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Physicochemical, Antioxidant and Sensory Quality of Brazilian Blueberry Wine

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ABSTRACT

Currently, Rio Grande do Sul state is the main producer of blueberry in Brazil. Practically all production is commercialized in fresh state and only a small portion is subject to processing. The blueberry wine making process is an alternative to expand the beverage industry and offers to the consumer a value-added product as well as a new market for Brazilian blueberry producers. The objectives of this study were to produce wines from blueberries and to evaluate the effect of deacidification (with calcium carbonate) and chaptalization (with glucose syrup or sucrose) on physicochemical characteristics, antioxidant content, and sensory parameters. Samples were analyzed for total soluble solids, pH, total titratable acidity, total sugar content, alcohol content, monomeric and total anthocyanin, total flavonols, total phenolic compounds and antioxidant activity by DPPH and FRAP methods. The use of calcium carbonate caused a reduction in total titratable acidity, while the use of glucose syrup resulted in wines with low alcohol content. The blueberries wine from Climax and Aliceblue cultivars had higher content of anthocyanin when produced with glucose syrup. The use of calcium carbonate and glucose syrup also provided wines more appreciated by tasters in relation to color. With regard to flavor, George and Aliceblue were the cultivars with lower preference under the control treatments (without carbonate and sugar). The presence of phenolic compounds may have provided a positive influence on wine flavor, once the more preferred wines presented the greater phenolic content.

Key-words: *Vaccinium ashei* Reade, anthocyanin, phenolic compounds, alcoholic beverage.

INTRODUCTION

Blueberries (*Vaccinium* sp.) are temperate fruits which require some winter chilling (Allemann and Young 2006). Native in several regions of Europe and the United States, blueberries were first introduced in Brazil in 1983 through a plant collection brought to Embrapa Clima Temperado (Pelotas, RS). Currently, Rio Grande do Sul is the main state in blueberry production in Brazil, with 45 growers cultivating 65 ha and 150 ton (Fachinello 2008). Itaara city is located in the center of Rio Grande do Sul state, at 425m of elevation

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above sea level. This climate is favorable for blueberries, with sufficient chilling hours, hard winters and dry summer (Itaaras's City Hall 2010).

Researchers from the Federal University of Rio Grande do Sul (UFRGS) found that the blueberry produced in Brazil has the same characteristics of the fruit cultivated in the United States and Europe, having the same antioxidant characteristics (Fachinello 2008). The antioxidant activity of this fruit is based on high content of phenolic compounds (Ehlenfeldt and Prior 2001, Castrejón et al. 2008) like anthocyanin, phenolic acids and tannins (Mcghie and Walton 2007). These compounds are a current interest due to their important biological and pharmacological properties including antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antiproliferative activities (Kim and Um 2011).

During wine processing, the fruits are pressed to release the sweet juice used for fermentation along with their skins and seeds. Like red grapes, anthocyanins of blueberries are present mainly in the skins (Riihinen et al. 2008), while tannins and phenolic acids predominate in the seeds. During fermentation process, these compounds are transferred to wine and contribute to the color, mouthfeel, and antioxidant properties of the wine.

Despite the similarity with grapes, some varieties of blueberries have less sugar and different organic acid composition, since the main acids in blueberries are both citric and malic acid (Dharmadhikari 1996). Although these acids can be used in acidification to enhance the total acidity of wines, they have an aggressive citric flavor. In order to reduce the acidity in a wine, potassium bicarbonate or calcium carbonate can be used (Jackson 2008). These additives reduce the acidity and increase the pH, in spite of this, they should not be used in high doses because they may lead to problems in stabilization (Rizzon and Gasparin 2005).

Based on these aspects, the aim of this work was to produce wine from blueberries cultivated in Brazil and study the effects of the deacidification and chaptalization on antioxidant, physicochemical and sensory parameters in the blueberry wines produced.

MATERIALS AND METHODS

SAMPLES AND FERMENTATION

Blueberries Aliceblue, Climax, and George cultivars (*Vaccinium ashei* Reade) from Itaara, RS, Brazil were cultivated at the same location (-29° 36' 1.31", -53° 49' 6.67") under the same environmental conditions and harvested at commercially mature stage from different plants, in December 2012. After harvest, fruits were immediately transported to laboratory where they were stored at 18 °C during 12 hours until use. For each fermentation unit, about 0.7 kg of fresh blueberries were used, which were divided into five lots. Each lot was treated by adding two types of sugar for chaptalization and calcium carbonate for deacidification as follows: (T1) control without sucrose and CaCO₃, (T2) sucrose + 0.9 g L⁻¹ CaCO₃, (T3) sucrose + 1.8 g L⁻¹ CaCO₃, (T4) glucose syrup + 0.9 g L⁻¹ CaCO₃, (T5) glucose syrup + 1.8 g L⁻¹ CaCO₃.

In order to adjust the level of sugar in the blueberry must, 30 g L⁻¹ of sugar previously set was added with the aim of increasing the alcohol content in 1.5% after fermentation, value allowed by legislation.

The experiment was guided with 3 cultivars, 2 types of sugar and 2 doses of carbonate with 3 repetitions, using the experimental outline totally randomized in a factorial scheme 3x2x2. After crushed, pectinolytic enzyme (3.5 g hL⁻¹) was added to the blueberry must and commercial yeast was inoculated (*Saccharomyces cerevisiae*, 20 g hL⁻¹). The maceration was carried out with skins and seeds for 12 days and total soluble solid content was checked periodically. The room temperature was maintained at 22 ± 2°C. When the

fermentation process was completed, a 50 ppm solution of sulphur dioxide was added. All treatments were made in triplicate.

PREPARATION OF EXTRACTS

Ten grams of blueberries from each cultivar were blended with 20 mL of distilled water and centrifuged (10 min, 1559.6 g). The supernatant was collected in order to determine pH, total titratable acidity and sugar content. The same procedure was used to determine total and monomeric anthocyanins, total flavonol and phenolic content. However, 70% ethanol acidified with 0.1% hydrochloric acid was used as extractor solvent.

TOTAL SOLUBLE SOLIDS, pH, TOTAL TITRATABLE ACIDITY, TOTAL SUGAR CONTENT AND ALCOHOL CONTENT

The total soluble solids (TSS) was measured in fruits with refractometer (Biobrix®) and expressed in °Brix. A pH meter (Digimed®) was used to measure the pH of each blueberry extract and blueberry wine and titratable acidity (TTA) was determined by titration with 0.1 N sodium hydroxide solution until pH 8.2 (determined by potentiometric method) and was expressed in terms of citric acid (%) (Adolfo Lutz Institute 2008). The total sugar content (TSC) was determined by Somogyi (1945) and Nelson (1944) method and alcohol content by Salleron-DuJardin Ebulliometer, with results expressed in degree Gay-Lussac (°GL) (Ribéreau-Gayon et al. 2006).

DETERMINATION OF MONOMERIC ANTHOCYANIN (MA) AND TOTAL ANTHOCYANIN (TA)

Monomeric anthocyanin (MA) and total anthocyanin (TA) content were determined by the pH-differential method (Giusti and Wrolstad 2001). The absorbance was measured at 700 nm and at the wavelength of maximum absorption. Absorbance (A) was calculated as showed in Equation 1 and monomeric anthocyanins in Equation 2. The total anthocyanins were calculated as showed in Equations 3 and 4. Results were expressed as cyanidin-3-glucoside per one hundred grams of fruit or liter of blueberry wine.

$$A = (A_{\lambda_{max}} - A_{700})_{pH\ 1.0} - (A_{\lambda_{max}} - A_{700})_{pH\ 4.5} \quad \text{Eq. 1}$$

$$\text{Monomeric Anthocyanins (mgL}^{-1}\text{)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad \text{Eq. 2}$$

$$A' = (A_{\lambda_{max}} - A_{700})_{pH\ 1.0} \quad \text{Eq. 3}$$

$$\text{Total Anthocyanins (mgL}^{-1}\text{)} = \frac{A' \times MW \times DF \times 1000}{\epsilon \times l} \quad \text{Eq. 4}$$

TOTAL FLAVONOL CONTENT AND TOTAL PHENOLIC CONTENT (TPC)

Total flavonol content was measured by the aluminum chloride colorimetric assay (Zhishen et al. 1999). It was expressed as mg quercetin equivalents (QE) per one hundred grams of fruit or liter of blueberry wine.

The total phenolic content of each extract was determined spectrophotometrically (FEMTO CIRRUS 80ST), according to the Folin-Ciocalteu method (Singleton and Rossi 1965). Absorbance was read at 765 nm and results were expressed in gallic acid equivalents (GAE) per one hundred grams of fruit or liter of blueberry wine.

ANTIOXIDANT ACTIVITY

DPPH method

DPPH radical scavenging activity was determined according to the method of Brand-Williams et al. (1995) with minor modifications. An aliquot of 3.9 mL of DPPH solution was mixed with 0.1 mL of the diluted samples (1:50 v/v) at three different concentrations and the absorbance was measured at 517 nm after 24 h in the dark. The scavenging effect (%) was estimated based on the percentage of DPPH radical scavenged as showed in Equation 5:

$$\text{Scavenging effect (\%)} = \frac{(\text{control abs}) - (\text{sample abs})}{(\text{control abs})} \times 100 \quad \text{Eq. 5}$$

The effective concentration of sample required to scavenge DPPH radical in 50% (EC_{50} value) was obtained by linear regression analysis, where concentration of sample were plotted against the percentages of DPPH radical scavenged. The lower EC_{50} means the higher antioxidant activity.

Frap method

To prepare the FRAP reagent, 200 mL of acetate buffer (0.3 M, pH 3.6) were mixed with 20 mL of $FeCl_3 \cdot 6H_2O$ 20 mM and 20 mL of TPTZ (10 mM dissolved in HCl 40mM). Aliquots (0.2 mL) of diluted extracts were mixed with 1.8 mL of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm after incubation at 37 °C for 10 min (Benzie and Strain 1996).

SENSORIAL ANALYSIS

For sensory analysis an ordering test was carried out with twenty assessors who are wine consumers. The assessors evaluated the samples for color and flavor. Five samples at 15 ± 2 °C were presented to assessors to be organized from the most preferred to the least preferred. The results were calculated by the sum of each sample ordering and were statistically evaluated by Friedman's test at 5% critical value using data from Newell and MacFarlane (1987) table (AOAC 1995). The research protocol was previously approved by the local Research Ethics Committee (16587313.5.0000.5346) and all the panelists gave their informed consent prior to the inclusion in the study.

STATISTICAL ANALYSIS

All analyses were carried out in triplicate and the results expressed as mean \pm standard deviation (SD). Statistical analysis was carried out using the Statistica software package version 9.0 (StatSoft, Inc., Tulsa, OK, USA). Differences between means were first analyzed using the one-way ANOVA test and then by the *post-hoc* Tukey test ($p < 0.05$). Pearson's correlation coefficient was calculated using the Statistic software.

RESULTS AND DISCUSSION

CHEMICAL COMPOSITION OF GEORGE, ALICEBLUE AND CLIMAX BLUEBERRIES

The physical and chemical characteristics of the studied cultivars are presented in Table I.

TABLE I
Chemical composition of selected cultivars of blueberry fruit from Itaara – RS.

	Cultivar		
	George	Aliceblue	Climax
TTA (g citric acid 100 g ⁻¹)	0.9 ± 0.0 ^a	0.5 ± 0.0 ^c	0.6 ± 0.0 ^b
pH	2.7 ± 0.0 ^b	3.2 ± 0.1 ^a	3.2 ± 0.1 ^a
TSS (°Brix)	12.9 ± 0.1 ^b	12.3 ± 0.3 ^b	17.6 ± 0.2 ^a
Total sugar (%)	10.4 ± 0.3 ^b	10.2 ± 2.9 ^b	14.0 ± 1.3 ^a
TA ¹	76.6 ± 20.9 ^c	117.1 ± 24.2 ^b	320.3 ± 19.8 ^a
MA ²	75.9 ± 5.4 ^c	116.0 ± 7.3 ^b	319.4 ± 5.9 ^a
Flavonols ³	356.8 ± 30.7 ^b	302.9 ± 54.8 ^b	594.9 ± 24.6 ^a
TPC ⁴	390.3 ± 34.9 ^b	432.0 ± 42.3 ^b	678.4 ± 60.0 ^a
DPPH (EC ₅₀) ⁵	64.2 ± 13.9 ^a	58.1 ± 12.2 ^a	21.1 ± 2.5 ^b
FRAP ⁶	3.3 ± 0.3 ^a	3.6 ± 0.1 ^a	3.6 ± 0.7 ^a

Treatment means of the ANOVA test (values are the mean value of three replicates). Values followed by the same letter at same line were not significantly different ($p < 0.05$) by Tukey's test. TTA= Total titratable acid, TSS= Total soluble solids, TA= Total Anthocyanin, MA= Monomeric Anthocyanin, TPC = Total Phenolic Content (^{1,2} mg cyanidin-3-glucoside equivalent 100g⁻¹ FW, ³ mg quercetin-3-rutinoside equivalent 100g⁻¹ FW, ⁴ mg of galic acid equivalent 100g⁻¹ FW, ⁵ mg FW, ⁶ mg trolox 100g⁻¹ FW.

The cultivar George showed the highest TTA and the lowest pH value compared to the other two cultivars (Table I). These characteristics could be related to the composition of organic acid content. Ehlenfeldt and Meredith (1994) show that the predominant organic acid in the highbush (*Vaccinium corymbosum* L.) cultivars was citric acid, while in rabbiteye (*V. ashei* Reade) cultivars, succinic and malic acid were found in greater quantities. These authors also showed that in Climax cv., these acids were present at an average level of 89%.

The highest TSS and total sugar content was detected in 'Climax', suggesting that the cultivar was more mature at the same time of harvest. Antunes et al. (2008) reported lower values to the same cultivar (12.81 °Brix). Other studies related significant differences in the soluble solids content, titratable acids and sugar content among different blueberry varieties. The values range from 8.5 to 13.32 °Brix, 0.4 to 1.2% citric acid and 7.6 to 9.3 g 100 g⁻¹ of total sugar (Skupien 2006, Šnĕ et al. 2011).

In this study Climax cv. showed the highest content of total phenolics, flavonols and anthocyanins. However, there were no significant differences in total flavonol and total phenolic content among 'George' and 'Aliceblue' ($p < 0.05$, Table I). The monomeric anthocyanin content ranged from 75.9 to 319.4 mg cyanidin-3-glucoside per 100 g FW, while the content of total anthocyanin ranged from 76.6 to 320.4 mg per 100 g FW (Table I). These values represent between 19% and 47% of total polyphenols, similarly as described by Bunea et al. (2011), who reported that anthocyanins represented between 23% and 37% percent of total polyphenols. The highest total phenolic content was recorded in 'Climax', at 678.4 mg GAE 100 g⁻¹ FW (Table I). Similar values were reported by Rodrigues et al. (2011) in the same cultivar. Nevertheless, Vizzoto et al. (2012) related that the cultivar 'Aliceblue' has higher phenolic content than 'Climax'. Among cultivars, a large variation was also observed from other researchers (167.6 - 677.8 mg cyanidin-3-glucoside per 100 g FW) (Kim et al. 2013). The total anthocyanins and phenolic content of blueberries are higher than other berries but similar to blackberry and elderberry (Nile and Park 2013).

The genus *Vaccinium*, member of the family *Ericaceae*, presents as the main phenolics the group of flavonols (lingonberry and cranberry) and hydroxycinnamic acids (blueberries and bilberry) (Häkkinen et al. 1999). In this study, the content of flavonols represents from 70% to 91% of total phenolic content (Table I).

The cultivar Climax exhibited significantly higher antioxidant activity (i.e. lower EC50 values) by DPPH method; however, it does not demonstrate statistical difference when analyzed by FRAP method (Table I).

The great antioxidant activity is in accordance with the high content of total phenolic (coefficient correlation = 0.999). Likewise Bunea et al. (2011), our results showed higher correlation between DPPH assay and phenolic content. All differences related about characteristics of cultivars as mentioned by different works occur probably due to different extraction methods, environmental growing conditions, and genotypes.

FERMENTATION OF BLUEBERRIES

Blueberry wines from all cultivars and treatments were tested periodically for °Brix. Figure 1 shows the decline of TSS during fermentation and reveals that the fermentation process occurs quickly until the 12th day and practically stagnates until the 30th day.

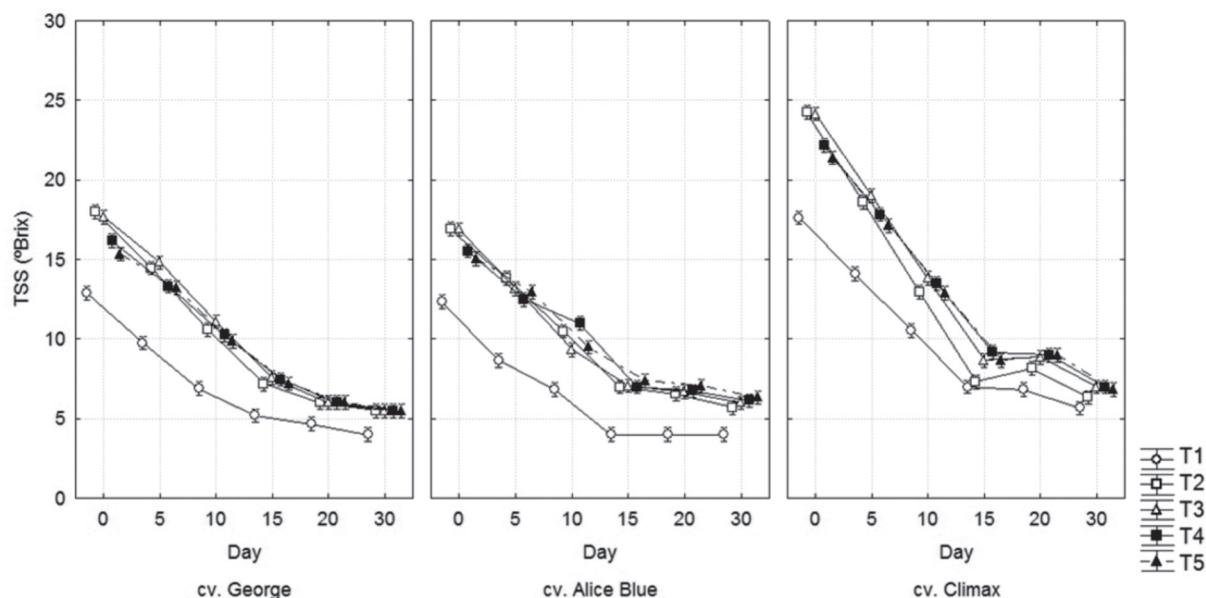


Figure 1 – Fermentation curves for each type of wine throughout time. (T1) control – without sucrose and CaCO_3 , (T2) sucrose + $0.9 \text{ gL}^{-1} \text{ CaCO}_3$, (T3) sucrose + $1.8 \text{ gL}^{-1} \text{ CaCO}_3$, (T4) glucose syrup + $0.9 \text{ gL}^{-1} \text{ CaCO}_3$, (T5) glucose syrup + $1.8 \text{ gL}^{-1} \text{ CaCO}_3$.

Fruits rich in sugars are preferred for fermentation processes because they provide higher alcohol content, contribute to the texture and make the drink more pleasant to taste (Hashizume 2001). The difference observed among cultivars could be due to different initial sugar content in blueberries used or due to an incomplete fermentation, resulting in more residual sugar in the final fermented wine.

PHYSICOCHEMICAL ANALYSIS OF BLUEBERRY WINES

In Table II, it can be observed that the use of calcium carbonate (T2 – T5) reduced the total titratable acid and increased pH values of all blueberry wines, except in George cultivar.

TABLE II
Physicochemical analysis of blueberry wines.

Cultivar	Treatment	TAA (g citric acid 100 mL ⁻¹)	pH	Total sugar (g L ⁻¹)	Alcohol (° GL)
George	T1	2.1 ± 0.5 ^a	3.3 ± 0.2 ^a	5.5 ± 0.2 ^c	5.2 ± 0.6 ^b
	T2	1.4 ± 0.1 ^b	3.6 ± 0.1 ^a	3.8 ± 1.6 ^{bc}	7.5 ± 0.9 ^a
	T3	1.5 ± 0.1 ^b	3.7 ± 0.1 ^a	1.9 ± 1.6 ^b	7.2 ± 0.3 ^a
	T4	1.6 ± 0.1 ^b	3.8 ± 0.1 ^a	6.9 ± 1.5 ^{ac}	6.2 ± 0.3 ^{ab}
	T5	1.8 ± 0.4 ^b	4.0 ± 0.1 ^a	10.1 ± 1.0 ^a	5.6 ± 0.4 ^b
Alicebblue	T1	1.7 ± 0.1 ^a	3.5 ± 0.0 ^b	1.7 ± 1.7 ^c	3.8 ± 0.4 ^b
	T2	1.5 ± 0.1 ^{ab}	4.0 ± 0.1 ^a	3.1 ± 1.7 ^c	7.2 ± 0.4 ^a
	T3	1.3 ± 0.3 ^{ab}	4.1 ± 0.1 ^a	5.5 ± 1.2 ^{bc}	7.4 ± 0.2 ^a
	T4	1.2 ± 0.1 ^b	3.9 ± 0.2 ^a	11.0 ± 2.6 ^a	6.0 ± 0.1 ^a
	T5	1.2 ± 0.5 ^b	4.1 ± 0.1 ^a	10.7 ± 2.5 ^{ab}	5.7 ± 0.6 ^a
Climax	T1	0.9 ± 0.1 ^a	3.6 ± 0.1 ^c	1.5 ± 1.9 ^c	7.4 ± 0.7 ^c
	T2	0.8 ± 0.1 ^{ab}	3.9 ± 0.2 ^{abc}	2.9 ± 1.5 ^{bc}	10.8 ± 0.8 ^a
	T3	0.6 ± 0.1 ^b	4.2 ± 0.1 ^{ab}	5.5 ± 4.4 ^{abc}	11.1 ± 0.4 ^a
	T4	0.6 ± 0.1 ^b	3.8 ± 0.1 ^{bc}	11.5 ± 1.1 ^a	9.9 ± 0.1 ^{ab}
	T5	0.6 ± 0.1 ^b	4.3 ± 0.2 ^a	9.9 ± 2.3 ^{ab}	9.2 ± 0.8 ^b

/(T1) control – without sucrose and CaCO₃, (T2) sucrose + 0.9 gL⁻¹ CaCO₃, (T3) sucrose + 1.8 gL⁻¹ CaCO₃, (T4) glucose syrup + 0.9 gL⁻¹ CaCO₃, (T5) glucose syrup + 1.8 gL⁻¹ CaCO₃. Treatment means of the ANOVA test (values are the mean value of three replications). Values followed by the same letter, within the same column, were not significantly different ($p < 0.05$) by Tukey's test, at the same cultivar. TTA= Total titratable acid.

Possibly George cultivar has a high content of malic acid which, combined with calcium carbonate during fermentation process, resulted in calcium malate. This salt needs at least six months for precipitation and reduction of the total acid content and increase of pH. In addition, wines from Alicebblue and Climax cultivars showed similar pH values.

These characteristics could be related to the composition of organic acid content and to the high content of potassium in berries. Ehlenfeldt and Meredith (1994) show that the predominant organic acid in the highbush (*Vaccinium corymbosum* L.) cultivars was citric acid, while in rabbiteye (*V. ashei* Reade) cultivars, succinic and malic acid were found in greater quantities. These authors also showed that in Climax cv., these acids were present at an average level of 89%.

The low acid content and high pH value observed in wines from Climax cv. can be due to a content of potassium, which may have contributed to high pH values without interfering on acid content. In grapes, it was observed that an increase of pH values is associated with high potassium content, resulting in wines with high pH (>3.7), on the other hand, it was also observed that pH is reduced during stabilization (Fogaça et al. 2007). High levels of pH can decrease the intensity of the color and the stability of wine (Daudt and Fogaça 2008).

The use of glucose (T4 – T5) resulted in wines with more amounts of residual sugar and low alcohol content (Table II). Polakowski (2008) related that yeast had the highest carbon dioxide formation rates using sucrose than all the other sugars studied. Although glucose syrup is composed primarily by glucose and water, it may contain some maltose or other sugars, and vanilla flavoring or preservatives that can affect fermentation.

TOTAL AND MONOMERIC ANTHOCYANIN, TOTAL FLAVONOLS, PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF BLUEBERRY WINES

Phenolic compounds and antioxidant activity presented by blueberry wines can be observed in Table III.

About total phenolic content, researchers have shown that blueberry wine has some similarity with grape wine and has more antioxidants than many fruit wine (Rupasinghe and Clegg 2007). The total phenolic content of George cv. wine does not show a significant difference between treatments, but in wines from Aliceblue and Climax cultivars, higher doses of calcium carbonate increased these values (Table III). Once anthocyanins are part of phenolic content, this increase can be related to high anthocyanin values. Using a similar method of vinification, Su and Chien (2007) reported lower values of both anthocyanins as total phenolic in blueberry wines (99.6 ± 0.11 mg of cyanidin-3-glucoside equivalents per liter and 1150 ± 3.06 mg gallic acid equivalents per liter of sample, respectively). Johnson et al. (2011) also reported lower values of phenolic content (590.0 ± 26.7 mg gallic acid equivalents (GAE) per liter of wine).

The blueberries wine from Climax and Aliceblue cultivars showed higher anthocyanin content when elaborated with glucose syrup. Although no significant difference had been observed in 'George', high values of anthocyanin were observed in blueberry wines with more residual sugar. Other approaches also showed that anthocyanin degradation decreased when sucrose concentration increased (Tsai et al. 2004) and in moderate concentration, sugar can preserve the berries anthocyanin (Nikkhah et al. 2007). Normally, anthocyanins seem to be more stable in acidic media at lower pH values than in alkaline solutions (He et al. 2012). Nevertheless, in this study no significant difference between doses of calcium carbonate was observed.

TABLE III

Total and monomeric anthocyanin, total flavonols, phenolic content, and antioxidant capacity of blueberry wines.

Cultivar	Treatment	TA ¹	MA ²	Total Flavonols ³	TPC ⁴	DPPH ⁵	FRAP ⁶
George	T1	110.5 ± 5.5 ^a	57.8 ± 12.3 ^a	1384.6 ± 348.0 ^b	1883.1 ± 158.1 ^a	3.7 ± 1.3 ^c	23.7 ± 1.6 ^a
	T2	105.6 ± 16.0 ^a	65.1 ± 8.8 ^a	1450.4 ± 200.0 ^{ab}	1939.5 ± 60.7 ^a	4.7 ± 0.4 ^{bc}	23.1 ± 0.6 ^a
	T3	92.7 ± 15.2 ^a	55.0 ± 9.6 ^a	1779.2 ± 32.9 ^{ab}	1877.5 ± 173.2 ^a	5.7 ± 0.8 ^{ab}	22.6 ± 2.6 ^a
	T4	105.6 ± 6.9 ^a	69.9 ± 1.7 ^a	2042.3 ± 205.4 ^a	1947.0 ± 53.2 ^a	4.8 ± 0.6 ^{abc}	22.3 ± 0.7 ^a
	T5	86.6 ± 6.7 ^a	54.1 ± 6.2 ^a	1275.0 ± 305.6 ^b	1862.0 ± 96.1 ^a	6.4 ± 0.5 ^a	23.7 ± 0.5 ^a
Aliceblue	T1	129.0 ± 24.0 ^b	69.4 ± 25.3 ^c	2141.0 ± 131.5 ^a	1676.6 ± 15.1 ^b	5.7 ± 0.6 ^a	22.9 ± 3.1 ^a
	T2	143.9 ± 22.2 ^{ab}	87.1 ± 22.4 ^{cb}	2162.9 ± 68.5 ^a	1752.9 ± 23.4 ^{ab}	6.4 ± 0.2 ^a	17.9 ± 0.1 ^a
	T3	152.0 ± 14.0 ^{ab}	96.9 ± 11.7 ^{abc}	1921.7 ± 242.4 ^a	1797.8 ± 83.7 ^{ab}	6.3 ± 1.2 ^a	19.9 ± 0.7 ^a
	T4	188.1 ± 6.1 ^a	128.4 ± 9.9 ^{ab}	1801.1 ± 214.0 ^a	1803.1 ± 36.3 ^{ab}	5.3 ± 0.9 ^a	20.8 ± 2.0 ^a
	T5	186.6 ± 15.7 ^a	134.0 ± 11.1 ^a	1142.3 ± 323.7 ^b	1899.2 ± 104.7 ^a	5.3 ± 0.8 ^a	21.9 ± 4.3 ^a
Climax	T1	108.0 ± 18.0 ^{cb}	54.7 ± 7.5 ^b	1187.3 ± 230.2 ^b	1672.2 ± 124.9 ^d	5.4 ± 0.7 ^a	19.7 ± 0.9 ^b
	T2	105.9 ± 8.2 ^c	59.3 ± 4.0 ^b	1384.6 ± 484.4 ^{ab}	2094.6 ± 65.0 ^c	4.4 ± 1.0 ^{ab}	20.7 ± 0.9 ^b
	T3	139.6 ± 8.0 ^b	82.8 ± 5.0 ^b	1845.0 ± 301.4 ^{ab}	2580.2 ± 96.0 ^{ab}	3.3 ± 0.1 ^b	32.4 ± 1.2 ^a
	T4	212.5 ± 11.5 ^a	123.0 ± 26.2 ^a	2130.0 ± 958.4 ^{ab}	2519.4 ± 111.8 ^b	3.6 ± 0.1 ^b	28.8 ± 0.6 ^a
	T5	213.2 ± 12.8 ^a	123.3 ± 15.5 ^a	2689.0 ± 189.9 ^a	2824.6 ± 54.1 ^a	3.3 ± 0.0 ^b	32.1 ± 3.8 ^a

(T1) control – without sucrose and CaCO₃, (T2) sucrose + 0.9 gL⁻¹ CaCO₃, (T3) sucrose + 1.8 gL⁻¹ CaCO₃, (T4) glucose syrup + 0.9 gL⁻¹ CaCO₃, (T5) glucose syrup + 1.8 gL⁻¹ CaCO₃. Treatment means of the ANOVA test (values are the mean value of three replications). Values followed by the same letter, within the same column, were not significantly different ($p < 0.05$) by Tukey's test, at the same cultivar. TA = Total Anthocyanin, MA= Monomeric Anthocyanin, TPC = Total Phenolics Content. ^{1,2} mg cyanidin3 – glucoside equivalent mg L⁻¹, ³ mg quercetin-3-rutinoside equivalent mg L⁻¹, ⁴ mg of galic acid equivalent mg L⁻¹, ⁵ EC₅₀, ⁶ mg trolox L⁻¹.

Although the content of total anthocyanins, total flavonols, and phenolic compounds in fruits (Table I) was different among cultivars, these values were similar in wines (Table III). Among treatments, little difference was noted. Nevertheless, in George cultivar higher antioxidant activity was observed in wines without calcium carbonate (Table III). The extraction of these compounds depends mainly on the maceration time and temperature, frequency and mode of cap punching, alcohol, and sulfur dioxide levels (Su and Chien 2007). Furthermore, the content of anthocyanins in wines depends also on the extractability from berries to wine (Romero-Cascales and Ortega-Regules 2005) and mechanical proprieties of berry (Zouid et al. 2010).

In blueberries fruits, a significant variation of individual anthocyanins and phenolic compounds between cultivars has been reported (Kim and Um 2011). The differences in antioxidant capacity behavior may be due to a different phenolic profile, once each phenolic compound has its own capacity and rate of scavenging DPPH free radical (Villaño et al. 2007). Chemical structure of flavan-3-ol family shows generally a good antioxidant response towards DPPH radical (Villaño et al. 2007). However, the stability of the monomeric anthocyanins depends on various factors, such as solution composition, pH value of wine, presence of substances such as ascorbic acid, sugars, sulfites, cofactors, and metallic ions (He et al. 2012). Possibly, this is a reason for EC_{50} to be greater when treated with calcium carbonate.

CORRELATION COEFFICIENTS (R^2) OF TOTAL AND MONOMERIC ANTHOCYANINS, FLAVONOLS, TOTAL PHENOLIC AND ANTIOXIDANT CAPACITY (FRAP AND DPPH)

Table IV presents the correlation coefficients (r^2), which indicate the possible correlation between polyphenol composition with different antioxidant activity and the correlation of different assays used with each other.

TABLE IV
Correlation coefficients (r^2) of total and monomeric anthocyanins, flavonols, total phenolic and antioxidant capacity (FRAP and DPPH).

	Source	TA ¹	MA ²	Flavonols ³	TP ⁴	DPPH ⁵
George	MA ¹	0.40669				
	Flavonols ²	0.036523	0.349987			
	TP ³	0.394232	0.955746	0.302559		
	DPPH ⁴	0.94541	0.196271	0.004644	0.185763	
	FRAP ⁵	0.00997	0.347093	0.916761	0.362815	0.002249
	Source	TA ¹	MA ²	Flavonols ³	TP ⁴	DPPH ⁵
Aliceblue	MA ¹	0.989116				
	Flavonols ²	0.632727	0.70527			
	TP ³	0.732534	0.819042	0.824352		
	DPPH ⁴	0.461354	0.413518	0.424763	0.157707	
	FRAP ⁵	0.00789	0.005196	0.129633	0.001019	0.526144
	Source	TA ¹	MA ²	Flavonols ³	TP ⁴	DPPH ⁵
Climax	MA ¹	0.990947				
	Flavonols ²	0.853037	0.880284			
	TP ³	0.63843	0.718598	0.857705		
	DPPH ⁴	0.508848	0.601243	0.698608	0.959582	
	FRAP ⁵	0.540208	0.61162	0.721833	0.880132	0.879428

TA = Total Anthocyanin, MA= Monomeric Anthocyanin, TP = Total Phenolic.

A correlation analysis was done among total anthocyanins, monomeric anthocyanins, total flavonols, total polyphenols, and the antioxidant activity values obtained. Apparently, high antioxidant activity of blueberry wine from George cv. is related to the presence of total anthocyanins ($r^2 = 0.945$), while blueberry wine from Climax cv. is related to content of total phenolic ($r^2 = 0.959$) by DPPH method. The differences observed are probably associated to composition of antioxidant compounds, once each compound has its own antioxidant activity (Iacopini et al. 2008).

SENSORY ANALYSIS

Table V shows the differences in the sum of each sample ordering, statistically evaluated by Friedman's test using data from Newell and MacFarlane (1987) table. The results obtained show that the chaptalization with glucose syrup and the reduction of acidity using low dose of calcium carbonate (treatment 4) promoted the highest sum despite the fact that there was no significant statistical difference, suggesting that this combination of chaptalization and deacidification improved the blueberry wine color, independent of the cultivar used (Table V). Interestingly, this treatment was the same with the higher anthocyanin content (Table II), suggesting a relationship between anthocyanin content and preference color by consumer.

TABLE V
Ranking sums of blueberry wine evaluated by the preference test.

Treatment	Cultivar	Color			Flavor		
		George	Aliceblue	Climax	George	Aliceblue	Climax
T1		74 ^a	71 ^{ab}	55 ^{bc}	30 ^b	39 ^b	58 ^a
T2		55 ^a	61 ^{ab}	39 ^c	62 ^a	78 ^a	57 ^a
T3		45 ^b	49 ^{ab}	49 ^{bc}	63 ^a	60 ^{ab}	54 ^a
T4		65 ^a	74 ^a	88 ^a	82 ^a	56 ^{ab}	59 ^a
T5		61 ^a	45 ^b	69 ^{ab}	64 ^a	66 ^{ab}	72 ^a

(T1) control – without sucrose and CaCO_3 , (T2) sucrose + $0.9 \text{ gL}^{-1} \text{ CaCO}_3$, (T3) sucrose + $1.8 \text{ gL}^{-1} \text{ CaCO}_3$, (T4) glucose syrup + $0.9 \text{ gL}^{-1} \text{ CaCO}_3$, (T5) glucose syrup + $1.8 \text{ gL}^{-1} \text{ CaCO}_3$. Values followed by the same letter at the same column were not significant different according to Newell and MacFarlane (1987) table. The critical value for the significance level of 5% is 28. The largest sum represents the most preferred.

Regarding flavor, the panelists did not perceive difference in 'Climax' wines. However, in George and Aliceblue cultivars the deacidification and chaptalization also resulted in most favorite wines, and no significant difference was observed among treatments. Our results are similar to other fruit wines. For example, elderberry wines elaborated with low sugar content and high total acidity were the least preferred, while wines with high total sugars and low total acidity were scored as the best for their taste and aroma (Garofulić et al. 2012). Besides improving the color, phenolic compounds may have influenced positively the flavor of wine, once the most preferred wine also contains higher phenolic content (Table II). These compounds can react with salivary proteins in mouth and are responsible for wine astringency and bitterness (Sun et al. 2011). This way, the deacidification with low doses of calcium carbonate and the residual sugar of chaptalization of glucose syrup can be suggested, once panelists demonstrate more preference by this treatment which resulted in wines with more content of anthocyanins comparing to others.

CONCLUSIONS

Based on the results obtained, it can be concluded that calcium carbonate reduces total titratable acidity, while the use of glucose syrup results in blueberry wines with high residual sugar content and low alcohol,

characteristics that were preferred by panelists in the sensorial analysis. At a first moment, the use of glucose syrup and 0.9 g L⁻¹ of calcium carbonate resulted in wines with higher anthocyanins content and a preferred color even though there was no significant differences between treatments in George and Aliceblue cultivars. Regarding the flavor, the blueberry wines from George and Aliceblue cultivars without added sugar and carbonate were the least favorites, while wines with high total sugars and low total acidity had higher approval.

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