Food Science and Technology ISSN 0101-2061

DOI: http://dx.doi.org/10.1590/1678-457X.6470

Characterization of blueberry fruits (Vaccinium spp.) and derived products

Priscilla Magro REQUE¹, Rosana Stroschoen STEFFENS¹, Alexandre MARTINS DA SILVA¹, André JABLONSKI², Simone Hickmann FLÔRES¹, Alessandro de Oliveira RIOS¹*, Erna VOGT DE JONG¹ in memoriam

Abstract

The objectives of this study were to physicochemically characterize and determine the antioxidant activities and anthocyanin contents of organic Rabbiteye blueberries grown in Southern Brazil and its derived products, in order to investigate the utility of food processing wastes as raw materials for developing products with beneficial health properties. The antioxidant capacity of the blueberries was superior to that of other fruits and juices. The pomace exhibited high activity, albeit lower than that of the fruit, while the flour and the dried blueberries lost 66% and 46% of the original antioxidant activity, respectively. The average anthocyanin contents of the fruits were moderate compared to other sources and species of blueberries. The pomace contains a large amount of anthocyanins while the flour and dried blueberries exhibited a 32% and 42% loss in anthocyanin content, respectively. The use of agro-industrial residues, in addition to adding value and minimizing the impact caused by the accumulation in the environment, can be directed toward the development of new products with bioactive properties.

Keywords: blueberry; processing; physicochemical characterization; antioxidant capacity; anthocyanins; HPLC.

Practical Application: Knowing that the blueberry is a fruit with high antioxidant capacity and great productive potential in southern Brazil, the characterization and determination of bioactive substances of blueberry fruits and its derived products showed that the utilization of agro-industrial wastes as raw materials can be an interesting alternative for developing new products with health promoting properties and technological applications, such as antioxidants and food dyes, besides minimizing the impact caused by their accumulation in the environment.

1 Introduction

The blueberry belongs to the family *Ericaceae*, subfamily *Vaccinoideae*, genus *Vaccinium*, and is native to North America and European regions, where it is widely cultivated and commercialized. In Brazil, this fruit is still relatively unknown, but it has great potential for growth, mainly in the state of Rio Grande do Sul (RS), due to the temperate climate, and the *Vaccinium ashei* has been considered the most promising species.

This fruit, like most berries, is rich in flavonoids, tannins and phenolic acids. Many studies have indicated that the blueberry has several beneficial health properties associated with the presence of such bioactive compounds, especially anthocyanins (Heinonen et al., 1998, Smith et al., 2000; Seeram, 2008).

The blueberry is known as a "longevity fruit" due to its high antioxidant capacity against free radicals and reactive species and it is considered one of the greatest sources of antioxidants among all fruits and vegetables (Prior et al., 1998). This activity is likely the main mechanism by which its consumption may reduces the risk of the development of several diseases, such as chronic non-communicable diseases (NCDs) that are stimulated by oxidative processes (Halliwell, 2006).

The main chronic diseases that the blueberry has been linked to the prevention and treatment are cancer (Smith et al., 2000; Katsube et al., 2003), *diabetes mellitus* (Martineau et al., 2006) and cardiovascular (Heinonen et al., 1998) and neurodegenerative (Joseph et al., 2003; Krikorian et al., 2010) diseases. Moreover, blueberries are also reported to have beneficial effects on vision (Kalt et al., 2010).

Agro-industrial wastes are composed primarily of organic matter, typically rich in sugars and fiber. These wastes have a high nutritional value but present an environmental problem when produced in large quantities. In addition, these residues may contain bioactive substances with health promoting properties and potential technological applications, such as antioxidants and even food dyes (Lee & Wrolstad, 2004). Thus, these wastes represent a potentially useful resource to be explored.

This study aimed to physicochemically characterize and determine the antioxidant activities and anthocyanin contents of blueberry fruit *in natura* and its derived products to encourage increase their production and trade, as well to investigate the utility of food processing wastes as raw materials for developing new products with beneficial health properties.

Received 25 Aug., 2014

Accepted 20 Nov., 2014 (006470)

¹Institute of Food Science and Technology, Federal University of Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brazil

 $^{^2}Department\ of\ Mining\ Engineering,\ Federal\ University\ of\ Rio\ Grande\ do\ Sul-UFRGS,\ Porto\ Alegre,\ RS,\ Brazil$

 $^{{\}rm *Corresponding~author:~alessandro.rios@ufrgs.br}$

2 Materials and methods

2.1 Samples

Organic blueberries of the cultivar Delite (Rabbiteye group) were supplied by the "Fazenda Viva o Verde" in the city of Camaquã, RS, Brazil (Latitude: 30° 46' 25.26" S and Longitude: 51° 42' 38.78" O). The berries were picked between December 2010 and January 2011 and stored at –18 °C until use.

Whole juice, dried blueberries and flour from the residue formed after the extraction of the blueberry juice (pomace) were produced. The whole juice was prepared using a domestic centrifugal-type extractor (Walita-Philips*), in which the liquid was collected and separated from the residue (pomace). The flour was produced by drying the pomace in a convection dryer at 80 °C for 2 hours and 30 minutes and subsequently grinding the dried pomace in a mill (Arbel, model MCF 55, São José do Rio Preto, Brazil). The dried blueberries were prepared by convective dehydration at 80 °C for 6 hours.

2.2 Physicochemical characterization

Total titratable acidity (TTA) was determined by titration, pH was measured using a pH meter (Quimis, model Q400A, Diadema, SP, Brazil) and the soluble solids (SS) were measured using a digital PAL-3 refractometer (Atago, model Pocket Pal-3, Ribeirão Preto, SP, Brazil). The protein concentration was determined by the Kjeldahl method using a conversion factor of 5.75. The lipid concentration was determined by the Soxhlet extraction method, and ash was analyzed in a muffle furnace controlled at 550 °C. Moisture content was measured by gravimetry, the total carbohydrate content was measured using the difference method and the reducing and non-reducing sugars were measured using the Eynon-Lane method. All analyses were carried out in three independent samples and according to AOAC procedures (Association of Official Analytical Chemists International, 2005).

2.3 Antioxidant activity

The DPPH method (Brand-Williams et al., 1995), based on the capture of free radicals by antioxidants, and the ABTS method (Re et al., 1999), in which the free radical is generated by a chemical reaction with potassium persulphate, were used (with minor modifications) to determine the antioxidant activity of each sample.

The extract was obtained from an approximately 10 g sample in 40 mL 50% aqueous methanol and 40 mL 70% aqueous acetone and centrifuged twice at $15000 \times g$ for 15 minutes (Hitachi, model Himac CR21E centrifuge, Tokyo, Japan). Three dilutions were prepared from the supernatant (1:5, 1:10 and 1:15).

For the DPPH method, a 100 μ L aliquot of each dilution was added to 3.9 mL of DPPH radical and read at 515 nm in an Ultrospec 3100 pro spectrophotometer (Amersham Pharmacia Biotech, Cambridge, UK) after 30 minutes, using methanol as the blank. For the ABTS method, an aliquot of 30 μ L of each dilution was added to 3.0 mL of ABTS radical, and the sample

was read in the spectrophotometer at 734 nm after 6 minutes of reaction, using ethanol as the blank and Trolox to generate a standard curve. The analyses of the extract were carried out in triplicate, and the results were presented in g or mL of sample/g of DPPH or in μM of Trolox equivalents (TE)/g or mL of sample.

2.4 Anthocyanin extraction, identification and quantification

The anthocyanins were exhaustively extracted from a sample of approximately 2 g using a 1% solution of HCl in methanol. The solution was then filtered and vacuum concentrated (T < 38 °C) in a rotary evaporator (Fisatom, model 801/802, São Paulo, SP, Brazil) (Francis, 1982). The concentrated crude extract was transferred to a 25 mL flask, and the volume was completed with the acidified methanol solution. A 1 mL aliquot of this solution was removed, dried under nitrogen gas (N $_2$) and stored frozen (T < -18 °C) until analysis. This extract, dried in N $_2$ (or 1 mL of previously filtered juice), was diluted in chromatographic grade methanol, homogenized in an ultrasound bath (Unique, model USC 1400, Indaiatuba, SP, Brazil) and filtered into a vial through a polyethylene membrane (Millex PTFE, Millipore) with a pore size of 0.45 mm and diameter of 13 mm.

The anthocyanins were quantified by High Performance Liquid Chromatography (HPLC) and identified in comparison with the appropriate standards. All of the solvents used in the HPLC separation were of chromatographic grade and were filtered through the Millipore vacuum filtration system using a 0.45 mm membrane for organic solvents prior to use (Millipore, Barueri, SP, Brazil).

An Agilent series 1100 chromatograph (Santa Clara, CA, USA) equipped with a quaternary solvent pumping system and UV/Vis detector was used for the HPLC analyses. The pigments were separated on a Shim-pak 5 mm C₁₈ CLC-ODS reverse phase column, 250 × 4.6 mm (Shimadzu, Kyoto, Japan). A linear gradient elution with a mobile phase of 4% aqueous phosphoric acid/acetonitrile, from 85:15 (v/v) to 20:80 (v/v) in 25 minutes in a chromatographic run of 15 minutes, was used, according to the conditions established experimentally by Zanatta et al. (2005). The flow rate of the mobile phase was 1.0 mL/min, and the injection volume was 5 μL or 10 μL (juice). The column temperature was maintained at 29 °C, and the chromatograms were processed at 520 nm. The anthocyanins were extracted and injected into the chromatograph in duplicate, and the compounds were identified by comparing the retention times (tR) of the samples with those of commercial standards obtained from Sigma-Aldrich® (St. Louis, MO, USA).

The anthocyanins were quantified by constructing standard curves with glycosylated anthocyanins (cyanidin-3-glucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3,5-diglucoside, malvidin-3-glucoside and malvidin-3,5-diglucoside) and aglycones or anthocyanidins (cyanidin, delphinidin, pelargonidin, malvidin and peonidin).

The limits of detection (LD) and quantification (LQ) were calculated according to Long & Winefordner (1983) as: 8.05×10^{-8} and 1.34×10^{-7} mg/kg (cyanidin-3-glucoside); 2.18×10^{-8} and 3.63×10^{-8} mg/kg (cyanidin-3,5-diglucoside); 2.84×10^{-7} and 4.74×10^{-7} mg/kg (delphinidin-3-glucoside); 9.75×10^{-8} and 1.63×10^{-7} mg/kg (pelargonidin-3-glucoside); 7.41×10^{-8} and 1.23×10^{-7} mg/kg (pelargonidin-3,5-diglucoside); 3.30×10^{-7} and 5.50×10^{-7} mg/kg (malvidin-3-glucoside); 1.73×10^{-6} and 2.89×10^{-6} mg/kg (malvidin-3,5-diglucoside); 1.51×10^{-7} and 2.52×10^{-7} mg/kg (aglycone cyanidin); 1.57×10^{-7} and 2.61×10^{-7} mg/kg (aglycone delphinidin); 9.57×10^{-8} and 1.59×10^{-7} mg/kg (aglycone pelargonidin); 7.12×10^{-8} and 1.9×10^{-7} mg/kg (aglycone malvidin); and 1.23×10^{-7} and 2.05×10^{-7} mg/kg (aglycone peonidin).

3 Results and discussion

Physicochemical characterization

The chemical composition (Table 1) of the blueberry fruits produced in RS (Brazil) demonstrated that they satisfied the quality parameters reported by Sousa et al. (2007). The blueberry has high water content (85.78%), making it very susceptible to deterioration due to microbial contamination. This amount of water in the tissues depends strongly on the soil water availability during harvest (Sousa et al., 2007). The pomace still has a high moisture content (81.45%) compared to the other products that have been subjected to a dehydration process, thus increasing their shelf life.

The dried blueberries are within the standard for dried fruits, with a moisture content below 25%. The blueberry flour had a moisture content of 5.9%, similar to that found by Hoye & Ross (2011) for flour produced from the dried seeds of "Merlot" grapes (7.4%).

Both the fruit and the products derived from blueberry have low lipid contents. The value found for fruit *in natura* (0.73%) was lower than that reported in the USDA database (U.S. Department of Agriculture, Agricultural Research Service,

2013) (2.09%) and that shown by Vasco et al. (2009) (5.26%) for blueberries grown in Ecuador.

The protein content of the dried blueberries (4.15%) was similar to the fruit (4.10%), as well of the flour (4.25%) compared to the pomace (4.67%). The protein concentration in the fruits was higher than that reported for a blueberry of Andean origin (Vasco et al., 2009) (3.68%) and similar to that reported in the USDA database (U.S. Department of Agriculture, Agricultural Research Service, 2013) (4.31%).

The fruits had had a higher ash content compared to the pomace, dried blueberries and flour, most likely due to differences in harvesting time and the mineral concentration in the soil for blueberries from the same batch. The ash values found in the fruit (1.80%) were higher than those in the USDA database (U.S. Department of Agriculture, Agricultural Research Service, 2013) (1.52%) but lower than those reported by Vasco et al. (2009) (2.11%).

The total carbohydrate content of the fruit (93.37%), calculated using the difference method, was higher than those reported by the USDA database (U.S. Department of Agriculture, Agricultural Research Service, 2013) (91.77%) and also by Vasco et al. (2009) (88.95%).

The blueberry has a sweet-sour or acid taste; therefore, sugar concentration and the pH are important parameters for assessing blueberry quality. The sugars are the most abundant soluble components in the blueberry, especially glucose and fructose (Sousa et al., 2007). Because these monosaccharides are reducing sugars, the values for total and reducing sugar were similar. The amount of total sugars in the fruit (70.65%) was higher than that reported in the USDA database (U.S. Department of Agriculture, Agricultural Research Service, 2013) (63.08%) and also higher than those measured in the products. The flour showed values below that of the dried blueberries, due to the lower concentration of sugars in the pomace. Moreover, both the flour and the dried blueberries may have been subjected

Table 1. Chemical composition of the Rabbiteye blueberry fruits, pomace, dried blueberries and flour.^a

	FRUIT	POMACE	DRIED BLUEBERRY	FLOUR
MOISTURE	85.78±0.62	81.45±0.46	24.36±0.83	5.91±0.19
TOTAL LIPID	0.73±0.06	0.67 ± 0.02	0.42 ± 0.04	1.04 ± 0.00
PROTEIN	4.10±0.08	4.67±0.19	4.15±0.21	4.25±0.16
ASH	1.80 ± 0.01	1.59±0.05	1.21±0.07	1.13 ± 0.04
REDUCING SUGARS	68.54±0.75	49.69±0.93	54.34±0.63	36.58±2.23
TOTAL SUGARS	70.65±1.55	51.39±1.37	58.04±1.81	41.24±2.48

 $^{^{\}rm a}$ Results presented as mean \pm standard deviation and expressed in g/100 g dry weight (DW).

Table 2. Quality parameters of the Rabbiteye blueberry fruits, pomace, dried blueberries, whole juice and flour.^a

	FRUIT	POMACE	DRIED BLUEBERRY	JUICE	FLOUR
pН	2.92±0.00	2.94±0.00	2.82±0.01	2.90±0.00	2.88±0.02
SS (°Brix)	10.67±0.29	10.50±0.00	ND	12.03±0.15	ND
TTA (%)	0.68 ± 0.01	0.40 ± 0.01	ND	0.50 ± 0.00	ND
RATIO SS/TTA	15.69	26.25	ND	24.06	ND

Abbreviations: SS (soluble solids), TTA (total titratable acidity), ND (not determined); *Results presented as mean ± standard deviation and expressed in fresh weight (FW).

to the Maillard reaction and caramelization during the drying process.

The pH values of the fruit and its derived products were low (Table 2), indicating that the blueberry can be considered acidic, an important factor in its preservation. The value for the fruit (2.92) was intermediate to those of Highbush cultivars (Molina et al., 2008) (2.80 to 3.20) and similar to those of other cultivars in the Rabbiteye group (Saftner et al., 2008) (2.80 and 3.00).

The blueberry juice had the highest SS content of the products measured, followed by fruit and pomace. Vázquez-Araújo et al. (2010) found values of 10.4, 12.9 and 10.8 °Brix in the juices of blueberry, blackberry and raspberry fruits, respectively. For mature Lowbush blueberries, Kalt & McDonald (1996) found a mean value of 11.15 °Brix, while Molina et al. (2008) showed an average of 11.63 °Brix for Highbush blueberries, and Saftner et al. (2008) reported an average of 11.75 °Brix for cultivars of the Rabbiteye group.

The TTA of the fruit was superior to that of the pomace and the blueberry juice, as well as those of the majority of the cultivars analyzed by Saftner et al. (2008). These authors reported that blueberries should have SS values above 10%, TTA values between 0.3 and 1.3%, pH values from 2.25 to 4.25 and an SS/TTA ratio between 10 and 33. Thus, the organic Rabbiteye blueberry, produced in RS, can be considered of commercial quality.

3.2 Antioxidant activity

Blueberries have a high antioxidant activity and are considered a major source of dietary antioxidants (Prior, 1998). The antioxidant capacity of our blueberry fruit (Table 3) was superior to that of many cultivars analyzed by Sellappan et al. (2002) using the ABTS assay (with the exception of the Premier),

as well as to most fruits (such as açaí, cashew apple, strawberry, passion fruit and mango) studied by Vasco et al. (2008) and Rufino et al. (2010) using the ABTS and DPPH methods.

The high antioxidant capacity of blueberries is more strongly correlated to its total phenolic content than to its anthocyanin content, and ascorbic acid also has a small contribution to antioxidant activity (Prior et al., 1998). According to these authors, fruit maturity at harvest positively affects antioxidant activity and total phenolic and anthocyanin contents, and variations exist between different varieties of blueberry. Several other factors influence the antioxidant activity, such as environmental conditions prior to harvest, stage of ripeness of the fruit after harvest, and storage and processing conditions (Connor et al., 2002).

The fruits had a higher antioxidant activity than any of the derived products. The juice extraction residue (pomace) contains approximately 52% of the antioxidant activity of the fruit (on a dry basis, measured by the ABTS method), showing that the compounds present in the peel contribute to the antioxidant properties of blueberry, likely due to the higher anthocyanin content (Lee & Wrolstad, 2004).

Blueberry juice has a low antioxidant capacity (2.79 g TE/L or 11.14 μM TE/mL), although it is higher than the antioxidant activity of bayberry (0.77 to 2.17 g TE/L) (Fang et al., 2009) and cranberry juices, and similar to açai juice (10.4 and 12.8 μM TE/mL, respectively) (Seeram et al., 2008). Some antioxidant compound losses may have occurred during the extraction of the juice; the release of enzymes such as polyphenol oxidase during the pressing process can cause oxidation reactions, degrading phenolic compounds (Skrede et al., 2000).

The flour obtained by drying the pomace lost approximately 66% of its antioxidant activity, expressed in dry weight and TE, likely due to thermal treatment and grinding, while the dried

Table 3. Antioxidant activity of the Rabbiteye blueberry fruits, pomace, dried blueberries, whole juice and flour.

	FRUIT	POMACE	DRIED BLUEBERRY	JUICE	FLOUR
ABTS ^a	35.39±0.84	22.74±0.33	85.89±0.11	11.14±0.18	39.48±0.34
$\mathrm{DPPH}^{\mathrm{a}}$	2938.05±14.38	4958±110.60	1246.74±9.45	12137.53±116.82	ND
$ABTS^b$	236.74±7.41	122.56±1.81	127.29±0.16	NA	41.93±0.36
$DPPH^b$	480.84±2.35	919.71±20.52	841.26±6.37	NA	ND

Abbreviations: TE (Trolox equivalents), NA (not applicable) e ND (not determined); *Results presented as mean \pm standard deviation and expressed in μ M TE/g (mL) FW, for ABTS, or in g (mL) FW/g DPPH; *B Results presented as mean \pm standard deviation and expressed in μ M TE/g (mL) DW, for ABTS, or in g (mL) DW/g DPPH.

Table 4. Anthocyanins of the Rabbiteye blueberry fruits, dried blueberries, whole juice, pomace and flour.^a

PEAK	TR	ANTHOCYANIN	FRUIT	DRIED BLUEBERRY	JUICE	POMACE	FLOUR
1, 2	3.57	Cy-3.5-Glu ^b	361.17±3.71	185.28±11.76	2.85±0.00	233.40±8.49	232.68±0.18
3	4.24	Dph-3-Glu	484.26±13.79	338.47±17.09	2.40 ± 0.04	375.48±14.53	178.09 ± 10.49
5	5.19	Cy-3-Glu ^c	261.21±1.38	125.13±6.23	4.95±0.05	231.48±11.65	173.59±11.84
8	5.92	Pg-3-Glu	208.36±0.73	101.40 ± 0.08	3.56±0.06	225.35±3.44	155.13±7.29
10	6.41	$Mv-3-Glu^d$	341.27±27.51	194.41±12.14	6.10±0.09	370.71±1.36	221.06±1.86
14	7.63	Cy Agl	119.48±2.44	83.37±5.92	0.75 ± 0.07	135.74±6.18	101.08±4.41
		Total	1775.75±8.26	1028.05±8.87	20.61±0.05	1572.15±7.61	1061.64±6.01

Abbreviations: tR (retention time of the standard, in minuts), Cy (cyanidin), Pg (pelargonidin), Mv (malvidin), Dph (delphinidin), Agl (aglycone) e Glu (glucoside); a Concentrations presented as mean \pm standard deviation and expressed in mg/100 g DW or mg/100 mL of juice. The concentrations of malvidin aglycone or peonidin aglycone were not considered because they were not found in all samples; b By injecting 1 μ L of this standard, the peak did not split, eluting in a single tR; c Concentrations in terms of cyanidin-3-glucoside, because it co-eluted with the malvidin-3,5-diglucoside; d Concentrations in terms of malvidin-3-glucoside, because it co-eluted with the delphinidin aglycone.

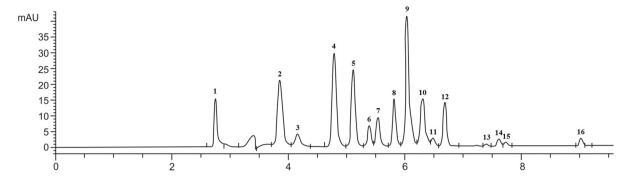


Figure 1. Chromatogram of the blueberry fruit, with the peaks numbered in order of elution.

blueberries lost approximately 46% of the antioxidant capacity of the fruit. Using the ORAC method, Kalt et al. (2000) found that only 16% of the antioxidant activity of the whole berry was present in the juice extraction residue from the Lowbush blueberry. The authors also observed that fruit subjected to more intense drying showed lower activity and that a commercial powder mixture contains approximately 14% of the ORAC of fresh fruit.

3.3 Anthocyanins

The profiles and concentrations of anthocyanins present in Rabbiteye blueberry fruit and derived products are shown in Table 4. A total of 16 chromatographic peaks were observed for the fruit (Figure 1), but only 8 of which were identified. The cyanidin-3,5-diglycoside split into two peaks, and malvidin aglycone, malvidin-3-glucoside and malvidin-3,5-diglycoside co-eluted with peonidin aglycone, delphinidin aglycone and cyanidin-3-glucoside, respectively. The anthocyanins pelargonidin aglycone and pelargonidin-3,5-diglycoside were not found. Pelargonidin-3-glucoside was identified in tR 5.8-5.9 min, and the concentrations were expressed in terms of this anthocyanin. However, as there are no reports of the presence of pelargonidin in other studies with blueberries, except in Koca & Karadeniz (2009), it is possible that this peak actually represents another compound of similar polarity.

According to Nicoué et al. (2007), co-elutions are common in reverse-phase liquid chromatography analysis of anthocyanins in blueberries because they contain a complex mixture of such compounds, and it is only possible to detect and distinguish these compounds by mass spectroscopy.

Kalt et al. (1999) identified 25 peaks representing simple or acylated anthocyanins in different species of blueberry, with delphinidin as the major compound. Gao & Mazza (1994) also reported 25 types of simple or acylated anthocyanins in Lowbush blueberries and 20 for Highbush blueberries, finding between 120 and 260 mg/100 g FW in cultivars of the first group and approximately 110 mg/100 g FW in the second group. These studies identified five anthocyanidins (cyanidin, delphinidin, malvidin, petunidin and peonidin) and its combinations with glucose, galactose and arabinose in position 3. Cho et al. (2004) reported a huge variety in the anthocyanin contents of

blueberries of different genotypes (1435.2 to 8227.3 mg/ kg FW) but similar profile distributions.

The total anthocyanin values found for the organic Rabbiteye blueberry produced in Southern Brazil were higher than those found in both Highbush and Lowbush blueberries (Gao & Mazza, 1994), in other Rabbiteye cultivars (Yousef et al., 2014) and in a blueberry native to Turkey (Lätti et al., 2009). The anthocyanin with the highest concentration was delphinidin-3-glucoside, with a mean of 288 mg/100 g DW in the fruits of Caucasian origin and 484 mg/100 g DW in the blueberries in this experiment. Wang et al. (2008) observed a higher total anthocyanin content in organic blueberries compared to conventionally produced fruits.

Kähkönen et al. (2003) reported high concentrations of anthocyanins in the bilberry, approximately 600 mg/100 g FW (the values in the present study were 290.62 mg/100 g FW; however, this number only includes the concentrations of the anthocyanins that were identified), although with concentrations of delphinidin-3-glucoside, cyanidin-3-glucoside and malvidin-3-glucoside very similar to those observed here.

Cranberry residue was studied by White et al. (2010, 2011), who found means between 121.4 and 362.5 mg/100 g DW and only identified six peaks, lower than the anthocyanin content in the blueberry pomace, as quantified by the sum of the six anthocyanins whose concentrations were determined.

Regarding the total anthocyanin content, calculated as the sum of the concentrations of the anthocyanins identified by HPLC, the blueberry pomace retained a high percentage relative to the whole fruit (approximately 88%). Lee & Wrolstad (2004) found that approximately 82% of total monomeric anthocyanins were contained in the peel of Highbush blueberries, as quantified by spectrophotometry. This result demonstrates that such compounds are preferably concentrated in the shell of the fruit, as suggested by its intense blue color in relation to the pulp.

The whole juice, in turn, had a low concentration of anthocyanins, with malvidin-3-glucoside as the major compound. According to Kalt et al. (1999), bilberry fruits, which are native to Europe, have anthocyanins both in the peel and the pulp; therefore, its juice is richer in these compounds, as reported by Müller et al. (2012) and Slatnar et al. (2012).

The flour produced by drying the pomace lost approximately 32% of the anthocyanin contents of its raw material, while the dried blueberries lost 42% of the anthocyanins present in fresh fruit. However, White et al. (2011) found no significant differences (p > 0.05) in anthocyanin content after drying cranberry pomace at 40, 60 or 80 °C.

4 Conclusions

The organic Rabbiteye blueberries grown in the state of Rio Grande do Sul, Brazil, can be considered of commercial quality, within the parameters analyzed. The products had a lower moisture content than the whole berries, thus increasing its shelf life, without incurring significant losses in their nutritional properties.

This blueberry demonstrated a higher antioxidant capacity than other fruits and juices from different sources. The pomace also exhibited high antioxidant activity, while the flour and the dried blueberries lost 66% and 46% of the antioxidant capacity of their raw materials, respectively.

The anthocyanin contents in the fruit were significant, and similarities in the profile were found for blueberries from other sources. The pomace contained a large amount of anthocyanins because these compounds are preferably concentrated in the peel of the Rabbiteye blueberry. The flour lost 32% of the anthocyanins contained in the pomace, while the dried blueberries lost 42% of the anthocyanins contained in the fruit.

Despite these losses, blueberry flour, dried blueberries and whole blueberry juice can all be considered good sources of nutrients and bioactive substances. Therefore, the use of these agro-industrial wastes, in addition to adding value and minimizing the impact caused by their accumulation in the environment, can also be used in the development of new products with beneficial health properties.

Acknowledgements

The authors are grateful to the financial support of CNPq (National Scientific and Technological Development Council) and Capes (Coordinator for Upgrading Graduate Level Personnel).

References

- Association of Official Analytical Chemists International AOAC. (2005). Official methods of analysis (18th ed.). Gaithersburg. Official Method 2005.08.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft Und-Technologie*, 28(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.
- Cho, M. J., Howard, L. R., Prior, R. L., & Clark, J. R. (2004). Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture*, 84(13), 1771-1782. http://dx.doi.org/10.1002/jsfa.1885.
- Connor, A. M., Luby, J. J., Hancock, J. F., Berkheimer, S., & Hanson, E. J. (2002). Changes in fruit antioxidant activity among blueberry

- cultivars during cold-temperature storage. *Journal of Agricultural and Food Chemistry*, 50(4), 893-898. http://dx.doi.org/10.1021/jf011212y. PMid:11829664
- Fang, Z., Zhang, Y., Lu, Y., Ma, G., Chen, J., Liu, D., & Ye, X. (2009). Phenolic compounds and antioxidant capacities of bayberry juices. *Journal of Agricultural and Food Chemistry*, 113, 884-888.
- Francis, F. J. (1982). Analysis of anthocyanins. In P. Markakis (Ed.), *Anthocyanins as food colors* (pp. 181-207). New York: Academic Press. http://dx.doi.org/10.1016/B978-0-12-472550-8.50011-1.
- Gao, L., & Mazza, G. (1994). Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *Journal of Food Science*, 59(5), 1057-1059. http://dx.doi.org/10.1111/j.1365-2621.1994.tb08189.x.
- Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry*, 97(6), 1634-1658. http://dx.doi.org/10.1111/j.1471-4159.2006.03907.x. PMid:16805774
- Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry*, 46(10), 4107-4112. http://dx.doi.org/10.1021/jf980181c.
- Hoye, C. Jr., & Ross, C. F. (2011). Total phenolic content, consumer acceptance, and instrumental analysis of bread made with grape seed flour. *Journal of Food Science*, 76(7), S428-S436. http://dx.doi.org/10.1111/j.1750-3841.2011.02324.x. PMid:22417561
- Joseph, J. A., Denisova, N. A., Arendash, G., Gordon, M., Diamond, D., Shukitt-Hale, B., & Morgan, D. (2003). Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutritional Neuroscience*, 6(3), 153-162. http:// dx.doi.org/10.1080/1028415031000111282. PMid:12793519
- Kähkönen, M. P., Heinämäki, J., Ollilainen, V., & Heinonen, M. (2003). Berry anthocyanins: isolation, identification and antioxidant activities. *Journal of the Science of Food and Agriculture*, 83, 1403-1411. http://dx.doi.org/10.1002/jsfa.1511.
- Kalt, W., Hanneken, A., Milbury, P., & Tremblay, F. (2010). Recent research on polyphenolics in vision and eye health. *Journal of Agricultural and Food Chemistry*, 58(7), 4001-4007. http://dx.doi.org/10.1021/jf903038r. PMid:20102149
- Kalt, W., & McDonald, J. E. (1996). Chemical composition of Lowbush blueberry cultivars. *Journal of the American Society for Horticultural Science*, 121, 142-146.
- Kalt, W., McDonald, J. E., & Donner, H. (2000). Anthocyanins, phenolics, and antioxidant capacity of processed Lowbush blueberry products. *Journal of Food Science*, 65(3), 390-393. http://dx.doi.org/10.1111/j.1365-2621.2000.tb16013.x.
- Kalt, W., McDonald, J. E., Ricker, R. D., & Lu, X. (1999). Anthocyanin content and profile within and among blueberry species. *Canadian Journal of Plant Science*, *79*(4), 617-623. http://dx.doi.org/10.4141/P99-009.
- Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., & Kobori, M. (2003). Induction of apoptosis in cancer cells by Bilberry (Vaccinium myrtillus) and the anthocyanins. *Journal of Agricultural and Food Chemistry*, *51*(1), 68-75. http://dx.doi.org/10.1021/jf025781x. PMid:12502387
- Koca, I., & Karadeniz, B. (2009). Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea region of Turkey. *Scientia Horticulturae*, 121(4), 447-450. http://dx.doi.org/10.1016/j. scienta.2009.03.015.
- Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., & Joseph, J. A. (2010). Blueberry supplementation improves memory in older adults. *Journal of Agricultural and Food Chemistry*, 58(7), 3996-4000. http://dx.doi.org/10.1021/jf9029332. PMid:20047325
- Lätti, A. K., Kainulainen, P. S., Hayirlioglu-Ayaz, S., Ayaz, F. A., & Riihinen, K. R. (2009). Characterization of anthocyanins in

- caucasian blueberries (Vaccinium arctostaphylos L.) native to Turkey. *Journal of Agricultural and Food Chemistry*, *57*(12), 5244-5249. http://dx.doi.org/10.1021/jf9005627. PMid:19480388
- Lee, J., & Wrolstad, R. E. (2004). Extraction of anthocyanins and polyphenolics from blueberry processing waste. *Journal of Food Science*, 69(7), 564-573. http://dx.doi.org/10.1111/j.1365-2621.2004. tb13651.x.
- Long, G. L., & Winefordner, J. D. (1983). Limit of detection: a closer look at the IUPAC definition. *Analytical Chemistry*, 55, 712-724.
- Martineau, L. C., Couture, A., Spoor, D., Benhaddou-Andaloussi, A., Harris, C., Meddah, B., Leduc, C., Burt, A., Vuong, T., Mai Le, P., Prentki, M., Bennett, S. A., Arnason, J. T., & Haddad, P. S. (2006). Anti-diabetic properties of the Canadian lowbush blueberry Vaccinium angustifolium Ait. *Phytomedicine*, 13(9-10), 612-623. http://dx.doi.org/10.1016/j.phymed.2006.08.005. PMid:16979328
- Molina, J. M., Calvo, D., Medina, J. J., Barrau, C., & Romero, F. (2008). Fruit quality parameters of some southern highbush blueberries (*Vaccinium xcorymbosum* L.) grown in Andalusia (Spain). *Spanish Journal of Agricultural Research*, 6(4), 671-676. http://dx.doi.org/10.5424/sjar/2008064-359.
- Müller, D., Schantz, M., & Richling, E. (2012). High performance liquid chromatography analysis of anthocyanins in bilberries (Vaccinium myrtillus L.), blueberries (Vaccinium corymbosum L.), and corresponding juices. *Journal of Food Science*, 77(4), C340-C345. http://dx.doi.org/10.1111/j.1750-3841.2011.02605.x. PMid:22394068
- Nicoué, E. E., Savard, S., & Belkacemi, K. (2007). Anthocyanins in wild blueberries of Quebec: extraction and identification. *Journal of Agricultural and Food Chemistry*, 55(14), 5626-5635. http://dx.doi.org/10.1021/jf0703304. PMid:17579428
- Prior, R. L. (1998). Antioxidant capacity and health benefits of fruits and vegetables: blueberries, the leader of the pack. In Proceedings of the 32nd Annual Open House North Carolina Blueberry Council (pp. 3-12). Portland: US Highbush Blueberry Council. Retrieved from http://www.blueberry.org/antioxidants.htm
- Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, W., Krewer, G., & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry*, 46(7), 2686-2693. http://dx.doi.org/10.1021/jf980145d.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26(9-10), 1231-1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3. PMid:10381194
- Rufino, M. S. M., Alves, R. E., de Brito, E. S., Pérez-Jiménez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996-1002. http://dx.doi.org/10.1016/j.foodchem.2010.01.037.
- Saftner, R., Polashock, J., Ehlenfeldt, M., & Vinyard, B. (2008). Instrumental and sensory quality characteristics of blueberry fruit from twelve cultivars. *Postharvest Biology and Technology*, 49(1), 19-26. http://dx.doi.org/10.1016/j.postharvbio.2008.01.008.
- Seeram, N. P. (2008). Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *Journal of Agricultural and Food Chemistry*, *56*(3), 627-629. http://dx.doi.org/10.1021/jf071988k. PMid:18211023
- Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Feng, L., Dreher, M., & Heber, D. (2008). Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. *Journal of Agricultural and Food Chemistry*, 56(4), 1415-1422. http://dx.doi.org/10.1021/jf073035s. PMid:18220345

- Sellappan, S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*, 50(8), 2432-2438. http://dx.doi.org/10.1021/jf011097r. PMid:11929309
- Skrede, G., Wrolstad, R. E., & Durst, R. W. (2000). Changes in anthocyanins and polyphenolics during juice processing of Highbush blueberries (*Vaccinium corymbosum* L.). *Journal of Food Science*, 65(2), 357-364. http://dx.doi.org/10.1111/j.1365-2621.2000. tb16007.x.
- Slatnar, A., Jakopic, J., Stampar, F., Veberic, R., & Jamnik, P. (2012). The effect of bioactive compounds on in vitro and in vivo antioxidant activity of different berry juices. *PLoS One*, *7*(10), e47880. http://dx.doi.org/10.1371/journal.pone.0047880. PMid:23110118
- Smith, M. A. L., Marley, K. A., Seigler, D., Singletary, K. W., & Meline, B. (2000). Bioactive properties of wild blueberry fruits. *Journal of Food Science*, 65(2), 352-356. http://dx.doi.org/10.1111/j.1365-2621.2000. tb16006.x.
- Sousa, M. B., Curado, T., Vasconcellos, F. N., & Trigo, M. J. (2007).
 Mirtilo: qualidade pós-colheita. Folhas de Divulgação AGRO, 556, 1-33.
- U.S. Department of Agriculture, Agricultural Research Service USDA. (2013). USDA National Nutrient Database for Standard Reference. Release 26. Beltsville: Nutrient Data Laboratory. Retrieved from http://www.ars.usda.gov/ba/bhnrc/ndl
- Vasco, C., Riihinen, K., Ruales, J., & Kamal-Eldin, A. (2009). Chemical composition and phenolic compound profile of mortino (Vaccinium floribundum Kunth). *Journal of Agricultural and Food Chemistry*, 57(18), 8274-8281. http://dx.doi.org/10.1021/jf9013586. PMid:19719139
- Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chemistry, 111(4), 816-823. http://dx.doi.org/10.1016/j. foodchem.2008.04.054.
- Vázquez-Araújo, L., Chambers, E. 4th, Adhikari, K., & Carbonell-Barrachina, A. A. (2010). Sensory and physicochemical characterization of juices made with pomegranate and blueberries, blackberries, or raspberries. *Journal of Food Science*, 75(7), S398-S404. http://dx.doi.org/10.1111/j.1750-3841.2010.01779.x. PMid:21535574
- Wang, S. Y., Chen, C. T., Sciarappa, W., Wang, C. Y., & Camp, M. J. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *Journal of Agricultural and Food Chemistry*, 56(14), 5788-5794. http://dx.doi.org/10.1021/jf703775r. PMid:18590274
- White, B. L., Howard, L. R., & Prior, R. L. (2010). Proximate and polyphenolic characterization of cranberry pomace. *Journal of Agricultural and Food Chemistry*, 58(7), 4030-4036. http://dx.doi.org/10.1021/jf902829g. PMid:20020689
- White, B. L., Howard, L. R., & Prior, R. L. (2011). Impact of different stages of juice processing on the anthocyanin, flavonol, and procyanidin contents of cranberries. *Journal of Agricultural and Food Chemistry*, 59(9), 4692-4698. http://dx.doi.org/10.1021/jf200149a. PMid:21438531
- Yousef, G. G., Lila, M. A., Guzman, I., Ballington, J. R., & Brown, A. (2014). Impact of interspecific introgression on anthocyanin profile of Southern Highbush bluberry. *Journal of the American Society for Horticultural Science*, 139, 1-14.
- Zanatta, C. F., Cuevas, E., Bobbio, F. O., Winterhalter, P., & Mercadante, A. Z. (2005). Determination of anthocyanins from camu-camu (Myrciaria dubia) by HPLC-PDA, HPLC-MS, and NMR. *Journal of Agricultural and Food Chemistry*, 53(24), 9531-9535. http://dx.doi. org/10.1021/jf051357v. PMid:16302773