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Bioactive compounds and juice quality from selected grape cultivars

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ABSTRACT: Grape juices have been valued due to their potential health benefits, which have demanded increased grape productivity and quality. Five grape cultivars grown in Brazil, Isabel Precoce, Carmem, Violeta, Concord and Bordo were evaluated in 2013 and 2014 seasons for bioactive components and also for juice processing quality traits. Production cycle was the longest for Carmem but lower and similar for Violeta, Isabel, Bordo and Concord. Isabel showed higher productivity ($5.4 \text{ kg} \cdot \text{plant}^{-1}$) but lowest soluble solids content (16.9°Brix), anthocyanins ($26.7 \text{ mg} \cdot 100 \text{ g}^{-1}$) and total phenolics ($110.7 \text{ mg} \cdot 100 \text{ g}^{-1}$). The highest anthocyanins contents were observed in Violeta ($189.9 \text{ mg} \cdot 100 \text{ g}^{-1}$) and Bordo ($133.8 \text{ mg} \cdot 100 \text{ g}^{-1}$). These

cultivars were also rich in phenolics (356.1 and $239.5 \text{ mg} \cdot 100 \text{ g}^{-1}$, respectively). The highest anthocyanin and total phenolics concentrations were found in Violeta juice (2.68 and $6.33 \text{ g} \cdot \text{L}^{-1}$) followed by Bordo (1.44 and $2.86 \text{ g} \cdot \text{L}^{-1}$). Isabel juice had the lowest content, 0.14 and $1.29 \text{ g} \cdot \text{L}^{-1}$, respectively. Biogenic amines were found at low concentrations only in the juices. Putrescine and spermidine were the major amines detected in juices. Phenylethylamine was detected only in Bordo juice from 2013 season and tryptamine was detected only in 2014 season. Blends were preferred over varietal juices except for Carmem.

Key words: *Vitis labrusca*, fruit juices, phenolics, amines, acceptance.

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INTRODUCTION

Recent studies have demonstrated, on the basis of short-term changes in biomarkers, the beneficial effects of some dietary polyphenols to human health. According to the American Dietetic Association (2004), the daily consumption of 250 to 500 mL of red grape juice, rich in procyanidins, has pronounced effects on the vascular system and show plasma antioxidant activity. Furthermore, they may protect the human body against oxidative stress, and reduce the risk of free radical damage and chronic diseases (O'Byrne et al. 2002; ADA 2004; Williamson and Manach 2005; Toaldo et al. 2015). Some of these health promoting effects attributed to phenolic compounds have also been associated with other food components, including bioactive amines, some of which are also present in grapes (Badria 2002; Gloria 2005).

Among bioactive amines, polyamines and tryptamines are known to have radical scavenging properties. Also, some biogenic amines have vaso- or neuro-activity. Besides their functional properties, bioactive amines are also relevant from quality and safety points of view. Some amines can be naturally present in grapes and others can be formed during contamination of grapes prior and during processing, and can be markers of poor hygienic-sanitary conditions. Furthermore, at high concentrations, some amines can cause adverse effects to human health (EFSA 2011; Bach et al. 2012).

Today's consumers are very concerned health wise. The potential health benefits associated with grape juice have attracted attention of children, elders and athletes, resulting in a 570% increase in grape juice commercialization worldwide in the last 10 years. This trend is expected to keep increasing in Brazil, especially because grape juice consumption is still small compared to other countries (IBRAVIN 2015). However, to respond to this increased demand, the productive sector will have to implement and upgrade vineyards toward grape processing. Furthermore, the development of grape cultivars with better juice characteristics is needed.

Characteristics such as color, aroma and taste, as well as short and long term cultivars to increase harvest season, are claimed by the industry. The main grape cultivars used for juice processing in Brazil are Isabel, Concord and Bordo; however, they do not meet desirable criteria. The Grape Breeding Program of Embrapa Grape and Wine

launched a group of grape cultivars for juice processing which should be evaluated under different climate and soil conditions to corroborate their agronomical and industrial performance. Among developed cultivars, some of the beneficial characteristics are: 'Concord Clone 30' and 'Isabel Precocé' have short term cycle, whereas 'BRS Carmem' has long term cycle; and 'BRS Violeta' has high sugar content and stronger color (Ritschel and Camargo 2007; Camargo et al. 2008). It is likely that the use of blends will be beneficial and their characterization is needed to attend consumers' preference.

The objective of the current study was to characterize the production cycle, productivity, and the composition of grapes and juices of Concord clone 30, Isabel Precocé, Violeta and Carmem cultivated in the south of Minas Gerais, Brazil, in the 2013 and 2014 seasons, and to compare with the traditional Bordo cultivar. In addition, different blends were prepared, physico-chemically characterized and submitted to consumer acceptance.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out during the 2013 and 2014 seasons in a vineyard set up in 2010 (and 2011 for Concord clone 30) in Caldas, MG, Brazil (lat 21°55'S, long 46°23'W, 1100 m a.s.l.), at a clayish soil. Concord Clone 30 (clonal selection of cultivar Concord), BRS Violeta (BRS Rubra × IAC 1398-21), Isabel Precocé (spontaneous somatic mutation of cultivar Isabel), BRS Carmem (Muscat Belly A × BRS Rubra) and the cultivar Bordo were grafted onto 1103 Paulsen rootstock and trained in vertical shoot position with bilateral cordons and pruned in two-node spurs in the same day. Vines and rows were spaced at 1.50 m and 2.50 m, respectively. The experimental design was in blocks with 8 plants in lines and 9 rows for each cultivar in a horizontal sequence in the same land, totalizing an experimental area of 360 plants with 72 plants of each cultivar.

Random grapes (total of 100) were harvested at maturity for each of the eight replicates per cultivar. Each sample was hand pressed and the musts were analyzed for titratable acidity, soluble solids and pH (Amerine and Ough 1980). Another eight randomized samples of 50 berries were collected for each treatment. Skins were separated manually,

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weighed, crushed in liquid nitrogen and stored at -80°C for analyses of anthocyanins and total phenolics.

Juices were prepared by steam juice extractor cooker (Stamp Inox, Caxias do Sul, RS, Brazil). Grapes from each cultivar were hand stripped and 20 kg of berries were placed in the extraction chamber. Juice extraction was accomplished with water vapor during 150 min. The juice was pasteurized at 80°C for 20 min and bottled at 80°C with no addition of preservatives. Four replicates of juice extraction were prepared for each cultivar.

Juice blends from the 2014 season were prepared by the mixture of equal parts of two varietal juices, except for Carmem. Violeta and Bordo were not mixed together due to their high content of phenolics. Juices and blends were analyzed for titratable and volatile acidities, pH, soluble solids, total sugars, ashes, color intensity, anthocyanins, and polyphenols. The blends and pure juices were also analyzed for consumer's preference.

Methods of analysis

Crop characterization

Production cycles were measured by the time length between pruning and harvest. Average vine production was estimated in the 2014 season multiplying the number of bunches per plant by average bunch weight of plot. Yield was obtained multiplying the average vine production by the number of plants per hectare (4,000).

Determination of physico-chemical characteristics

Musts were analyzed for soluble solids (SS; $^{\circ}\text{Brix}$), titratable acidity (TA; g tartaric acid $\cdot 100\text{ mL}^{-1}$) and pH (Amerine and Ough 1980). The juices were analyzed for titratable acidity, volatile acidity (Vac; g acetic acid $\cdot 100\text{ mL}^{-1}$), pH, soluble solids ($^{\circ}\text{Brix}$), ratio (SS/TA), total sugars (g $\cdot\text{L}^{-1}$), ashes (g $\cdot\text{L}^{-1}$) (Amerine and Ough 1980); color intensity (420 nm + 520 nm + 620 nm) and total polyphenols were determined in a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto) according to Ribéreau-Gayon et al. (2006).

Determination of bioactive compounds

Anthocyanins and total phenolic compounds

Skins and juices were analyzed for anthocyanins and total phenolic compounds. A 150 mg sample of powdered

skin was extracted with acidified methanol (10 mL $\cdot\text{L}^{-1}$ HCl) in an Ultra Turrax disperser T-18 basic (IKA, Wilmington, NC, USA) and the extract was used for the analysis of anthocyanins and total phenolics. Total phenolic compounds were determined by the Folin-Ciocalteu method (Amerine and Ough, 1980). Grape skin extracts or grape juice samples were treated with 10% diluted Folin-Ciocalteu reagent followed by addition of 7.5% sodium carbonate. The mixture was kept at 50°C for 5 min and the absorbance determined at 765 nm on an UV-Vis Q-108U spectrophotometer (Quimis, São Paulo, Brazil). The results were expressed as mg gallic acid $\cdot 100\text{ g}^{-1}$ berry or g gallic acid $\cdot\text{L}^{-1}$ juice calculated by interpolation in a gallic acid calibration curve ($R^2 \geq 0.9998$).

The concentration of anthocyanins was determined by the pH differential method (Giusti and Wrolstad, 2001) using buffer solutions of potassium chloride (pH 1.0) and sodium acetate (pH 4.5). Absorbance was determined at 520 nm and 700 nm and the results were expressed as mg malvidin-3-glucoside $\cdot 100\text{ g}^{-1}$ berry or g malvidin-3-glucoside $\cdot\text{L}^{-1}$ juice, considering a molecular mass of 529 g $\cdot\text{mol}^{-1}$ and molar absorption coefficient of 28000 L $\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$.

Free bioactive amines

Free bioactive amines were determined by ion-pair reverse phase liquid chromatography (HPLC), followed by post-column derivatization with *o*-phthaldehyde (OPA) and fluorimetric detection at Laboratório de Bioquímica de Alimentos, Faculdade de Farmácia, UFMG, which is accredited under ISO 17025. Grape samples (10 randomly selected berries) were crushed and centrifuged. The corresponding must, or juice, was filtered through a cellulose hydrophilic membrane filter (HAWP, Millipore, Bedford, MA, USA) and used for analysis. Determination of ten amines followed the method described by Santiago-Silva et al. (2011). A LC-10 AD system connected to a RF-10AXL spectrofluorometric detector at 340 and 450 nm of excitation and emission (Shimadzu, Kyoto, Japan) was utilized. A Novapak C18 column, 300 \times 3.9 mm i.d., 10 μm , was used with a Novapak C18 guard-pak insert (Waters, Milford, MA, USA). The mobile phases were: A – solution of 0.2 mol $\cdot\text{L}^{-1}$ sodium acetate and 15 mmol $\cdot\text{L}^{-1}$ 1-octanesulfonic acid sodium salt adjusted to pH 4.9 with acetic acid, and B – 100% acetonitrile, at gradient elution (Table 1). Identification of amines was performed



by comparison of retention times in samples to those of standard solutions and also by addition of the suspect amine to the sample. Quantification was accomplished by direct interpolation in standard curves for each amine and the concentrations were expressed as $\text{mg} \cdot 100 \text{ g}^{-1}$ or $\text{mg} \cdot \text{L}^{-1}$.

Table 1. Gradient elution program.

Elution time (min)	A (%)	B (%)
0	100	0
0.01	95	5
21	80	20
22	95	5
25	95	5
40	76	24
45	76	24
50	65	35
51	95	5
60	100	0

Consumer preference test

Varietal juices and blends from the 2014 season were submitted to preference ordering tests by 32 untrained panelists (male and female consumers, aged 17 to 60 years old, average 34.4 years of age). The samples were codified with random three digit numbers and offered in small plastic cups along with a cup of water, cracker and an evaluation form. Because of the high number of combinations, samples were offered in three panels of six samples each, with 10 min interval between them. Consumers were asked to rank the samples according to their preference, from the most liked (score 1) to the least liked (score 6). Results for each sample were added and the most liked sample had the lowest value (Ferreira et al. 2000).

Table 2. Average vine production of cultivars Isabel Precoce, Concord, BRS Carmem, Bordo and BRS Violeta grown in Caldas, MG, Brazil, during the 2014 season.

Cultivar	Bunches (n°)	Bunch weight (g)	Vine production (kg)	Yield ($\text{ton} \cdot \text{ha}^{-1}$)
Carmem	27.440 b	148.635 a	4.104 ab	16.416 ab
Concord	10.440 c	64.584 d	0.671 c	2.683 c
Bordo	48.330 a	67.280 cd	3.262 b	13.046 b
Isabel Precoce	49.220 a	107.240 b	5.390 a	21.559 a
Violeta	37.000 ab	88.180 bc	3.196 b	12.784 b
CV	26.4	172	34.1	34.1

Mean values with different letters in the same row are significantly different (Tukey test, $p \leq 0.05$).

RESULTS AND DISCUSSION

Agronomical characteristics

Isabel Precoce was the most productive cultivar with yield of $21.6 \text{ ton} \cdot \text{ha}^{-1}$, followed by Carmem with $16.4 \text{ ton} \cdot \text{ha}^{-1}$. Bordo and Violeta showed similar yield, 13.0 and 12.8, respectively, while Concord was the least productive with only $2.7 \text{ ton} \cdot \text{ha}^{-1}$ (Table 2).

Carmem had the longest production cycle, 204 days from pruning to harvest, whereas Violeta and Bordo showed the shortest cycle, average of 150 days. Concord and Isabel Precoce had intermediate production cycles of 160 days in average. The harvest times were similar to those reported by Camargo et al. (2008) in Serra Gaúcha (RS), except for Bordo and Violeta, which were harvested about 10 days earlier in Caldas (MG). The longest production cycle for Carmem is mainly due to a higher flowering-veraison period. Phenological data shows that it is possible to adjust pruning date to harvest cultivars Violeta, Isabel Precoce, Bordo and Concord in the same day to make suitable blends in Caldas, MG.

Grape composition

Results from 2013 and 2014 seasons were grouped and the mean data of physico-chemical characteristics of the five different cultivars of Labrusca grapes are presented on Table 3. Cultivation practices and conditions were similar, except that in 2014 there was higher precipitation during harvesting. Soluble solids content varied from 16.9 to 18.7°Brix with higher values observed for Carmem and Concord and the lowest for Isabel Precoce and Bordo. Mean pH values ranged from 3.42 to 3.76, and Isabel Precoce, Bordo and Concord were the ones with lower pH, and Carmem with the highest. Titratable acidity ranged from

0.43 to 0.58 g·100 mL⁻¹ with the lowest values for Isabel Precoce and Bordo.

Soluble solids and titratable acidity varied from those reported for the same cultivars in Serra Gaucha, RS, Brasil (Camargo et al. 2008), and Petrolina, PE, Brasil (Lima et al. 2015). It is probable that climatic conditions prevalent at these different locations affected acidity and sugar content of the cultivars.

Anthocyanins contents ranged from 26.7 to 190.0 mg·100 g⁻¹. Violeta had the highest content followed by Carmem and Bordo, with intermediate contents. Low contents were found in Isabel Precoce and Concord.

Total phenolics content followed a similar pattern compared to anthocyanins and ranged from 110.7 mg·100 g⁻¹, in Isabel Precoce grapes, to 356.1 mg·100 g⁻¹ in Violeta. Carmem and Concord had similar intermediate contents.

Anthocyanins contents were similar to those reported for other cultivars grown in Minas Gerais, Brazil (Abe et al. 2007; Miotto et al. 2014), and also within values reported for red grapes, – 30 to 750 mg·100 g⁻¹ (Malacrida and Motta 2006), the variability being possibly associated with variety, season, environment and management practices (Jackson and Lombard 1993). Concord showed lower anthocyanin content than that reported by Malacrida and Motta (2006). Concord vineyard was set up in 2011 and showed lower development than the other cultivars. The lowest production reflects the smaller size of the plants, which may have contributed to impair phenolic biosynthesis (Borsani et al. 2010). Total phenolics values

were within literature results (Abe et al. 2007; Miotto et al. 2014).

No amines were detected in grapes from the current study, even though there are reports on the presence of bioactive amines in grapes with the predominance of putrescine, spermidine and spermine (Shiozaki et al. 2000; Bauza et al. 2007), and also the occurrence of histamine, cadaverine and 2-phenylethylamine in red and white grape native to Trentino, Italy (Nicolini et al. 2003).

Grape juice composition

The physico-chemical characteristics of juices prepared with grapes from different cultivars are indicated in Table 4. Soluble solids content ranged from 12.4 °Brix in Bordo juice to 15 °Brix in Violeta and Carmem juice. Titratable acidity ranged from 0.63 g·100 mL⁻¹ (Isabel Precoce and Carmem juices) up to 0.86 g·100 mL⁻¹ (Bordo). Likewise, the ratio SS/TA for the juices ranged from 14.4 to 23.5 with higher values observed in Carmen juice.

Among additional characteristics analyzed, volatile acidity varied from 0.01 to 0.03 g·100 mL⁻¹, the higher values observed for Carmem. Ashes ranged from 3.11 to 4.44 g·L⁻¹ with higher values observed for Carmem and Violeta.

Soluble solids and titratable acidity values were within literature reports (Burin et al. 2010; Toaldo et al. 2015; Dal Magro et al. 2016). The ratio SS/TA values were within limits of 14 to 45 established by Brazilian legislation for integral juice (Brasil 2004).

Table 3. Physicochemical characteristics (pH, soluble solids, titratable acidity) and bioactive components (anthocyanin, total phenolics and bioactive amines) content of five grape cultivars grown in Caldas, MG, Brazil, during the 2013 and 2014 seasons.

Variable	Grape cultivar (mean values ± standard deviation)					CV
	Isabel Precoce	BRS Carmem	Bordo	BRS Violeta	Concord clone 30	
Soluble solids (°Brix)	16.9 ± 0.39 d	18.7 ± 0.60 a	17.4 ± 0.41 c	18.1 ± 0.41 b	18.5 ± 0.27 ab	2.44
pH	3.4 ± 0.06 c	3.8 ± 0.11 a	3.4 ± 0.05 c	3.7 ± 0.04 b	3.4 ± 0.04 c	1.89
Titratable acidity (g tartaric acid·100·mL ⁻¹)	0.4 ± 0.02 b	0.6 ± 0.05 a	0.4 ± 0.07 b	0.6 ± 0.05 a	0.6 ± 0.07 a	10.16
Anthocyanin (mg malvidin-3-glucoside·100 g ⁻¹)	26.7 ± 7.04 e	94.2 ± 22.07 c	133.8 ± 22.17 b	190.0 ± 40.53 a	54.0 ± 7.92 d	22.71
Total phenolic (mg gallic acid·100 g ⁻¹)	110.7 ± 24.89 d	199.7 ± 46.70 c	239.5 ± 30.38 b	356.1 ± 78.79 a	188.3 ± 49.12 c	16.78
Total bioactive amines* (mg·100 g ⁻¹)	nd	Nd	nd	nd	nd	-

*Total bioactive amines (nd ≤ 0.4 mg·100 g⁻¹ of each of ten amines). Mean values with different letters in the same line are significantly different (Tukey test, p ≤ 0.05).

Table 4. Physicochemical characteristics and bioactive components of juices from five grape cultivars grown in Caldas, MG, Brazil, during the 2013 and 2014 seasons.

Physicochemical characteristics						
Variable	Grape cultivar (mean values \pm standard deviation)					CV
	Isabel Precoce	BRS Carmem	Bordo	BRS Violeta	Concord clone 30	
Soluble solids ($^{\circ}$ Brix)	13.2 \pm 0.35 b	14.8 \pm 0.80 a	12.4 \pm 1.76 b	15.0 \pm 0.63 a	13.6 \pm 1.12 ab	7.39
Total sugars (g·L ⁻¹)	118.1 \pm 2.02 ab	127.6 \pm 5.03 a	98.5 \pm 13.17 c	125.4 \pm 4.92 a	112.8 \pm 9.17 b	6.77
pH	3.4 \pm 0.05 c	3.9 \pm 0.03 a	3.4 \pm 0.04 d	3.7 \pm 0.01 b	3.5 \pm 0.02 c	0.91
Titrateable acidity - TA (g tartaric acid·100 mL ⁻¹)	0.7 \pm 0.04 cd	0.6 \pm 0.06 d	0.9 \pm 0.10 a	0.7 \pm 0.02 bc	0.8 \pm 0.13 ab	8.87
Ratio TSS/TA	19.8 \pm 1.03 bc	23.8 \pm 3.44 a	14.4 \pm 0.52 d	20.3 \pm 0.69 b	17.7 \pm 1.84 c	8.88
Volatile acidity (g acetic acid·100 mL ⁻¹)	0.01 \pm 0.000 b	0.03 \pm 0.011 a	0.01 \pm 0.003 b	0.01 \pm 0.003 b	0.01 \pm 0.002 b	35.85
Ash (g·L ⁻¹)	3.1 \pm 0.25 b	4.4 \pm 0.35 a	3.5 \pm 1.08 b	4.2 \pm 0.33 a	3.5 \pm 0.46 b	9.60
Color intensity*	9.6 \pm 1.29 d	28.0 \pm 4.43 c	40.8 \pm 8.68 b	70.1 \pm 2.49 a	28.7 \pm 2.71 c	13.46
Bioactive components						
Variable	Grape cultivar (mean values \pm standard deviation)					CV
	Isabel Precoce	BRS Carmem	Bordo	BRS Violeta	Concord clone 30	
Anthocyanin (g malvidin-3-glucoside·L ⁻¹)	0.1 \pm 0.05 e	1.2 \pm 0.14 c	1.4 \pm 0.19 b	2.7 \pm 0.25 a	0.5 \pm 0.04 d	13.09
Total phenolic (g gallic acid·L ⁻¹)	1.3 \pm 0.18 c	2.5 \pm 0.17 b	2.9 \pm 0.56 b	6.3 \pm 0.41 a	2.7 \pm 0.83 b	12.91
Total polyphenols (l 280 nm)	37.1 \pm 2.45 c	87.1 \pm 8.80 b	95.9 \pm 12.31 b	185.8 \pm 18.06 a	81.7 \pm 24.45 b	13.80

*Absorbance at (420 nm + 520 nm + 620 nm). Mean values with different letters in the same line are significantly different (Tukey test, $p \leq 0.05$).

When comparing results obtained with the respective grapes, which originated the juices, lower soluble solids and higher titrateable acidity were observed in the juices, which can be explained by the method applied for juice extraction. The use of high temperatures can cause plasmolysis of the membrane and rupture on the fruit cell wall, facilitating the liberation of water (Lima et al. 2015). However, it also facilitates extraction of acids from skin and seeds which contributes to higher titrateable acidity (Ribéreau-Gayon et al. 2006).

During cultivation, the high vigor of Carmem vine provided reduced sun exposure of bunches and increased incidence of berries rot. This may have contributed to the increased juice volatile acidity, which was above the limit of 0.025 g·100 mL⁻¹ established by Brazilian legislation (Brasil 2004). The observed value, however, is similar to values (0.03 g·100 mL⁻¹) reported by Rizzon and Miele (2012).

The higher ashes values observed in Carmem and Violeta juices may have contributed to the observed higher pH values in these two cultivars. Studies involving scion and rootstocks show strong positive correlations between grape juice potassium and pH (Walker and Clingeleffer

2009). pH and ashes values found in Bordo, Concord and Isabel Precoce juices are in accordance with mean pH values of 3.31 and ashes of 3.24 g·L⁻¹ observed by Rizzon and Miele (2012) in commercial whole grape juices.

Violeta juice showed the highest anthocyanins (2.68 g·L⁻¹), phenolic content (6.33 g·L⁻¹) and total polyphenols index (185.92). Color intensity of prepared juices ranged from 9.6 in Isabel Precoce to 70.1 in Violeta.

Red grapes are natural sources of phenolic compounds with antioxidant capacity, among them anthocyanins, which can be beneficial to human diet (Abe et al. 2007). Phenolic composition of Violeta cultivar was higher than the values described in the literature for both anthocyanins and phenolics for cultivars such as Isabel Precoce, BRS Cora, Bordo, Isabel or Concord (Lima et al. 2015; Toaldo et al. 2015; Dal Magro et al. 2016).

When compared to the results observed for the respective berries, there was an increase in total phenolic content in the juice, especially for Violeta and Concord. This is probably due to extraction of phenolic compounds from seeds. Seed tannins, however, can provide astringency to the juice (Ribéreau-Gayon et al. 2006). Furthermore,

the use of heat during extraction can favor phenolics extraction (Lima et al. 2015), which are heat resistant (Teófilo et al. 2011).

Color is the most important attribute used by consumers to select grape juice and it is directly dependent on the phenolic composition (Abe et al. 2007). Except for Isabel Precoce, the varietal juices from Caldas, MG, Brazil, showed higher anthocyanin, total polyphenol index and color intensity compared to those reported by Burin et al. (2010) or Rizzon and Miele (2012).

Only four amines were detected in the juices, including putrescine, spermidine, phenylethylamine and tryptamine. Spermidine and putrescine were found in juices from both seasons, however higher values of both amines were observed in 2013 season. Putrescine ranged from 0.48 mg·L⁻¹ in Bordo juice to 2.38 mg·L⁻¹ in Violeta juice in 2013 season, but was detected only in Violeta juice in 2014 season at 0.57 mg·L⁻¹. In 2013 season, spermidine content ranged from 1.01 mg·L⁻¹ in Bordo juice to 2.03 mg·L⁻¹ in Violeta juice. In 2014, however, Violeta juice showed the lowest content, 0.36 mg·L⁻¹, while Bordo juice had the highest level, 0.61 mg·L⁻¹. Phenylethylamine and tryptamine were only found in juices from the 2013 and the 2014 seasons, respectively. Phenylethylamine was only detected in Bordo juice at 0.31 mg·L⁻¹ and tryptamine was detected in Violeta (0.46 mg·L⁻¹), Isabel Precoce (2.77 mg·L⁻¹) and Bordo (2.95 mg·L⁻¹) (Figure 1). Different from results for

physico-chemical traits, the season did affect the profile and contents of amines in the grape juices, reason why results were presented separately for each season.

Amines can be present in plants freely or conjugated to other components such as phenolic acids. Conjugated amines also play roles in plant defense response to pathogens and abiotic stresses (Kang et al. 2009).

Even though amines were not detected in the grapes, they were found in the grape juices, suggesting that amines were present in the grape in conjugated forms or that they were formed during processing, probably by thermal or enzymatic decarboxylation of free amino acids (Gloria 2005). It is also likely that amines from seeds and peel (Bauza et al. 2007) could have been released into the juice during processing (Gloria 2005). This is consistent with reports in the literature for different fruits (Santiago-Silva et al. 2011).

The occurrence of the polyamine spermidine and its precursor putrescine in fruits and vegetables is associated with many developmental processes such as cell division, root formation, embryogenesis, floral initiation, leaf senescence, fruit development and ripening. Polyamines are also important in membrane stabilization by acting as free radical scavengers and inhibiting phospholipid oxidation. Moreover, polyamines may delay ripening and are also involved in the control and modulation of stress tolerance in plants (Fariduddin et al. 2013; Kalac 2014; Pathak et al.

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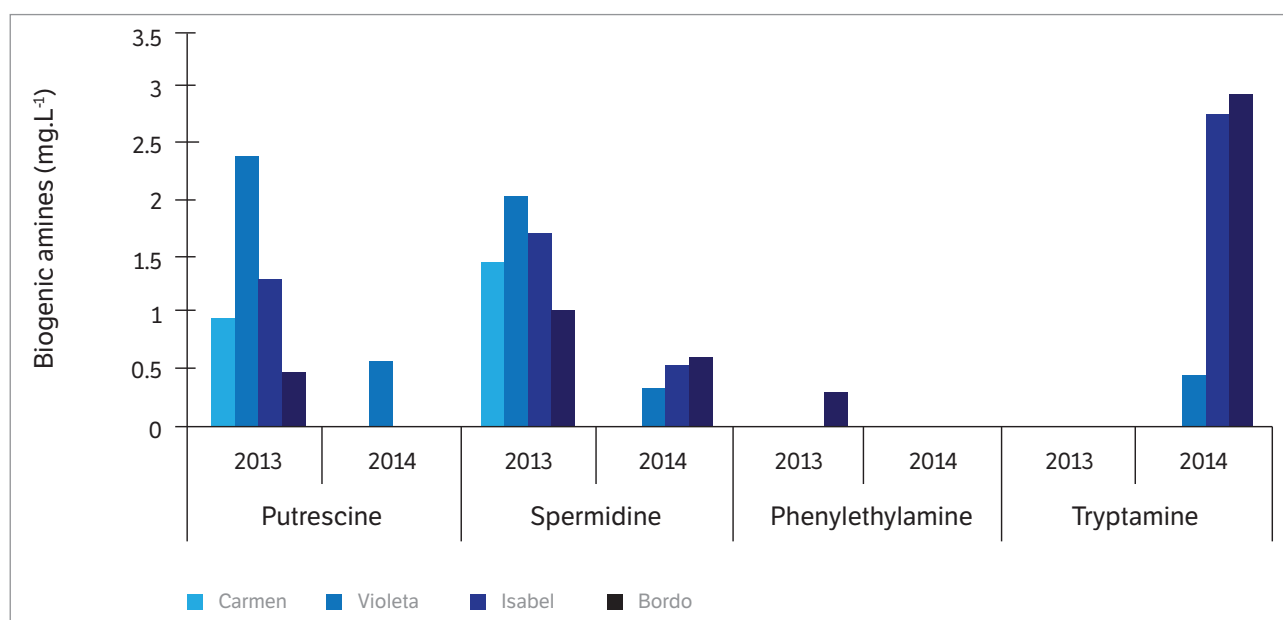


Figure 1. Biogenic amines of grape juices obtained from the cultivars Isabel Precoce, BRS Carmem, Bordo and BRS Violeta grown in Caldas, MG, Brazil, during the 2013 and 2014 seasons.

2014). Biogenic amines can also be present in plants – some are inherent and others synthesized as response to stress, as protection factors (Gloria 2005). Tryptamine is precursor of the growth hormone indole acetic acid, radical scavenger, antioxidant and radioprotective agent, may act as phytoalexins and activate immune responses (Badria 2002). Phenylethylamine is formed through the action of decarboxylase enzymes produced by microorganisms from phenylalanine. The amino acid concentration depends on grape variety, nitrogenous fertilization, grape maturity and climatic conditions (Ancín-Azpilicueta et al. 2008).

Similar contents of putrescine and spermidine were detected in Isabel Precoco juices from Petrolina, PE, Brazil (Nassur et al. 2014). The highest contents of putrescine and spermidine were found in Violeta juice, and the lowest in Bordo, and during the 2013 season. This higher polyamines and putrescine contents could be associated with inherent cultivar resistance to stress or abiotic conditions which required improved resistance (Fariduddin et al. 2013; Pathak et al. 2014).

It is interesting to observe that tryptamine and phenylethylamine were only detected in juices from the 2013 and 2014 seasons, respectively. Since these amines are formed from the respective amino acids tryptophan and phenylalanine, which are produced via shikimic acid pathway (Gloria 2005), it is likely that an abiotic condition might have directed the pathway towards one amine or the other. This deserves further investigation.

The presence of either amine – tryptamine or phenylethylamine – in grape juice is desirable as these amines can be associated with some health benefits (Badria 2002; Gloria 2005). However, at high concentration ($\geq 3 \text{ mg} \cdot 100 \text{ g}^{-1}$), phenylethylamine can induce headache in susceptible individuals. The biogenic amines – histamine and tyramine – which are known to cause adverse effects to human health, were not found in the juices. Amine quantification in the grape juices from *Vitis labrusca* cultivars grown in the south of Minas Gerais, Brazil, showed that at the concentration found, there is no risk for human health (EFSA 2011).

Juice blends

The juices obtained in the 2014 season were used to prepare blends, which would have the advantage of enhancing juice quality. The physicochemical characteristics, phenolic content and color of the blends were compared to individual juices as indicated in Table 5.

The mixture with Isabel Precoco contributed to reduce volatile and total acidity of the Violeta, Concord and Bordo juices. Isabel Precoco showed low concentration of phenolic compounds, but when mixed with Violeta, Concord or Bordo, the phenolic composition of the blend reached acceptable values. Lima et al. (2014) also observed increase in color and phenolic content of Isabel Precoco juice when blended with Violeta juice.

The cut with Isabel Precoco decreased anthocyanin and total phenolic content of Violeta juice to values close to Carmem and Bordo juices, respectively. The lowest anthocyanin content and color index were observed with the blend Concord and Isabel Precoco. The blend Violeta and Concord had the highest phenolic composition and color intensity with values close to those observed in Violeta juice.

Consumer preference test

Results from the consumer preference test (Figure 2) indicated that Carmem by itself ranked as the most liked juice. It can also be observed that the other juices were not well appreciated by consumers, indicating that they will be better used by means of blends. In fact, the next most accepted blends were CON \times ISA and VIO \times ISA. Although the blend CON \times ISA showed the same ratio and lower color intensity compared to VIO \times CON, the later showed lower acceptance probably due to higher concentration of total phenolics ($4.26 \text{ g gallic acid} \cdot \text{L}^{-1}$).

The preference for Carmem varietal juice confirms its appropriateness for juice production. Bordo juice had low sugar and high acidity content which may have impaired its acceptance, while Isabel Precoco juice lacked color. In general blends were preferred over varietal juices. Combinations of Violeta \times Concord and Bordo \times Concord were better accepted than the varietal juices, but less preferred than the combinations with Isabel Precoco. The cut with Isabel Precoco decreased total phenolic content of Violeta juice, which may have contributed to the increase in consumer acceptance of the blend over the varietal juice. The blend Bordo \times Isabel Precoco was the least preferred combination tested with Isabel Precoco. Besides acidity decrease, there was no increase in sugars, which may have contributed to reduce consumer's preference for this blend.

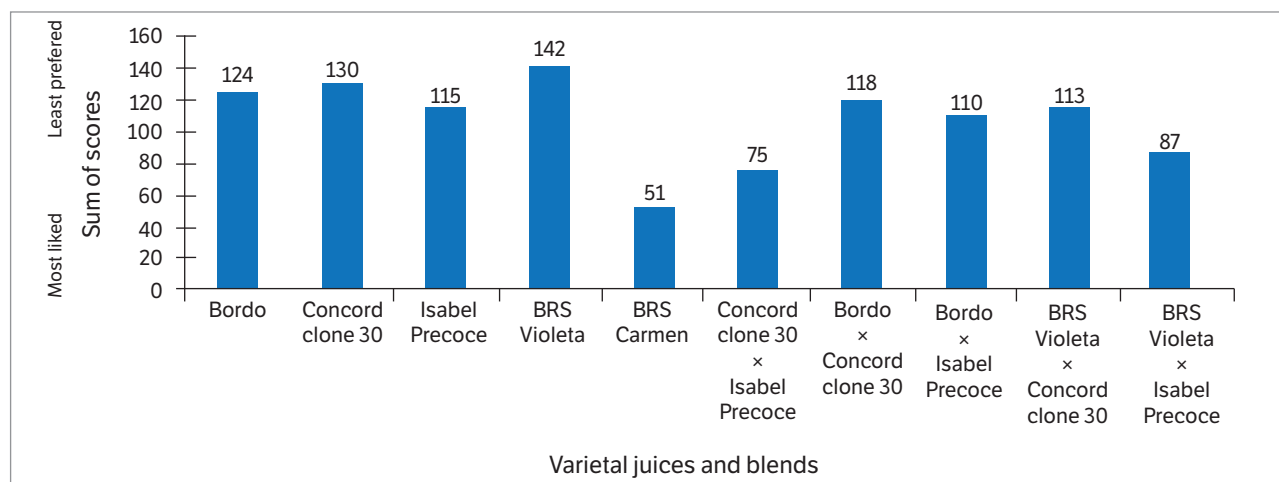
The balance between sugar, acidity and phenolic content may play an important role in consumer's acceptance of the juice. Indeed, acidity is responsible for the freshness flavor

Table 5. Physicochemical characteristics and composition of juices from different *Vitis labrusca* cultivars and their blends. Caldas, 2014 season.

Parameters	Mean values (juice or blend)					CV
	Violeta	Isabel	Concord	Bordo	Carmem	
Volatile acidity (g acetic acid·100 mL ⁻¹)	0.012 b	0.004 h	0.008 cd	0.006 fg	0.031 a	4.94
Titrateable acidity (TA - g tartaric acid·100mL ⁻¹)	0.76 f	0.69 h	0.91 c	0.98 a	0.73 g	0.93
pH	3.63 b	3.39 e	3.34 f	3.35 f	3.71 a	0.21
Soluble Solids (°Brix)	15.02 a	13.19 j	14.67 d	13.76 h	14.74 c	0.05
Ratio (TSS/TA)	19.69 b	19.12 c	16.03 e	14.04 g	20.19 a	0.94
Total sugars (g·L ⁻¹)	123.80 a	118.80 b	117.08 b	109.58 f	123.37 a	0.55
Anthocyanins (g malvidin-3-glucoside·L ⁻¹)	1.72 a	0.07 g	0.23 f	0.98 b	0.86 cd	4.33
Total phenolic (g gallic acid·L ⁻¹)	5.48 a	1.23 i	3.15 d	3.03 e	2.42 f	1.17
Total polyphenols (l 280nm)	163.53 a	38.63 f	92.33 d	105.20 c	89.60 d	3.89
Color intensity†	58.30 a	9.20 h	25.78 e	38.97 c	25.19 e	1.02
Ash (g·L ⁻¹)	4.35 ab	3.29 f	3.83 bcde	3.75 cdef	4.80 a	4.67

Parameters	Mean values (juice or blend)					CV
	VIO × ISA*	VIO × CON	BOR × ISA	BOR × CON	CON × ISA	
Volatile acidity (g acetic acid·100 mL ⁻¹)	0.007 de	0.009 c	0.005 gh	0.006 fg	0.006 ef	4.94
Titrateable acidity (TA - g tartaric acid·100mL ⁻¹)	0.74 g	0.86 d	0.82 e	0.94 b	0.81 e	0.93
pH	3.53 c	3.48 d	3.35 f	3.35 f	3.36 f	0.21
Soluble Solids (°Brix)	14.09 f	14.77 b	13.47 i	14.25 e	13.96 g	0.05
Ratio (TSS/TA)	19.04 c	17.08 d	16.43 e	15.16 f	17.13 d	0.94
Total sugars (g·L ⁻¹)	112.00 de	114.20 c	110.43 ef	114.20 c	113.75 cd	0.55
Anthocyanins (g malvidin-3-glucoside·L ⁻¹)	0.81 d	0.92 bc	0.54 e	0.61 e	0.15 fg	4.33
Total phenolic (g gallic acid·L ⁻¹)	3.37 c	4.26 b	2.16 h	3.04 e	2.30 g	1.17
Total polyphenols (l 280nm)	96.37 cd	130.27 b	70.53 e	98.20 cd	63.13 e	3.89
Color intensity**	31.57 d	41.56 b	22.13 f	31.49 d	17.86 g	1.02
Ash (g·L ⁻¹)	3.65 def	4.26 bc	3.32 ef	3.87bcd	3.73 cdef	4.67

*BOR – Bordo; CON – Concord (clone 30); ISA – Isabel (Precoco); VIO – (BRS) Violeta. **Absorbance at (420 nm + 520 nm + 620 nm). Mean values with different letters in the same line are significantly different (Tukey test, $p \leq 0.05$).

**Figure 2.** Consumer preference of varietal or blend grape juices obtained from cultivars Bordo, BRS Carmem, Isabel Precoco, BRS Violeta and Concord clone 30 grown in Caldas, MG, Brazil during the 2014 season.

of the juice, therefore there must be a balance of sweetness to acidity in the range of 15 to 45.50 (Brasil 2004). High phenolic content can be responsible for astringency and bitterness of the juice (Ribéreau-Gayon et al. 2006), but there is no legislation about phenolic compounds limits in juices.

Blends are a good option for the industry because they are preferred by consumers over varietal juices. Isabel Precoco, with low phenolic content but high productivity, is a good option to be used in blends with Bordo, Violeta or Concord. On the other hand, Carmem juice showed high ratio and intermediate total phenolics and anthocyanins content. These characteristics were appreciated by the consumers. Due to its longer production cycle and high productivity, Carmem can be an option to increase harvest period.

Correlation among variables

Sensorial characters such as ratio, total phenolics and color intensity were submitted to Pearson correlation test with the physico-chemical characteristics of the juices. Titratable acidity showed strong negative correlation with ratio, while pH and total sugars were positively correlated. Ratio corresponds to the division of soluble solids with acidity. High acidity levels decrease pH and also the ratio. It is related to unripe grapes but also to heat extraction during processing.

Soluble solids content had strong positive correlation with total phenolics and color intensity. These characters also showed strong correlation with anthocyanins and total polyphenols. Phenolic maturation, the biosynthesis and accumulation of anthocyanins and phenolic compounds in grape skins, starts at veraison concomitant with the increasement in soluble solids and reduction in acidity levels, mainly malic acid (Ribéreau-Gayon et al. 2006). The extractability of anthocyanins and phenolics compounds from grape skins increases throughout ripening as consequence

of berry softening due to the degradation of the cellular wall. Therefore, not only soluble solid content is relevant to anthocyanin content but also the physiological stage (Hernández-Hierro et al. 2012).

CONCLUSION

Among Labrusca grapes investigated, Isabel Precoco provided the highest productivity among cultivars and is a good option to be used as blend with Bordo, Violeta or Concord. Carmem showed high productivity and the longest harvest season. It also showed high soluble solids and anthocyanins, which are desirable for grape juice processing.

Heat processing of grapes to produce juice increased titratable acidity, especially in Bordo. It also increased total phenolics content in juices of Violeta and Concord clone 30.

No amine was detected in the grapes, however spermidine was present in every juice from both seasons. The season affected the occurrence of putrescine in some cultivars. Phenylethylamine and tryptamine occurred sporadically, the first in 2013 and the second in 2014.

Juice's total phenolics and color have strong positive correlation with soluble solids, anthocyanins and total polyphenols.

Carmem is the most liked juice, followed by the blend Concord × Isabel Precoco. Violeta and Concord juices were the least preferred.

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