Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v39i1.28413

Antioxidant activity and total phenol content of blackberries cultivated in a highland tropical climate

Mayara Neves Santos Guedes[1], Rafael Pio[2*], Luana Aparecida Castilho Maro[3], Fabíola Fonseca Lage[4], Celeste Maria Patto de Abreu[4] and Adelir Aparecida Saczk[4]

[1]*Departamento de Agricultura, Universidade Federal de São João del-Rei, Sete Lagoas, Minas Gerais, Brazil.* [2]*Departamento de Agricultura, Universidade Federal de Lavras, Cx. Postal 3037, 37200-000, Lavras, Minas Gerais,Brazil.* [3]*Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Itajaí, Santa Catarina, Brazil.* [4]*Departamento de Química, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. *Author for correspondence. E-mail: rafaelpio@dag.ufla.br*

ABSTRACT. Blackberries are an important option for the diversification of fruit crops. However, there is currently no literature regarding plant cultivation in high-altitude tropical climates. Knowledge of the phenolic composition of blackberries is essential because variations in the levels of these components may exist between cultivars and may depend on environmental conditions. High performance liquid chromatography (HPLC) was used to evaluate the total phenol content of different blackberry cultivars (Arapaho, Brazos, Cainguangue, Cherokee, Choctaw, Comanche, Ébano, Guarani, Tupy and Xavante). Free radical scavenging activity in these cultivars was assayed using a DPPH test. The HPLC-UV chromatogram of blackberry fruit extracts at 280 nm revealed the presence of phenolic compounds. The results showed significant differences in the levels of phenolic compounds in the blackberry cultivars tested. Antioxidant activity was evaluated using the ABTS free radical and ranged from 2.7 ± 0.1 to 19 ± 2 μmole of Trolox equivalents per gram of sample (b.u.). These results are in good correlation with the phenolic contents of the blackberries tested. The Xavante blackberry cultivar had the highest levels of polyphenols that could be individually identified. Catechin polyphenols were found to be the main component in the blackberry varieties tested.

Keywords: total phenols, free radicals, HPLC, *Rubus* spp.

Atividade antioxidante e fenóis totais de amoras-pretas cultivadas em clima tropical de altitude

RESUMO. Amoras-pretas são uma opção importante para a diversificação da fruticultura, no entanto, as informações são restritas sobre plantas cultivadas no clima tropical de altitude. Conhecimento da composição dos fenóis antioxidantes na composição das amoras-pretas é essencial, pois pode haver variações nos níveis destes componentes entre as cultivares e em favor das condições ambientais. A cromatografia líquida de alta eficiência (HPLC) foi utilizada para avaliar o teor de fenóis totais em frutos de diferentes cultivares de amora-preta (Arapaho, Brazos, Cainguangue, Cherokee, Choctaw, Comanche, Ébano, Guarani, Tupy e Xavante) e os potenciais radicais livres pelo teste DPPH. O esboço de extratos das amoras-pretas por HPLC-UV a 280 nm revelou a presença de compostos fenólicos, com tempos de retenção e absorção nas características de UV-Vis. Os resultados mostraram que existem diferenças nos níveis de compostos fenólicos das amoras-pretas testadas. A atividade antioxidante foi avaliada por varrimento de ABTS de radicais livres, que variou de 2,7 ± 0,1 e 19 ± 2 μmoles equivalentes de trolox por grama de amostra (b.u.), que está em boa correlação com o conteúdo de compostos fenólicos. A cultivar Xavante se destacou aos mais altos níveis de polifenóis identificados individualmente e polifenóis foi o principal componente nas cultivares.

Palavras-chave: fenóis totais, radicais livres, HPLC, *Rubus* spp.

Introduction

Small red fruits orberries are characterized by the presence of high concentrations of a variety of bioactive compounds, including anthocyanins, phenolic compounds, organic acids, tannins and flavonoids (Szajdek & Borowska, 2008; Zozio, Dominique, & Dornier, 2011; Maro, Pio, Guedes,Abreu, & Curi, 2013).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, Pio, Moura, Lima, & Valle, 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).In addition, the chemical composition of the fruit is influenced by multiple genetic factors, which limits quality and consumer acceptance (Scalzo, Battino, Costantini, & Mezzetti, 2005).

Blackberries come from deciduous fruit-bearing trees of temperate climates. However, recently new orchards have been established in subtropical and tropical regions (Campagnolo & Pio, 2012; Curi et al., 2015; Caproni et al., 2016).The fruit chemical composition of a specific cultivar may be affected by several factors, and environmental conditions are among the most important factors that influence chemical composition (Maro et al., 2014).

The blackberry fruits grown in high-altitude tropical climates that are characterized by milder conditions require study to evaluate the chemical quality of the fruits. Therefore, the purpose of this study is to assess the quality and content of phenolic compounds in different cultivars of blackberries being grown in a high-altitude tropical climate.

Material and methods

Fruits from 10 blackberry cultivars were collected manually and randomly from different positions and orientations on thirty blackberry plants. The plants were at the commercial maturity stage. The orchard experiences a high-altitude tropical climate. The city is located at 21°14' S 45°00' W at an average altitude of 918 m in Brazil. According to Köeppen’s classification, the climate is type Cwb, mild temperate (mesothermal). This climate is characterized by a dry winter and rainy summer (Dantas, Carvalho, & Ferreira, 2007).

For each cultivar, a total of 2,000 g of fruit was collected randomly from thirty plants. Of this amount, we selected 1,200 g. The fruits were transported to the biochemistry laboratory, where fruits were selected to form experimental units.Four replicates of 300 g were analyzed for each cultivar. Fruits were stored in a freezer at -18°C with 90-95% relative humidity until the time of analysis. The experimental design was completely randomized and contained four replications. The independent variable was the blackberry cultivar(Arapaho, Brazos, Cainguangue, Cherokee, Choctaw, Comanche, Ébano, Guarani, Tupy and Xavante), and the total phenol content and antioxidant of each cultivar was analyzed.

Reagents and standards

Sodium hypochlorite, ethanol, hydrochloric acid and acetone were purchased from Vetec Chemistry. Methanol was purchased from JT Baker Chemical Co. (Phillipsburg, NJ), and Trolox was purchased from Sigma - Aldrich (St. Louis, MO, USA). The mobile phase used for HPLC analysis consisted of ultrapure Milli-Q water (Millipore, Billerica, MA, USA), acetic acid and methanol (Merck, Darmstadt, Germany). The HPLC standards for gallic acid, *p*-coumaric acid, ferulic acid, ellagic acid, 3,4-dihydroxybenzoic acid, salicylic acid and syringic acid, monomers of condensed tannins gallocatechin, catechin and epigallocatechin gallate and resveratrol were acquired from Sigma-Aldrich (St. Louis, MO, USA). The standards for m- and o-cumaric acid and vanillic acid were obtained from Fluka (St. Louis, MO, USA). Stock standard solutions were prepared in dimethyl sulfoxide and/or methanol (Merck).

Extraction

Natural extractions of blackberry samples were performed as described by Arabbi, Genovese, and Lajolo (2004) with some modifications. Samples were extracted with a mixture of methanol/water/acetic acid (70:30:5) in a 1:15 (m:v) ratiowith ultrasonication for 10 minutes in an ice bath. The homogenized sample was filtered through paper filter (Whatman nº 1). The filtrate was then evaporated in a plate at 80ºC until a volumeof 10 mL remained. This final volume was diluted with Milli-Q water to a volume of 15 mL. A 2 mL aliquot was filtered through a polyethylene filter with a 22μm pore size membrane (Millipore) and collected in a 5 ml vial. Extractions were performed in quadruplicate.

Separation, identification and quantification of phenolic compounds

Chromatography was performed using an Agilent Model 1100 HPLC.Compounds were best detected at a wavelength of 280 nm. Phenolic compound extracts and standards were injected into an Ascentis C18 column (25 cm x 4.6 mm x 5 μm) connected to a Supelguard Ascentis C18 pre-column (2 cm x 4.0 mm x 5 μm). The mobile phase consisted of 2% acetic acid (A) and methanol: water: acetic acid (70:28:2 v/v/v) (B). The flow rateused in all analyses was 100 mL min.$^{-1}$, and the injection volume was 20 μL. Eachrun was 65 minutes longwith a gradient as follows: 100% of solvent A for 5 minutes, 70% of solvent A for 20 minutes, 60% of solvent A for 18 minutes, 55% of solvent A for 7 minutes, and 0% of solvent A for 10 minutes. Solvent A (100%) was then added to equilibrate the column until the end of the run. The column was maintained at a temperature of 45°C.

Phenolic compounds present in blackberry fruit were identified by comparing the retention times of peaks in the extracts with those of standard compounds. Compound identities were confirmed co-chromatography of extracts and standards. To quantify phenolic compounds, an external standard was used. Standard curves of the compounds found in samples were obtained by diluting concentrated solutions to produce a concentration range of $5.0x10^{-7}$ to $1.5x10^{-3}$ mol L^{-1}. Calibration curves were constructed by plotting the average detector response (n = 3) for the peak area according to concentration.

The addition of standards toextracts was also used for identification. Quantification was performed using external standards with the following stock concentrations: galic acid (85.06 mg L^{-1}), gallocatechin (153.13 mg L^{-1}), 3,4 dihydroxybenzoic acid (77.06 mg L^{-1}), catechin (145.14 mg L^{-1}), chlorogenic acid (177.15 mg L^{-1}), epigallocatechin gallate (229.18 mg L^{-1}), vanillic acid (84.07 mg L^{-1}), epicatechin (145.13 mg L^{-1}), meta and ortho-coumaric (82.08 mg L^{-1}), ferulic acid (97.09 mg L^{-1}), resveratrol (114.12 mg L^{-1}), ellagic acid (151.09 mg L^{-1}) andsalicylic acid (69.06 mg L^{-1}). Each solution was injected three times into the HPLC system to obtain the average concentration and retention time of each compound.

Determination of antioxidant activity

Total antioxidant activity was measured according to the $ABTS^+$ [2,2azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)] method established by Maro et al. (2013). Extracts were obtained according to the method described by Arabbi et al. (2004). The resultsare expressed as μmol Trolox equivalent per gram of sample.

Statistical Analysis

The data were subjected to an analysis of variance, and means were compared using the Scott-Knott test with a 5% probability of error. Data were analyzed to identify correlations between individual phenolic compounds and antioxidant activity.

Results and discussion

Figure 1 shows the UV chromatogram at 280 nm obtained from HPLC analysis of a 20 μL aliquot of a 5 x 10^{-5} mol L^{-1} standard solution of phenolic compounds.

Phenolic acids (syringic acid and salicylic acid), acids derived from the hydrolysis of hydrolysable tannins (gallic acid and ellagic acid) and condensed tannins monomers (catechin and gallocatechin), were identified and quantified in fruits from various blackberry cultivars using HPLC. These analyses showed high reproducibility (Figure 2).

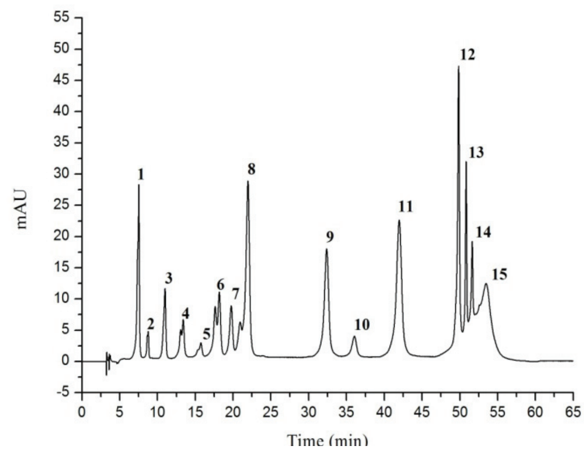

Figure 1. Chromatogram of the phenolic acid standard solution with spectrophotometric detection at 280 nm. Identified peaks: 1 = Gallic acid; 2 = gallocatechin; 3 = 3,4 dihydroxy phenolic 4 = catechin; 5 = chlorogenic acid; 6 = epigallocatechin; 7 = vanillic acid; 8= syringic acid; 9 = P-coumaric acid; 10 = ferulic acid; 11 = M-coumaric acid; 12 = O-coumaric acid; 13 = Resveratrol; 14 = ellagic acid and 15 = Salicylic acid.

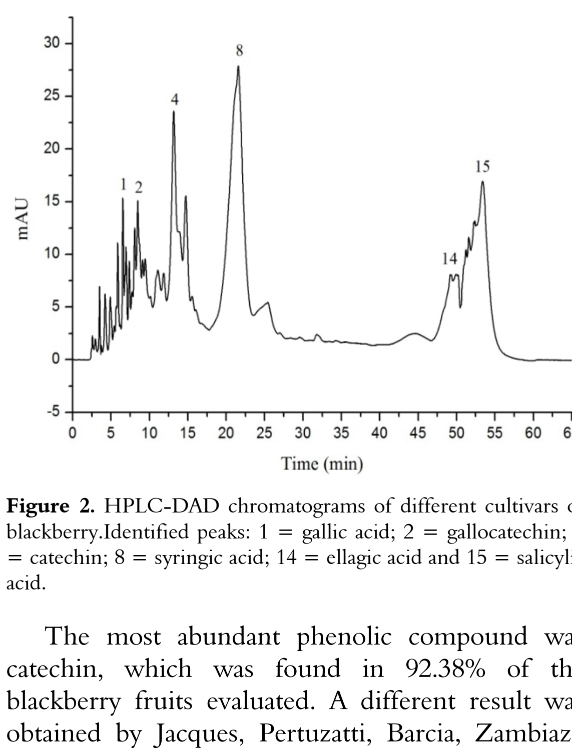

Figure 2. HPLC-DAD chromatograms of different cultivars of blackberry.Identified peaks: 1 = gallic acid; 2 = gallocatechin; 4 = catechin; 8 = syringic acid; 14 = ellagic acid and 15 = salicylic acid.

The most abundant phenolic compound was catechin, which was found in 92.38% of the blackberry fruits evaluated. A different result was obtained by Jacques, Pertuzatti, Barcia, Zambiazi, and Chim (2010), who reported that gallic acid was the most abundant phenolic compound. The content of catechin found in the Xavante cultivar was 258.58 mg per 100 g, which was higher than theother cultivars analyzed in this study (Table 1).

Table 1. Phenolic compound contents (mg per 100 gfresh weight) and antioxidant capacity (Trolox equivalent per g sample) of blackberries cultivars.

Blackberry cultivars	Phenolic compounds(1)						ABTS
	Ellagic acid	Gallic acid	Catechin	Gallocatechin	Syringic acid	Salicylic acid	
Arapaho	1.39c	0.58 g	156.95 c	1.06 c	2.01 d	17.14 d	51.35 a
Brazos	1.10 e	0.84 a	56.35 f	0.98 e	1.01 h	17.08 d	18.55 e
Caingangue	1.17 d	0.60 f	60.32 e	1.00 d	1.51 e	20.32 a	15.15 h
Cherokee	0.98 f	0.53 h	23.06 h	0.87 h	1.99 d	17.12 d	29.79 d
Choctaw	0.97 f	0.63 e	10.35 i	0.89 g	1.39 f	19.49 b	17.50 f
Comanche	1.16 d	0.53 h	38.28 g	0.92 f	2.80 b	16.46 d	33.05 c
Ébano	1.44 b	0.65 d	222.56 b	1.14 b	3.19 a	18.15 c	33.17 c
Guarani	0.91 g	0.70 c	23.77 h	0.86 i	1.23 g	19.20 b	17.10 f
Tupy	0.78 h	0.54 h	64.58 d	0.75 j	1.38 f	14.78 e	16.06 g
Xavante	3.08 a	0.77 b	258.58 a	1.20 a	2.59 c	16.73 d	50.19 b
Average	1.29	0.64	92.38	0.96	1.91	17.64	28.19
CV %	2.52	1.39	1.47	0.34	0.72	2.91	1.28
R^2	0.99	0.99	0.99	0.99	0.96	0.99	

(1) Means followed by the same small letter in the column do not differ from one another by the Scott-Knott test($p \leq 0.05$).

Catechin is a flavonoid compound. Flavonoids are present in fruits and vegetables and are considered to be therapeutic agents due to their beneficial effects on health. The protective properties of flavonoids against certain cancers, cardiovascular diseases and aging are all potentially beneficial for human health (Hidalgo, Sánchez-Moreno, & Pascual-Teresa, 2010; Carvalho, Cavaco, & Brodelius, 2011). The ellagic acid content of the10 black berry cultivars studied in this work ranged from 0.78 to 3.08 mg per 100 g fresh weight. The Xavante cultivar showed the highest levels of ellagic acid, followed by the Ebano cultivar. The lowest level of ellagic acid was observed in the Tupy cultivar (Table 1). Ellagic acid values in this study were lower than those obtained by Siriwoharn, Wrolstad, and Durst (2005) (50 mg per 100 g).

Recent interest in ellagic acid has been due to the compound's phytonutrient properties, which include disease prevention and antioxidant, antimutagenic, and anticarcinogenic effects (Maro et al., 2013; Souza et al., 2014).

Of the phenolic compounds identified and quantified, gallic acid was found at the lowest concentration, ranging from 0.53 to 0.84 mg per 100 g fresh weight. The highest amount of gallic acid was found in the Brazos cultivar, followed by the Shavante cultivar (Table 1). A higher gallic acid content (350.49 mg per 100 g fresh weight) was observed by Jacques et al. (2010) in Tupy black mulberry fruits cultivated in temperate climates. These discrepancies may be related to differences in species and cultivars as well as differences in extraction methodology.

Difference in gallocatechin contents were observed between cultivars, varying from 0.75 mg per 100 gin the Tupy cultivar to 1.20 mg per 100 g^{-1} in the Xavante cultivar. An average of 0.96 mg 100 g (Table 1) was observed. Previous studies have shown gallocatechinto aid in preventing hypoglycemia and in lowering cholesterol (Lee, Kim, Kim, Shin, & Baik, 2008).

The phenolic content of syringic and salicylic acids also varied among the blackberry cultivars studied. Syringic and salicylic acid contents averaged 1.91 and 17.64 mg per 100 g, respectively (Table 1). Phenol concentration sare influenced by factors such as the cultivar type, pre-harvest environmental and climatic conditions, plant diseases, harvesting procedure, point of maturity at harvest, geographic location, exposure to sunlight, post-harvest storage conditions, fruit processing, extraction method and quantification method (Cordenunsi, Nascimento, Genovese, & Lajolo, 2002; Vizzotto & Pereira, 2011; Souza et al., 2015).

The quantification data of individual phenolic compounds in domestic blackberry cultivars in highland tropical climate has not been reported previously; therefore, these results are important for the selection of varieties with superior properties for consumption and breeding of superior cultivars.

The antioxidant activity of blackberry fruits was determined using the ABTS method and reported in equivalents of Trolox, a water-soluble analog of vitamin E. Fruits from the Arapaho and Xavante cultivarsexhibited antioxidant activities of 51.35 and 50.19 μmol of Trolox equivalent per g of sample, respectively. These activities were 34-70% higher than the activities of the other cultivars.

These results corroborate the results obtained by Silva, Vendruscolo, and Toralhes (2011) for blackberry fruits cultivated in temperate conditions: the cultivar that showed the highest antioxidant activity (Xavante) also showed the highest total phenol content.

Correlations between antioxidant activity (ABTS) and individual polyphenol contents in blackberry fruits are shown in Table 2. Mean antioxidant activities correlate positively with the average valuesof most of individual polyphenols evaluated in this study. Correlations between ellagic acid, catechin, gallocatechin and syringic acid contents with antioxidant activity were observed (Table 2). On the other hand,there was no significant correlation (5% probability) between gallic acid content and antioxidant activity (Table 2). These results indicate that variations in phenolic compound contents can lead to different biological responses. Although the catechins represent potential constituents, other compounds may act synergistically, increasing the beneficial effects associated with the consumption of fruits and their blackberry derivatives.

Table 2. Pearson Correlation of ellagic acid, galic acid, catechin, gallocatechin, syringic acid, salicylic acid with antioxidant activity.

Variables	Correlation
Ellagic acid	0.71*
Galic acid	0.00ns
Catechin	0.73*
Gallocatechin	0.69*
Syringic acid	0.67*
Salicylic acid	0.32ns

* = F significant at 5%; ns = F not significant at 5%.

Conclusion

Fruits from blackberry cultivars grown under tropical high-altitude conditions exhibit varying levels of phytochemicals, which may contribute to their natural antioxidant properties.

The polyphenol catechin was the major component of the blackberry cultivars studied. In addition, the Xavante cultivar had higher individual levels of the polyphenols studied in this work.

References

Arabbi, P. R., Genovese, M. I., & Lajolo, F. M. (2004). Flavonoids in vegetable foods commonly consumed in Brasil and estimated ingestion by the Brazilian population. *Journal of Agricultural and Food Chemistry*, *52*(5), 1124-1131. doi: 10.1021/jf0499525

Campagnolo, M. A., & Pio, R. (2012). Phenological and yield performance of black and redberry cultivars in western Paraná State. *Acta Scientiarum. Agronomy*, *34*(4), 439-444. doi: 10.4025/actasciagron.v34i4.15528

Caproni, C. M., Curi, P. N., Moura, P. H. A., Pio, R., Gonçalves, E. D., & Pasqual, M. (2016). Blackberry and redberry production in crop and intercrop in Pouso Alegre, southern Minas Gerais, Brazil. *Ciência Rural, 46*(10), 1723-1728. doi: 10.1590/0103-8478cr 20150623

Carvalho, I. S., Cavaco, T., & Brodelius, M. (2011). Phenolic composition and antioxidant capacity of six artemisia species. *Industrial Crops and Products*, *33*(2), 382-388. doi: 10.1016/j.indcrop.2010.11.005

Cordenunsi, B. R., Nascimento, J. R. O., Genovese, M. I., & Lajolo, F. M. (2002). Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal Agriculture of Food Chemistry*, *50*(9), 2581-2586. doi: 10.1021/jf011421i

Curi, P. N., Pio, R., Moura, P. H. A., Lima, L. C. O., & Valle, M. H. R. (2014). Qualidade de framboesas sem cobertura ou cobertas sobre o dossel e em diferentes espaçamentos. *Revista Brasileira de Fruticultura*, *36*(1), 199-205. doi: 10.1590/0100-2945-234/13

Curi, P. N., Pio, R., Moura, P. H. A., Tadeu, M. H., Nogueira, P. V., & Pasqual, M. (2015). Produção de amora-preta e amora-vermelha em Lavras-MG. *Ciência Rural*, *45*(8), 1368-1374. doi: 10.1590/0103-8478cr20131572

Dantas, A. A. A., Carvalho, L. G., & Ferreira, E. (2007). Classificação e tendências climáticas em Lavras, MG. *Ciência e Agrotecnologia*, *31*(6), 1862-1866. doi: 10.1590/ S1413-70542007000600039

Guedes, M. N. S., Abreu, C. M. P., Maro, L. A. C., Pio, R., Abreu, J. R., & Oliveira, J. O. (2013). Chemical characterization and mineral levels in the fruits of blackberry cultivars grown in a tropical climate at an elevation. *Acta Scientiarum. Agronomy*, *35*(2), 191-196. doi: 10.4025/actasciagron.v35i2.16630

Hidalgo, M., Sánchez-Moreno, C., & Pascual-Teresa, S. (2010). Flavonoid-flavonoid interaction and its effect on their antioxidant activity. *Food Chemistry*, *121*(3), 691-696. doi: 10.1016/j.foodchem.2009.12.097

Jacques, A. C., Pertuzatti, P. B., Barcia, M. T., Zambiazi, R. C., & Chim, J. F. (2010). Estabilidade de compostos bioativos em polpa congelada de amora-preta (*Rubus fruticosus*) cv. Tupy. *Química Nova*, *33*(8), 1720-1725. doi: 10.1590/S0100-40422010000800019

Lee, S. M., Kim, C. W., Kim, J. K., Shin, H. J., & Baik, H. J. (2008). GCG-Rich tea catechins are effective in lowering cholesterol and triglyceride concentrations in hyperlipidemic rats. *Lipids*, *43*(5), 419-429. doi: 10.1007/s11745-008-3167-4

Maro, L. A. C., Pio, R., Guedes, M. N. S., Abreu, C. M. P., & Curi, P. N. (2013). Bioactive compounds, antioxidant activity and mineral composition of fruits of raspberry cultivars grown in subtropical areas in Brazil. *Fruits*, *68*(3), 209-217. doi: 10.1051/fruits/ 2013068

Maro, L. A. C., Pio, R., Guedes, M. N. S., Abreu, C. M. P., & Moura, P. H. A. (2014). Environmental and genetic variation in the post-harvest quality of raspberries in subtropical areas in Brazil. *Acta Scientiarum. Agronomy*, *36*(3), 323-328. doi: 10.4025/ actasciagron.v36i3.18050

Scalzo, J., Battino, M., Costantini, E., & Mezzetti, B. Breeding and biotechnology for improving berry nutritional quality. (2005). *BioFactors*, *23*(4), 213-220.

Silva, R. S., Vendruscolo, J. L., & Toralhes, R. P. (2011). Avaliação da capacidade antioxidante em frutas produzidas na região Sul do RS. *Revista Brasileira de Agrociência*, *17*(3-4), 392-400.

Siriwoharn, T., Wrolstad, R. E., & Durst, R. W. (2005). Identification of ellagic acid in blackberry juice sediment. *Journal of Food Science*, *70*(3), 189-197. doi: 10.1111/j.1365-2621.2005.tb07124.x

Souza, V. R., Pereira, P. A. P., Silva, T. L. T., Lima, L. C. O., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry*, *156*(8), 362-368. doi: 10.1016/j.food chem.2014.01.125

Souza, V. R., Pereira, P. A. P., Teixeira, T. R., Silva, T. L. T., Pio, R., & Queiroz, F. (2015). Influence of processing on the antioxidant capacity and bioactive compounds in jellies from different blackberry cultivars. *International Journal of Food Science & Technology*, *50*(7), 1658-1665. doi: 10.1111/ijfs.12819

Szajdek, A., & Borowska, E. J. (2008). Bioactive compounds and health-promoting properties of berry fruits: Review. *Plant Foods for Human Nutrition*, *63*(4), 147-156.

Vizzotto, M., & Pereira, M. C. (2011). Amora-preta (*Rubus* sp.): otimização do processo de extração para determinação de compostos fenólicos antioxidantes. *Revista Brasileira de Fruticultura*, *33*(4), 1209-1214. doi: 10.1590/S0100-29452011000400020

Zozio, S., Dominique, P., & Dornier, M. (2011). Evaluation of anthocyanin stability during storage of a coloured drink made from extracts of the Andean blackberry (*Rubus glaucus* Benth.), açai (*Euterpe oleracea* Mart.) and black carrot (*Daucus carota* L.). *Fruits, 66*(3), 203-215. doi: 10.1051/fruits/2011030

Received on July 3, 2015.
Accepted on December 13, 2015.