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ORIGINAL ARTICLE

Effects of blackberries (*Rupus* sp.; cv. Xavante) processing on its physicochemical properties, phenolic contents and antioxidant activity

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Abstract Beneficial health effects are attributed to the wide array of polyphenols such as anthocyanins present in berries. Blackberries have been improved genetically to be cultivated in different climate such as Brazil. Thus, distinctive cultivars were created. However, antioxidant properties of these have not been evaluated. This study aimed to investigate the availability of phenolic compounds in Brazilian cultivar blackberries (cv. Xavante) after processing into purées, *coulis* and jam changes during storage. Physicochemical, total phenolic compounds (TPC), anthocyanins and antioxidant activity of frozen and processed food products were evaluated. The pH values of jam and frozen pulp increased to a greater extent during storage. Pureé A (70 °C for 20 min) showed the highest value for lightness and redness on day 1 with a decrease after 30 days of storage. Redness values decreased to a greater extent for purée A, indicating less stable product over time whereas c*oulis* presented optimal stability shown by the smaller color difference value. No differences in TPC were observed for frozen pulp, purée A, purée B, and *coulis*. Significantly lower amount of TPC was present in the jam indicating degradation of phenolic compounds upon heating and concentration process involved in jam making. An increase in antioxidant activity (ABTS assay) was observed in processed products after storage, which might be related to the development of new compounds with greater antioxidant activity. Therefore, processing of blackberries into food products is an alternative to prolong the accessibility of those fruits without extensive loss of antioxidant activities.

Keywords Blackberries cv Xavante · Processing · Antioxidant activity · Phenolic compounds

Introduction

The consumption of vegetables and fruits has been related to heath benefits such as protection against diseases associated with oxidative stress, e.g., chronic diseases (Brownmiller et al. 2008). Those effects have been generally attributed to the extensive array of polyphenols such as anthocyanins, flavan-3-ols, proanthocyanidins, and flavonols present in vegetables and fruits (Hervert-Hernández et al. 2009).

Blackberry fruits (*Rubu*s sp) are great sources of natural antioxidants, particularly anthocyanins and phenolic acids (Wang and Lin 2000), that have proven to be associated with health benefits such as antioxidant, anticonvulsant, and anti-inflammatory properties (Nogueira and Vassilieff 2000; Wang and Lin 2000; Cuevas-Rodríguez et al. 2010; Sariburun et al. 2010).

Brazil has a great biological diversity of fruits in which their antioxidants properties are still unrevealed and need to be potentially explored. Blackberries are native to Asia, Europe, North and South America and most cultivars have specific growing profile related to the edaphology and climate of specific regions (Hirsch et al. 2013).

Blackberries were introduced in Brazil in the 1970s by the Brazilian Agricultural Research Corporation (Embrapa), and

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have been improved genetically originating adapted cultivars, e.g., Xavante, Guarani, Caingangue, and Tupy (Denardin et al. 2015). Those cultivars have been widely commercialized on South of Brazil, nonetheless, due to its high moisture and tissue fragility, blackberries are very perishable fruits and have to be consumed within some days (Temocico et al. 2008) which may cause losses. Thus, processing the fruits into food products such as juice, purée, preserved canned fruits, *coulis*, and jam can be an alternative to prolong the accessibility and consumption of blackberries.

In the other hand, processing and storage can have noticeable effects on the phenolic content of fruits and, therefore, may reduce or even increase their health benefits properties (Zafrilla et al. 2001; Arkoub-Djermoune et al. 2016). Gil et al. (2000) have shown that processing of pomegranates into juices increases their antioxidant capacity and total phenolic content (Gil et al. 2000). Contrary, strawberry processing into jams affected the flavonoids content by a decrease in 20% (Häkkinen et al. 2000). Likewise, red raspberries flavonoids content decreased slightly with processing and more markedly during 6 months of storage (Zafrilla et al. 2001).

Only a few studies have investigated the antioxidants compounds and antioxidant activity of Brazilian blackberry cultivar Xavante (Hirsch et al. 2013; Oliveira et al. 2014; Denardin et al. 2015). Guedes et al. (2017) showed that the Xavante blackberry cultivar had the highest levels of polyphenols in comparison with different blackberry cultivars such as Arapaho, Brazos, Cainguangue, Cherokee, Choctaw, Comanche, Ébano, Guarani and Tupy. However, none of them have dealt with the effect of processing into food products on the phenolic compounds and antioxidant activity. Thus, we aimed to investigate the availability of phenolic compounds from blackberries (*Rubus* spp.; cv. Xavante) after different processing conditions translated into different food products after 1 and 30 days of storage.

Materials and methods

Materials

Blackberries (*Rupus* sp.; cv. Xavante) were obtained from a farm in South of Brazil (25°23′42″S 51°27′28″O). Samples were collected, frozen at − 20 °C until processing. Chemical reagents were purchased from Sigma Aldrich, Brazil. Sugar was purchased on local Market. All other reagents were of analytical grade (Sigma Aldrich, Brazil).

Food products processing

Fruits were sanitized with chlorine water (200 ppm) for 5 min and washed with distilled water. A fruit extractor was used to obtain the pulp. Three products were produced using blackberry pulp based on standards methods for each of them. Purée which consist of pulp pasteurization (70° C purée A or 80° C-purée B) for 20 min by indirect heating; *Coulis* was prepared by mixing pulp and sugar (70:30) follow by indirect heating at 60° C for 8 min, addition of citric acid (0.2% *w/w*) and heating for 2 min; and jam that was prepared by mixing pulp and sugar (1:1), heating up to 90° C and adding citric acid (0.36%). The jam was concentrated to 65° Brix (approximately 30 min). Table 1 shows the overview of each product processing.

Physicochemical measurements

Samples were evaluated for pH value, total soluble solids (TSS, °Brix), total titratable acidity (TTA), TSS/TTA ratio and CIELab color parameters (L: lightness, a:redness and b:yellowness). The pH was measured using a digital pH meter (Tecnopon®, Piracicaba, Brazil). The total soluble solids content were measured using a digital refractometer (HI96801-Hanna®-instruments, São Paulo, Brazil). The titratable acidity was carried out using the method described by Instituto Adolfo Lutz (2008) and results were expressed as percentage of citric acid.

Color parameters L (lightness), a (redness) and b (yellowness) were determined using a D65 Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). The color difference (ΔE) was calculated according to Eq. (1). The colorimeter was standardized using the white calibration plate. All analyses were carried out in triplicate.

$$\Delta \mathrm{E} = \sqrt{\mathrm{L}^2 + a^2 + b^2} \qquad (1)$$

Quantification of phenolic compounds

Total phenolic compounds

The total phenolic compounds (TPC) content was measured based on Singleton et al. (1965) method with slight modifications. The pulp/products were dispersed in methanol considering the concentration of pulp added in each product. A 125-µL aliquot of the methanolic solution was mixed with 125 µL of Folin-Ciocalteu reagent (diluted 1:1 in water), 2250 µL of a sodium carbonate solution (28 g/L). After 30 min in darkness at room temperature, the absorbance of the samples was measured at 725 nm in a spectrophotometer. Gallic acid was used to prepare the standard curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (mg of GAE/g).

Table 1 Processing parameters and added ingredients

Samples	Temperature (°C)	Time (min)	Added ingredients
Frozen pulp	–	–	–
Pureé A	70	20	–
Pureé B	80	20	–
Coulis	60	10	Sugar (30%), citric acid (0.20%)
Jam	70–90	30	Sugar (50%), citric acid (0.36%)

Anthocyanins

Anthocyanins content was measured based on spectrophotometric method (Fuleki and Francis 1968). Ethanol (70%) was acidified with HCl 0.1% (30%) to pH = 2.0. Samples (1 g) were mixed with 3 mL of acidified solution (pH = 2) and kept under storage for 24 h at 4 °C, centrifuged (3000 rpm/10 min) and mixed with 2 mL of supernatant and 4 mL of acidified solution. The aliquot was diluted in methanol and the absorbance was measured at 535 nm as previously described (Fuleki and Francis 1968). Results were expressed in mg anthocyanin/100 g sample.

Antioxidant activity

DPPH scavenging

Samples were evaluated for their free-radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of El-Massry et al. (2002) with modifications. Methanol was used as extractor and various volumes were mixed with 2.0 mL of DPPH methanolic solution (60 μM) and kept in darkness for 30 min. Absorbance was measured at 517 nm against pure methanol (blank) using a spectrophotometer. Percentage inhibition of the DPPH radical was calculated as follows:

$$\%\ \text{Inhibition DPPH}* = \left(\text{Abs}_{\text{DPPH}*} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{DPPH}*}) * 100$$

where Abs_{DPPH} is the absorbance of the methanolic solution of DPPH, and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH solution mixed with the methanolic solution. Percentage inhibition was plotted against the extract dispersion concentration, and 50% of DPPH inhibition (IC50) was determined by linear interpolation.

ABTS scavenging

Samples were evaluated also for their free-radical-scavenging activity of 2,2 azino bis 3-ethylbenzo thiazoline 6-sulfonic acid diammoninum salt (ABTS). A 5 mL aliquot of ABTS solution (7 mM) was mixed with 88 μL of potassium persulfate (140 mM) and kept overnight. The solution was diluted with ethanol up to an absorbance of 0.70 ± 0.02. Samples methanolic solutions were added and radical scavenging (%) was measured at 734 nm after 6 min of reaction. The percentage inhibition was calculated as follows (Eq. 2):

$$\%\ \text{Inhibition ABTS} = \left(\left(\text{Abs}_{\text{ABTS}} - \text{Abs}_{\text{sample t=6min}}\right)/\text{Abs}_{\text{ABTS}}\right) * 100 \quad (2)$$

where Abs_{ABTS} is the absorbance of the ethanolic solution of ABTS, and $\text{Abs}_{\text{sample t = 6min}}$ is the absorbance of ABTS solution mixed with a methanolic solution of the sample after 6 min of reaction.

Results and discussions

Physicochemical characteristics

In Table 2 a range of primordial physicochemical properties of the frozen and processed products are presented. *Coulis* and jam products presented lower pH values, as expected, compared to purées A and B, due to the addition of citric acid during the production. Total titratable acidity values confirmed, to some extent, the difference in pH values. An increase in pH values was also observed for all products after 30 days of storage, while no differences were observed for total soluble solids, total titratable acidity and, consequently the ratio TSS/TTA. As observed, the pH values of jam and frozen pulp have increased to a greater extent during storage. Thus, storage has affected significantly mainly the pH of the product.

Color changes are shown in Table 3. Color is an important quality factor for foods, mostly for fruit-based products such as jam. Thus, the color of the products should not change during storage. However, processing may alter color features of fruit-based products due to the enhancement of reactions during heat, dissolution or concentration. Pureé A has shown the highest value for lightness (L) at day 1 with a significant decrease after 30 days of storage followed by purée B, while *Coulis* has presented the lower L value. No differences were observed for the other samples studied regarding lightness. Redness (a) has

Table 2 Physicochemical properties of frozen blackberry pulp and processed food products

Samples	PH		Total soluble solids (°Brix)		Total titratable acidity (% citric acid)		TSS/TTA	
Storage time	Day 1	Day 30	Day 1	Day 30	Day 1	Day 30	Day 1	Day 30
Frozen pulp	3.07 ± 0.110^{bBA}	3.32 ± 0.084^{aB}	9.60 ± 0.78^{aC}	9.40 ± 0.26^{aC}	1.37 ± 0.028^{aB}	1.42 ± 0.023^{aA}	7.00 ± 0.55^{aC}	6.61 ± 0.42^{aC}
Pureé A	3.15 ± 0.067^{bA}	3.31 ± 0.065^{aB}	9.55 ± 0.65^{aC}	9.25 ± 0.49^{aC}	1.33 ± 0.043^{aB}	1.29 ± 0.056^{aC}	7.18 ± 0.90^{aC}	7.17 ± 0.30^{aC}
Pureé B	3.15 ± 0.048^{bA}	3.36 ± 0.067^{aA}	8.35 ± 0.89^{aC}	9.50 ± 0.67^{aC}	1.39 ± 0.050^{aB}	1.36 ± 0.026^{aB}	6.00 ± 0.34^{bC}	6.98 ± 0.31^{aC}
Coulis	3.00 ± 0.043^{bBC}	3.16 ± 0.083^{aC}	34.30 ± 0.24^{aB}	34.15 ± 0.50^{aB}	1.27 ± 0.046^{aC}	1.28 ± 0.046^{aC}	27.00 ± 0.16^{aB}	26.67 ± 0.42^{aB}
Jam	2.91 ± 0.070^{bC}	3.30 ± 0.061^{aB}	69.65 ± 0.67^{aA}	69.15 ± 0.82^{aA}	1.96 ± 0.087^{aA}	1.00 ± 0.081^{bD}	35.53 ± 0.25^{bA}	69.15 ± 0.48^{aA}

Means with different small letters in the same row for each parameter are significantly different within same analysis ($P < 0.05$). Means with different capital letters in the same column are significantly different within same storage time ($P < 0.05$). Results are expressed as mean ± standard deviation

Table 3 Color parameters of frozen blackberry pulp and processed food products

Samples	Lightness (L)		Redness (a)		Yellowness (b)		
	Storage time						
	Day 1	Day 30	Day 1	Day 30	Day 1	Day 30	ΔE
Frozen pulp	17.59 ± 0.76^{aBC}	16.70 ± 0.61^{aC}	24.43 ± 0.42^{aC}	20.74 ± 0.67^{bC}	-6.85 ± 0.08^{aB}	-2.54 ± 0.076^{bC}	8.61 ± 0.75^{C}
Pureé A	21.43 ± 0.32^{aA}	18.47 ± 0.26^{bA}	29.89 ± 0.70^{aA}	24.92 ± 0.47^{bA}	-2.29 ± 0.08^{aD}	-0.07 ± 0.03^{bA}	12.80 ± 1.10^{A}
Pureé B	18.70 ± 0.30^{aB}	18.02 ± 0.35^{aAB}	28.11 ± 0.58^{aB}	24.95 ± 0.5^{bA}	-4.73 ± 0.067^{aC}	-0.15 ± 0.023^{bB}	10.59 ± 1.0^{B}
Coulis	16.83 ± 0.43^{aC}	16.85 ± 0.28^{aB}	23.20 ± 0.62^{aD}	22.21 ± 0.31^{bB}	-8.51 ± 0.076^{aA}	-3.62 ± 0.04^{bD}	6.42 ± 1.12^{D}
Jam	17.89 ± 0.51^{aBC}	17.68 ± 0.61^{aAB}	24.56 ± 0.67^{aC}	21.55 ± 0.44^{bBC}	-9.14 ± 0.06^{aA}	-5.17 ± 0.056^{bE}	7.51 ± 1.33^{CD}

Means with different small letters in the same row for each parameter are significantly different within same analysis ($P < 0.05$). Means with different capital letters in the same column are significantly different within same storage time ($P < 0.05$). Results are expressed as mean ± standard deviation been reported to be positively correlated with anthocyanins and their stability in blackberries (Jiménez-Aguilar et al. 2011; Yamashita et al. 2017). In this study, purée A has presented the highest redness (a) value. Also, redness has decreased significantly for all products after 30 days, however, redness (a) values decreased to a greater extent for purée A (ΔE = 12.8 ± 1.10), indicating that this product is less stable over time whereas *Coulis* has shown an optimal stability showed by the smaller color difference (ΔE) value (Table 3).

The color of the products after processing and during storage may influence consumer acceptability. Color stability and antioxidants compounds content in red fruits and their processed products can be influenced by many factors such as temperature and time of processing, pH, oxygen, water activity and storage conditions. Degradation of anthocyanin during heating can lead to changes in color (Poiana et al. 2011).

Quantification of antioxidants compounds

Figure 1 shows the results obtained for total phenolic compound (TPC) and anthocyanins presented in the samples after 1 and 30 days of storage. At day 1 no differences in total phenolic compounds (TPC) were observed for frozen pulp, purée A, purée B, and *coulis*, while a significantly lower amount of TPC was present in the jam sample (Fig. 1a). This might be related to the degradation of phenolic compounds with temperature and increasing solid content during heating (Yamashita et al. 2017). After 30 days of storage, the amount of quantified TPC in the frozen pulp decreased. A similar decrease was previously observed after strawberry jam processing (Poiana et al. 2011). However, a statistically significant increase in TPC values was observed for purée B and *coulis*.

Anthocyanins were also quantified in the samples (Fig. 1b). A mild heat treatment (70 °C for 20 min) did not affect the amount of anthocyanin in purée A, however, a more intensive heat treatment of purée B (80 °C for

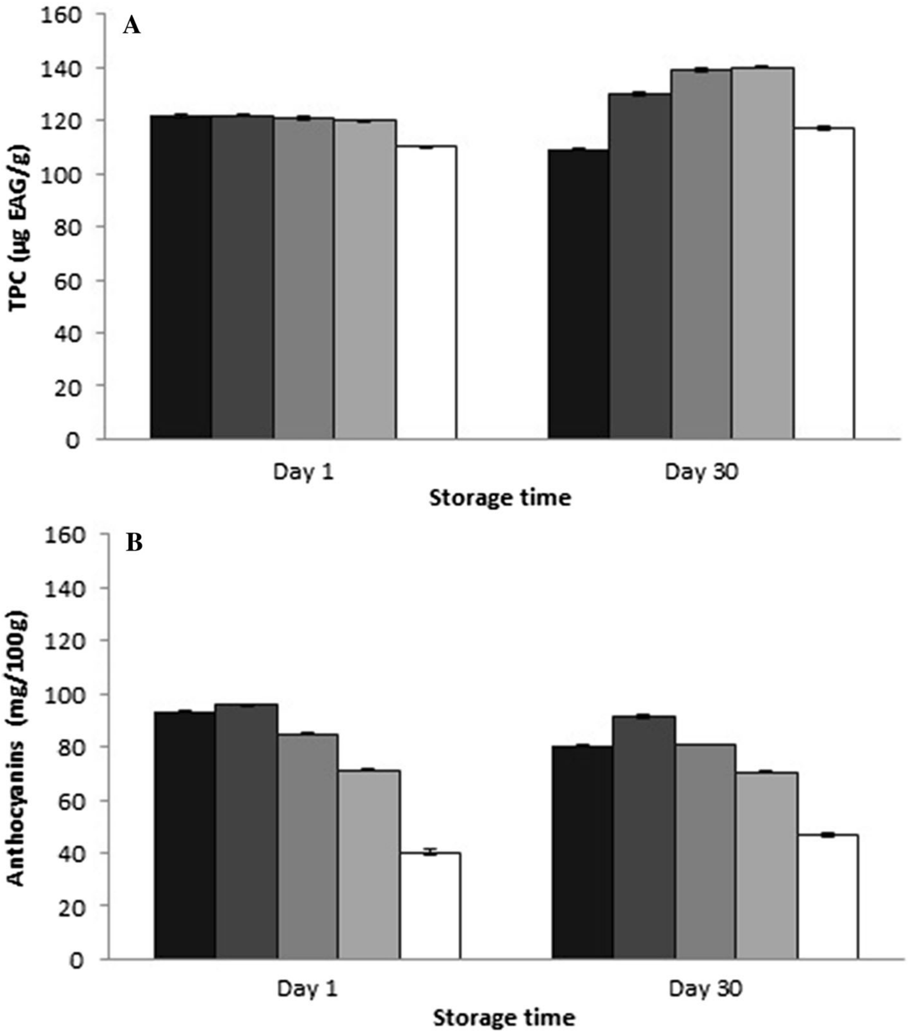

Fig. 1 Total phenolic compounds (**a**) and anthocyanins content (**b**) of frozen pulp (■), purée A (■), purée B (■), *coulis* (■) and jam (□) after 1 and 30 days of storage at 5 °C

20 min) seems to degrade the anthocyanin that together with the concentration factor (*coulis* and jam samples) may enhance the degradation of anthocyanin due to the closeness of reacting molecules in a concentrated system (Yamashita et al. 2017). Likewise, jam presented the lower amount of anthocyanins at both days 1 and 30. In accordance with previous studies, the highest anthocyanin content present in purée A correlates with the higher redness value of it presented in Table 3 (García-Viguera et al. 1998; Zafrilla et al. 2001). A decreased in anthocyanin was observed for frozen pulp and purée A after 30 days of storage, but no significant difference was noticed for purée B, *coulis* and jam samples.

Hager et al. (2010) studied the changes in blackberry (Apache cv) ellagitannin (one of the phenolic compounds) composition after juicing, pureeing, canning, and freezing as well as changes in processed products. Canning, pureeing, and freezing had little effect on ellagitannins, but a significant decrease in ellagitannin was observed after juicing, while only slight changes were observed during storage of thermally processed products (Hager et al.2010).

Antioxidant activity

Antioxidant activity was evaluated using the DPPH and ABTS assays. Several methods can be used to determine the antioxidant activity in foods and plants due to the presence of numerous bioactive compounds and antioxidant mechanisms. Therefore, two or more methods are commonly used to evaluate antioxidant activity. Among the methods that have been used by researchers, DPPH free radical scavenging and ABTS are the most common due to the facility of analysis and good correlation with other methodologies (Sousa and Vieira 2011). The concentration required to inhibit 50% of DPPH radicals (IC50) is shown in Fig. 2a, while ABTS assays show the percentage of inhibition after 6 min (Fig. 2b). IC50 is usually inversely correlated with the phenolic concentration in the samples (Felix da Silva et al. 2015); i.e., the higher the amount of

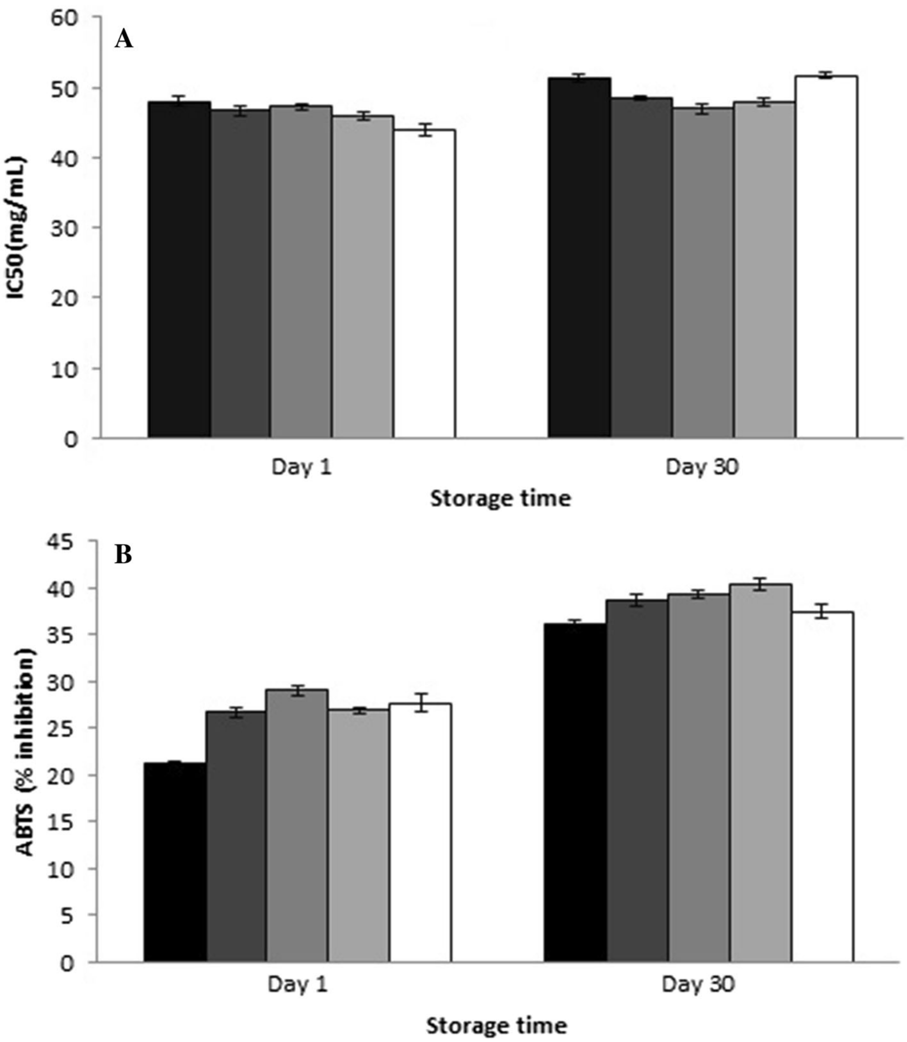

Fig. 2 Antioxidant activity by concentration for 50% inhibition (IC50) using DPPH scavenging (**a**) and percentage of inhibition (**b**) using ABTS scavenging. Frozen pulp (■), purée A (■), purée B (■), *coulis* (■) and jam (□) after 1 and 30 days of storage at 5 °C

phenolic compounds the lower the IC50 value. However, even though jam sample has shown a significantly lower amount of TPC, no significant difference in IC50 values was observed between the samples (Fig. 2a) at day 1. Thus, after 30 days the IC50 prominently increased for frozen pulp and jam samples, indicating a loss of antioxidant activity, but no significant differences were observed for purée A, purée B, and *coulis*. Although total phenolic compounds are the major potential responsible for the antioxidant properties of fruits jams, antioxidant activity is not limited to these, once it has been proved that some degradation products of anthocyanins may also present antioxidant activity (Tsai and Huang 2004; Tsai et al. 2004; Brownmiller et al. 2008; Poiana et al. 2011).

In this way, distinct results were observed using ABTS assay (Fig. 2b). Processed products exhibited significantly higher percentage of inhibition compared to frozen pulp both times analyzed. Antioxidant activity of samples increased with storage time (day 30), nevertheless no differences were observed between processed food products. These differences underline that different radicals have different antioxidant kinetics potentials when reacting with phenolic compounds. Similarly, as discussed above, the degradation of bioactive compounds can originate compounds with antioxidant activity upon storage. Therefore, higher antioxidant activity was measured after day 30. Fu et al. (2017) have also observed an increase in the antioxidant activity of Chinese tangerine (Pericarpium Citri Reticulatae, PCR) over storage. Likewise, the antioxidant capacity of tomatoes products has been reported to be higher than the fresh tomatoes (Re et al. 2002). In other studies, the antioxidant activity of processed foods derived from tomato maintained or even increased due to the development of new compounds with antioxidant activity (Re et al. 2002). It was also previously reported that total phenolic compounds decreased significantly after dehydration of fresh plums, while antioxidant activity in dried plums increased (Piga et al. 2003). This may be explained by the formation of new compounds with antioxidant activity during heat treatment and by hypothesizing that intermediate phenolic formed during processing may have greater antioxidant activity than intact phenolic compounds (Piga et al. 2003).

Conclusion

This study reported the effect of processing of Xavante blackberries on its antioxidant activity and changes during storage. The results showed that the blackberry Brazilian cultivar Xavante is a great source of phenolic compounds, including anthocyanins, with a substantial antioxidant activity. The antioxidant activity increased upon processing. Therefore it suggests that processing of blackberries into products can be an alternative to prolong the accessibility and consumption without extensive loss of its antioxidant effects. However, a better understanding of the development of intermediate compounds formed during processing is still needed to improve the final properties blackberries-based processed products. Such investigations are on-going in our laboratories and will be communicated in due course.

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