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Biochemical characterization of blackberry fruit (Rubus sp) and jellies

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Abstract

The present study aimed to quantify the phenolic compounds, carotenoids, anthocyanins and antioxidant activity using 2 different methods in blackberry fruits harvested at 3 different harvest points along the storage time and jellies made from these fruits and the quantification of ascorbic acid in the fruits. The harvesting points used were defined visually, being: 100% red (T1); 50% red and 50% black (T2) and 100% black (T3). After harvest, the fruits were selected, washed and packed in PET boxes with capacity for 200g of fruit and stored for 15 days at 2ºC, being evaluated every 3 days and for the manufacture of the jellies, the fruits were first pulped in stainless steel pulping machine being the jellies prepared with 50:50 (fruit: commercial crystal sugar) in open pan. The results show that the loss of phenolic compounds, anthocyanins and carotenoids was small when fruits and jelly were compared, as well as the antioxidant activity, and this shows that the processing of this fruit does not cause losses in relation to these evaluated parameters.

Keywords: anthocyanins, flavonoids, food processing, harvesting point, *Rubus* sp.
Abbreviations: AA_L-ascorbic acid; DPPH_Method for evaluation of antioxidant capacity; FRAP_Iron reduction method; TEAC_ Trolox Equivalent Antioxidant Capacity.

Introduction

The biological activities of red fruits, such as blackberry, are partly attributed to their high content of a wide range of phytochemicals such as flavonoids (anthocyanins, flavones, and flavans), tannins (proanthocyanidins, ellagitannins and gallotannins), stilbenoids (such as resveratrol), phenolic acids (derivatives of hydroxybenzoic and hydroxycinnamic acids) and lignans (Seeram, 2006).

Phenolic compounds are substances having at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group. These chemical compounds belong to a heterogeneous group with approximately 10,000 compounds (Hermann and Weaver, 1999). They can be divided into two major groups: flavonoids subdivided into flavones, flavanols, flavonols, flavanones, isoflavones and anthocyanidins, and non-flavonoids, which comprise the groups of phenolic acids, lignans and stilbenes (Li et al., 2009).

Polyphenols are secondary metabolites naturally present in fruits and vegetables, and in red fruits, especially blackberry fruits, are important sources of these compounds in human food (Sellappan et al., 2002; Zheng and Wang, 2003). Phenolic compounds are formed by plants under stress conditions such as infections, injuries, exposure to UV radiation, among others (Naczk and Shahidi, 2006), protecting against pathogens and predators (Bravo, 1998).

These compounds exhibit potent antioxidant activity and are widely necessary to prevent the deterioration of oxidizable products such as cosmetics and food products. In addition, these compounds are beneficial to human health (Curi et al., 2014).

In this group of small fruits, blackberries (*Rubus* spp.) are considered excellent sources of phenolic compounds. However, there is a great diversity between blackberry cultivar results in fruits with different characteristics, both in flavor and staining; these differences are associated with the polyphenol content and profile of the fruit (Guedes et al., 2013; Curi et al., 2015).

The importance of polyphenols in human nutrition is generally related to health promotion and possible prevention of some diseases (Gibney et al., 2006). Among its potentially beneficial effects on health are highlighted anti-inflammatory, antiviral, antimicrobial and antioxidant activity (Narayana et al., 2001; Liu, 2003).The objective of the experiment was to perform the biochemical characterization from the evaluation of total phenolic compounds, carotenoids, anthocyanins and antioxidant activity using 2 different methods in blackberry fruits and jelly and ascorbic acid in blackberry fruits harvested in 3 different collection points along the storage time, thus evaluating the influence of refrigerated storage time of fruits and jellies and processing on the analyzed biochemical parameters.

Results and Discussion

The loss of astringency is one of the main changes that occur during the fruits ripening in general and is directly related to the presence of phenolics (Chitarra and Chitarra, 2005), which, with its reduction, would improve the fruit palatability characteristics.

Phenolic compounds

Regarding the storage environment Antunes et al. 2006 observed that, at room temperature, there was an increase in the total phenolic compounds until the sixth day, decreasing from there until the twelfth day, reaching 3.70 mg g^{-1} in the refrigerated environment, there was a slight increase until the ninth day, reaching 4.67 mg g^{-1}, after which there was a small decrease of 0.12 mg g^{-1} until the twelfth day. It can be seen from the results presented in Table 1 that the behavior of the phenolic compounds content in the blackberry fruits did not present any pattern. Other authors found lower phenolic content in blackberry (about 0.21 mg Gallic acid g^{-1}) in different genotypes (Wang and Mazza, 2002). The cultivars 'Tupy' and 'Guarani', when cultivated in a tropical climate (central region of Brazil), presented lower levels (0.37 and 0.43 mg of Gallic acid g^{-1}), respectively (Hassimoto et al., 2008), when compared with the results of the present study. Results similar to these authors were found in 'Cherokee' cultivated in Turkey (0.30 mg of Gallic acid g^{-1}) (Koca and Karadeniz, 2009).

Comparing blackberry with other fruits of the same botanical family as raspberry (30 mg Gallic acid 100 g^{-1} fruit) and strawberry (80 mg Gallic acid 100 g^{-1} fruit) (Agar et al., 1997), it is observed that the blackberry content comprises higher content of total phenolic compounds. The total phenol content was higher in red-berries (1,093.22 mg 100 g^{-1}); and the lowest, in the 'Guarani' cultivar (147.86 mg 100 g^{-1}), differing statistically from the other cultivars evaluated by Guedes et al., (2014). Tosun *et al*., (2008) reported mean contents of total phenols of 938.8 ± 125.9 mg 100 g^{-1} in blackberry fruits produced in Turkey.

Table 2 shows values of phenolic compounds in blackberry jellies elaborated with 3 harvest points. It can be verified that when compared with the data of phenolic compounds of fruits, the jellies presented increase in the contents during the time of storage. In blackberry nectar, Araujo et al., (2009) found values that ranged from 1.91 to 1.73 mg of Gallic acid g^{-1}.

In Table 3, we can verify that the anthocyanin and carotenoid contents were decreasing throughout the evaluation period in all evaluated treatments.

Anthocyanins and carotenoids

Meneguel et al., (2008) studying edible coatings on the conservation of blackberry fruits found values at the beginning of storage of 53 ± 3 µg g^{-1} of fruits and linear increase in total anthocyanin content with storage time in all treatments used. The authors verified that the increase rate of the anthocyanin content in the control fruits was 21 µg g^{-1} day^{-1}. Mota (2006), working with blackberry juice cultivar 'Comanche' obtained a concentration of total anthocyanins of 1210 µg g^{-1} of juice and after 30 days of storage at 16 to 18ºC dropped to 530 µg g^{-1}.

Comparing with the studies described above, with the exception of Mota (2006), which shows a decrease in the anthocyanin content in the fruits, the others show an increase in the contents over the storage period. These values do not agree with the values found in the present experiment. The decrease may be explained by the consumption of these compounds during storage.

In blackberry, due to the high content of total anthocyanins, the yellowish coloration characteristic of the carotene presence is not representative as in other fruits.
The total carotenoids content in blackberry (87.7 µg β-carotene g^{-1} fruit) is reported to be higher than the acerola (*Malpighia punicifolia*) (53 µg β-carotene g^{-1} fruit), that, like blackberry has a predominance of anthocyanins (Araújo et al., 2007). According to Marinova and Ribarova (2007), the blackberry has several carotenoids, including lutein (0.3 mg 100 g^{-1} of fruit), zeaxanthin (29.0 mg 100 g^{-1} of fruit), β-crytoxanthin (30.1 mg 100g^{-1} of fruit), α-Carotene (9.2 mg 100 g^{-1} of fruit) and β-carotene (101.4 mg 100 g^{-1} of fruit).

In Table 3, we can verify that the content of carotenoids presented decrease during the storage period. Carotenoids are mostly thermolabile, and one of the major causes of color loss during storage is oxidation, which is accelerated by light, temperature and presence of metal catalysts (Sarantópoulos et al*.,* 2001). The results of the experiment show that the loss was more pronounced in the treatment which had 100% red fruits.

Throughout the storage there has been a significant decrease in the jellies anthocyanin content in the treatment which was used 100% of red fruits, in agreement with the studies on jellies of seven conventional blackberry cultivars realized by Mota (2006), which observed a mean reduction of 57% in the initial content of total anthocyanins over 90 days of storage at room temperature. The decrease in the content of these pigments during the storage period may be due to the presence of oxygen inside the package that can cause oxidative reactions, which can generate unstable products from anthocyanins, thus causing loss of color (Wrolstad and Skrede, 2002). The other treatments presented a decrease in the intermediate days of evaluation and increase at the end of the evaluation period.

Studies conducted by Wang and Lin (2000) on blackberry, raspberry and strawberry fruits indicate that ripe black raspberry and blackberry fruits constitute a rich source of anthocyanins (197.2 and 152.8 µg g^{-1} respectively) when compared to mature fruits of red raspberry (68.0 µg g^{-1}) and strawberry (31.9 µg g^{-1}). It should be noted that these fruits, like the blackberry, belong to the Rosaceae botanical family.
When verifying the study that shows the anthocyanins contents (and other pigments), it is possible to verify that there is no pattern of their increasing or decreasing, being the behavior different among fruits of different cultivars. Based on the values reported in the literature on anthocyanin content, and the great variation between the different genetic materials, there is great potential in the production of blackberry in order to obtain a natural dye for the food industry and for medicines (Embrapa Clima Temperado, 2008).

The blackberry jam retains a good part of the anthocyanins found in the fruits and is a source to be considered of these

Table 1. Phenolic Compound values (mg of Gallic acid g^{-1}) of blackberry fruits harvested at 3 different harvest points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
T1	7.33 ± 0.71abB	7.18 ± 0.21abB	7.16 ± 0.18aB	7.40 ± 0.16aB	10.41 ± 0.15aA	7.55 ± 1.23aB
T2	7.90 ± 0.74aAB	8.37 ± 1.01aAB	7.62 ± 0.72aB	9.26 ± 0.58aA	8.32 ± 1.99bAB	8.54 ± 0.71aAB
T3	8.87 ± 0.79aABC	9.61 ± 0.92aAB	8.19 ± 0.50aBCD	9.79 ± 0.28aA	6.87 ± 0.08cD	7.76 ± 0.17aCD

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 2. Phenolic Compound values (mg of Gallic acid g^{-1}) in jellies made from blackberry fruits harvested at 3 different collection points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
T1	7.18 ± 0.08aB	4.61 ± 0.19bC	6.68 ± 0.75aB	4.80 ± 1.02cC	10.47 ± 1.49bA	10.43 ± 1.30bA
T2	7.14 ± 0.08aB	6.64 ± 0.57aB	6.58 ± 0.54aB	8.00 ± 0.46bB	11.23 ± 0.89abA	12.53 ± 0.4aA
T3	5.04 ± 0.27bC	4.88 ± 0.10bC	7.37 ± 1.29aB	12.42 ± 0.70aA	12.27 ± 1.02aA	12.53 ± 0.44aA

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 3. Values of Anthocyanin (µg g^{-1}) and Carotenoids (µg g^{-1}) in blackberry fruits harvested at 3 different collection points along storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
Anthocyanin						
T1	1423.20 ± 575.90aA	321.68 ± 109.08aB	92.23 ± 12.12aB	98.97 ± 44.49aB	30.11 ± 9.12aB	45.65 ± 10.98aB
T2	829.74 ± 139.06bA	265.69 ± 142.39aB	111.30 ± 27.75aB	95.74 ± 9.01aB	53.43 ± 5.64aB	136.28 ± 21.36aB
T3	719.67 ± 213.20bA	377.14 ± 162.94aB	145.30 ± 74.05aB	121.30 ± 9.16aB	80.24 ± 21.80aB	114.77 ± 36.12aB
Carotenoids						
T1	222.15 ± 121.17aA	35.11 ± 9.16aB	22.69 ± 1.50aB	20.53 ± 7.11aB	8.01 ± 1.54aB	12.06 ± 3.08aB
T2	128.62 ± 17.51bA	49.51 ± 19.87aB	22.87 ± 3.50aB	18.59 ± 1.88aB	10.76 ± 0.75aB	23.90 ± 3.48aB
T3	122.78 ± 34.33bA	64.53 ± 24.86aB	30.79 ± 7.24aB	21.83 ± 1.97aB	15.35 ± 3.44aB	20.30 ± 6.34aB

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 4. Values of Anthocyanin (µg g^{-1}) and Carotenoids (µg g^{-1}) in jellies prepared from blackberry fruits harvested at 3 different collection points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
Anthocyanin						
T1	347.55 ± 85.51aA	131.09 ± 21.50bB	120.41 ± 5.61bB	54.12 ± 8.66bB	143.76 ± 65.72aB	94.83 ± 21.12bB
T2	445.36 ±77.70aB	706.38 ± 103.27aA	98.21 ± 8.95bC	82.82 ± 10.44bC	225.02 ± 60.69aC	547.37 ± 45.26aAB
T3	109.93 ±20.08bC	78.11 ± 9.01bC	512.66 ± 77.52aB	982.98 ± 373.74aA	148.67 ± 46.85aC	625.14 ± 77.58aB
Carotenoids						
T1	128.36 ± 31.40aA	22.81 ± 3.77bB	23.77 ± 1.27bB	11.52 ± 1.70bB	23.28 ± 10.21bB	16.14 ± 3.63bB
T2	107.92 ± 20.80aA	119.94 ±17.99aA	19.20 ± 2.40bB	15.76 ± 2.21bB	43.06 ± 12.98bB	92.47 ± 8.01aA
T3	21.05 ±5.08bC	17.90 ± 2.28bC	83.36 ± 11.99aB	167.44 ± 62.72aA	181.28 ± 10.94aA	105.96 ± 12.94aB

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 5. Antioxidant activity values (TEAC -TROLOX Equivalent Antioxidant Capacity - µmol g^{-1}) in blackberry fruits harvested at 3 different collection points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days						Average
	0	3	6	9	12	15	
T1	0.31 ± 0.17	0.32 ± 0.02	0.31 ± 0.05	1.50 ± 1.63	0.39 ± 0.02	0.44 ± 0.01	0.55ab
T2	0.35 ± 0.05	0.30 ± 0.04	0.27 ± 0.06	2.61 ± 2.02	1.94 ± 2.16	1.17 ± 1.67	1.11a
T3	0.29 ± 0.04	0.33 ± 0.02	0.26 ± 0.10	1.16 ± 1.70	0.41 ± 0.05	0.42 ± 0.02	0.48b
Average	0.32B	0.32B	0.28B	1.76A	0.92AB	0.68B	

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 6. Antioxidant Activity Values (TEAC - TROLOX Equivalent Antioxidant Capacity - µmol g^{-1}) in jellies prepared from blackberry fruits harvested at 3 different collection points along storage time on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
T1	0.45 ± 0.01	0.41 ± 0.04	0.36 ± 0.08	0.41 ± 0.02	0.40 ± 0.04	0.41 ± 0.03
T2	0.41 ± 0.01	0.39 ± 0.04	0.39 ± 0.02	0.42 ± 0.04	0.39 ± 0.06	0.34 ± 0.03
T3	0.36 ± 0.09	0.43 ± 0.04	0.35 ± 0.10	0.40 ± 0.03	0.37 ± 0.05	0.38 ± 0.03

T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 7. Antioxidant activity values (FRAP - µM ferrous sulphate g^{-1}) in blackberry fruits harvested at 3 different collection points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage days					
	0	3	6	9	12	15
T1	0.39 ± 0.10aB	0.45 ± 0.04aAB	0.51 ± 0.06aAB	0.40 ± 0.03aB	0.56 ± 0.04aA	0.52 ± 0.05aAB
T2	0.41 ± 0.08aB	0.54 ± 0.12aA	0.40 ± 0.08bB	0.37 ± 0.08aB	0.37 ± 0.08bB	0.42 ± 0.06aAB
T3	0.48 ± 0.12aA	0.48 ± 0.04aA	0.44 ± 0.07abAB	0.34 ± 0.02aB	0.39 ± 0.05bAB	0.46 ± 0.03aAB

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 8. Antioxidant Activity Values (FRAP - µM ferrous sulphate g^{-1}) in jellies prepared from blackberry fruits harvested at 3 different collection points along storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage Days					
	0	3	6	9	12	15
T1	0.48 ± 0.10aA	0.37 ± 0.02bA	0.38 ± 0.08bA	0.37 ± 0.05aA	0.30 ± 0.05aA	0.34 ± 0.02bA
T2	0.44 ± 0.07aB	0.77 ± 0.17aA	0.25 ± 0.11bB	0.38 ± 0.04aB	0.39 ± 0.0aB	0.44 ± 0.08abB
T3	0.35 ± 0.03aBC	0.28 ± 0.04bC	1.04 ± 0.46aA	0.54 ± 0.16aB	0.39 ± 0.02aBC	0.58 ± 0.02aB

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

Table 9. Ascorbic Acid values (mg 100 g^{-1}) in blackberry fruits harvested at 3 different collection points along the storage time, on 0; 3; 6; 9; 12 and 15 days after harvest. Cerqueira César-SP, 2013.

Treat.	Storage Days					
	0	3	6	9	12	15
T1	3.95 ± 0.25aC	3.62 ± 0.22aC	3.86 ± 0.32aC	3.32 ± 0.48aC	7.13 ± 0.71aB	9.11 ± 0.16aA
T2	4.16 ± 0.45aB	3.35 ± 0.47aBC	3.84 ± 0.55aB	2.24 ± 0.71aC	6.63 ± 1.12aA	6.77 ± 1.80bA
T3	3.20 ± 0.15aBC	2.62 ± 0.76aBC	4.04 ± 0.71aB	2.30 ± 0.29aC	7.40 ± 1.58A	3.90 ± 0.62cB

Averages followed by the same lowercase letter in the column and upper case in the row did not differ statistically from each other by Tukey test (P> 0.05). T1-Fruits 100% Red; T2-Fruits 50% Red and 50% Black and T3-Fruits 100% Black.

compounds, since the contents present in this product are superior to those found in the in *natura* fruits of other red fruits such as strawberry (30 to 60 µg g^{-1}) (Cordenunsi et al*.,* 2003), Surinam cherry (16,23 µg g^{-1}) (Lima et al., 2005) or acerola (3.79 g to 59,74 µg g^{-1}) (Lima et al., 2003).

The jellies presented different behaviors for the carotenoid content. The jellies prepared from 100% of red fruit showed sharp decline. The intermediate jellies showed a decrease followed by an increase in the final periods of evaluation, while the jellies elaborated with 100% of black fruit showed an increase up to the 12th day and after that period, it declined. The values of anthocyanins and carotenoids in blackberry jellies are presented in Table 4.

Antioxidant activity

Table 5 shows the values of Antioxidant Activity Values (TEAC - TROLOX Equivalent Antioxidant Capacity - µmol g^{-1}) found for the 3 collection points evaluated. It can be verified that there is no pattern among the fruits evaluated. It is noticed that when evaluating the fruits average in each day of analysis, there is an increase at the 9 and 12 days after the harvest followed by a decrease. The results of this study are similar to that presented in the study by Kuskoski *et al.*, (2005) in blackberry fruits, which report 82.6% inhibition. Silva (2007) cites the 'Guarani', 'Tupy' and 'Brazos' blackberry cultivars, 12.03; 9.89; and 11.48 µmol g^{-1}, respectively.

Table 6 shows the Antioxidant Activity value (Trolox equivalent) of the jellies. It is noticed that, unlike the fruits, the jelly behavior did not show statistically significant differences neither during storage nor according to the collection points of the same ones. The reduction in the antioxidant capacity of jellies can be explained by the losses of the bioactive compounds during the processing of the jellies, such as total phenols, vitamin C and tocopherols, but mainly in relation to the greater losses of total anthocyanins. The DPPH and FRAP evaluations are indicated as simple and rapid methods to evaluate the antioxidant activity of fruits and vegetables (Antolovich et al., 2001; Bagetti et al., 2009).

In the FRAP assay the antioxidant capacity is measured as the ability to reduce Fe^{3+} - TPTZ complex to Fe^{2+} (Benzie and Strain, 1996), whereas the DPPH assay involves a rapid electron transfer process from phenolic compounds to the radical DPPH (Brand-Williams et al., 1995, Martins et al., 2009). Despite some differences, the analyses of FRAP and DPPH showed similar results.

As in the case of the Antioxidant Activity by the DPPH method described above, it can be verified that little variation occurred between the treatments and the days of analysis. It can also be noticed that when we compare the values found in fruits and jellies, the results show little variation in most cases (Table 8).

Ascorbic acid

The treatments evaluated in this study presented lower ascorbic acid contents than those mentioned in the international literature for blackberry. Agar, et al., (1997) reported vitamin C content of 21 mg 100 g^{-1} of fresh fruit and Pantelidis et al., (2007) report average levels of 14 mg 100 g^{-1}. These results can be explained by the quantification of vitamin C, since some methods quantify only the most active form, L-ascorbic acid (AA), and it does not measure the other compounds chemical forms, isomers and oxidized forms, which represent the Vitamin C.

Table 9 shows that as for the ascorbic acid contents, the fruits presented an increase in the contents the longer their storage time. This pattern was verified in treatments 100% Red and 50% Red and 50% Black. On the fruits in the last treatment (fruits 100% Black) there is an increase in the 12th day of evaluation and drop at the 15th day. This can be explained by the degradation that occurs due to the senescence of the fruits.

According to Pantelidis et al., (2007), the content of ascorbic acid present in blackberry (0.9 mg of ascorbic acid in 100 g^{-1} fruit) is low when compared to other fruits studied by Hernandéz, Lobo and Gonzáles (2006) and Araújo et al., (2007) as the content in the papaya (86,0 mg of ascorbic acid in 100 g^{-1} fruit), in mango (89,0 mg of ascorbic acid in 100 g^{-1} fruit) and acerola (183 mg 100g^{-1}).

Studies carried out by Pantelidis et al., (2007) reported large variations in vitamin C content in several cultivars of blackberry and raspberry, both from the Rosaceae family and the 'Rubus' genus, ranging from 14.3 to 103.3 mg of ascorbic acid in 100 g^{-1} fresh fruit.

Materials and Methods

Plant Material

Blackberry fruits of the Tupy cultivar were harvested manually and randomly from different positions and orientations on thirty blackberry plants. The plants were at the commercial maturity stage at a commercial farm in the municipality of Cerqueira César-SP (latitude 23°02'08" S, longitude 49°09'58" W and altitude of 737 meters) and transported immediately to the Fruit and Vegetable Laboratory, of the Department of Horticulture, at the Faculty of Agronomic Sciences, UNESP Botucatu Campus. This method of harvesting was chosen because it is the most used by the producers for this type of fruit since the fruits present different stages of maturation in the same plant (Souza, 2015). The blackberries were washed in running water and immersed for 20 minutes in chlorinated solution at 20 ppm and then exposed to air to dry naturally on smooth surface benches.

Treatments

The harvesting points that constituted the treatments were defined visually, being: 100% red -T1; 50% red and 50% black -T2 and 100% black -T3, being the fruits evaluated at the time of harvest and at 3, 6, 9, 12 and 15 days after harvest and the jellies were elaborated from these fruits.

For the manufacture of the jellies (Brasil, 1978) the fruits were first pulped in a discontinuous stainless steel pulper with 0.5 mm mesh sieve and prepared with 50:50 (fruit: commercial crystal sugar). For the gelling, commercial pectin (0.5% in relation to pulp weight) was used, with no citric acid added due to the acidity of the fruits. The cooking was done in an open copper pot with a maximum capacity of 8L, with continuous manual stirring. The determination of the jelly end point was performed using a refractometer, setting the value of 65º Brix as the standard.

Experimental design and biochemical analyses

The evaluations of the jelly were carried out after 24 hours of the manufacture so that could have the stabilization of the texture and coloration. The evaluations realized were: Total Phenolic Compounds in the fresh matter, according to the Folin-Ciocalteu spectrophotometric method (Singleton et al*.,* 1999) with results compared to the standard curve of the Gallic acid, since this phenolic acid is the most used as standard in the scientific literature and the results expressed in mg of Gallic acid g^{-1}; Carotenoids and Anthocyanins performed in the fresh matter according to the method validated by Sims and Gamon (2002) and the absorbance values converted to µg g^{-1}; Determination of the DPPH radical scavenging activity having the measurement performed with absorbance at 517 nm and determined using a spectrophotometer (Singleton et al*.,* 1999) having the results expressed in equivalent antioxidant capacity of TROLOX-TEAC in µmol g^{-1} of sample according to standard curve (Brand-Williams et al*.,* 1995); Determination of the total antioxidant activity by iron reduction method (FRAP) and the reading performed at 595 nm (Rufino et al., 2006) and the results expressed in uM ferrous sulfate g^{-1} of fruit and Ascorbic Acid through the method described by Terada *et al.*, (1978) with some modifications.

Statistical analysis

Each treatment consisted of five replicates formed by trays with about 200 g of fruit and glass jars with about 200g of jelly. The results were submitted to analysis of variance and the means were compared by the Tukey test at the 5% probability level.

Conclusion

The experiment showed that the blackberry fruits present high levels of phenolic compounds when compared to other fruits. Another important fact is the low or no loss of phenolic compounds, anthocyanins and carotenoids and the stability in relation to the antioxidant activity by the methods used when comparing fruits at the starting point and the jellies manufactured at this point and at the end of the storage period. Processing losses were also small. A pre-test was performed to check ascorbic acid in the jellies, but the amount found was insignificant. This shows that in relation to this parameter, the losses due to processing are large.

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