higher values ( $317 \mu\text{mol trolox g}^{-1}$ ) when compared with the results of the present study. Secondary metabolites are responsible for the antioxidant activity of fruits, and variations during the stages of development are related to the physiological maturation of fruits (Taiz & Zeiger, 2006) or even in response to factors such as environmental stresses, which involve temperature, oxygen, and pathogens (Roussos et al., 2007).

Moreover, the difference in the extracts may be due to the extraction steps involving different solvents. Further studies on

the appropriate solvent are necessary for selective extraction of natural antioxidants. In the present study, the various solvents provide maximum solubilization of the antioxidants in the sample. The use of three solvents with different polarities ethyl ether (2.9), ethanol (5.2), and distilled water (9) allowed the solubilization of more polar compounds (aqueous extract), with intermediate polarity (ethanolic extract), and nonpolar compounds (ether extract) (Borguini & Torres, 2009).

With respect to the vitamin C content (Figure 5a), a slight reduction was observed up to 14 DAA, followed by elevation to full ripening of fruits, ranging from  $476.21 \text{ mg ascorbic acid } 100\text{g}^{-1}$  at DAA 10 to  $1178.91 \text{ mg ascorbic acid } 100\text{g}^{-1}$  at 34 DAA. These results indicate that the synthesis of ascorbic acid was higher during the beginning of ripening, as also observed by Neves et al. (2015) in Camu-Camu fruit. The degradation of cell wall polysaccharide possibly resulted in increased galactose, which is one of precursors of ascorbic acid biosynthesis (Wheeler et al., 1998; Smirnoff et al., 2001).

Rufino et al. (2010) studied the vitamin C content in mature jaboticabas and found lower values ( $2.38 \text{ mg ascorbic acid } 100\text{g}^{-1}$ ) when compared to this study. This variation may be due to the ascorbic acid, besides influenced by the species, also suffers variations according to the plant genotype, maturation stage, soil and climatic conditions, since it has various functions in abiotic stress response (Ishikawa et al., 2006).

When compared the vitamin C levels of mature jaboticaba fruit var. *Pingo de mel* of the present study ( $1178.91 \text{ mg ascorbic acid } 100\text{g}^{-1}$ ) with the results found by Assis et al. (2001) and Rufino et al. (2010) in mature acerola ( $957 \text{ mg}$  and  $1357 \text{ mg ascorbic acid } 100\text{g}^{-1}$ , respectively), it can be stated that the jaboticaba is a source of this vitamin, as it contains vitamin C levels close to those of acerola, which is recognized as a fruit rich in vitamin C.

The hydrolysed tannin contents of jaboticaba fruits var. *Pingo de mel* (Figure 5b) were  $1103.76 \text{ mg gallic acid } 100\text{g}^{-1}$  at 10 DAA and  $247.22 \text{ mg gallic acid } 100\text{g}^{-1}$  at the end of ripening. A marked decrease in the levels of hydrolysed tannins was observed from

10 DAA to 14 DAA, followed by a tendency to stabilization, since a higher astringency was observed in the initial period of fruit development. Robichaud & Noble (1990) argue that tannins are compounds responsible for fruit astringency, but with more recent and detailed studies Tavares et al. (2016) suggest that hydrolysable tannins may be the main phenolic compounds responsible for the reported astringency. The disappearance of astringency is probably due to the condensation of the tannins, rendering them insoluble (condensed) and therefore incapable of forming complexes.

#### 4 Conclusion

Jaboticaba fruit var. *Pingo de mel* exhibited high antioxidant activity and higher phenolic compounds levels in the green stage (10 DAA), though it was unfit for consumption and industrialization. Considering that the lower the hydrolysed tannins, the better the fruit sensory quality, in addition to the health benefits of vitamin C and antioxidant activity, jaboticaba fruit harvesting should take place in 34 days after anthesis for better use of compounds.

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