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ORIGINAL PAPER

Biogenic Amines and the Antioxidant Capacity of Juice and Wine from Brazilian Hybrid Grapevines

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Abstract
Some biogenic amines (BAs) are used as quality markers in grape-derived products. The prolife of 9 BAs was determined in juices and wines elaborated from hybrid grapes. Low levels of histamine, tyramine and cadaverine were found. Juices elaborated from 'BRS Rúbea' showed the highest tyramine levels (1.56 mg/L), while no histamine was found in wines elaborated from 'Seleção 34' and its higher content was detected in 'BRS Carmem' (3.55 mg/L). Juices elaborated from 'BRS Violeta' showed elevated content (472.88 mg/L) of total phenolic compounds (TPC) and mono-hydrated serotonin (6.20 mg/L), and wines elaborated from 'Violeta' presented a high serotonin mono-hydrate content (23.63 mg/L) and high antioxidant activity with FRAP test (77.24 mmol $FeSO_4$/L). Juices elaborated from hybrid grapes 'BRS Violeta' and wines from 'BRS Violeta', 'Seleção 34' and 'Seleção 13' had high levels of bioactive compounds, emphasizing the great potential of these cultivars for winemaking.

Keywords Histamine · Tyramine · Dopamine · Serotonin; antioxidant activity

Introduction

The wines and juices elaborated from American and hybrid grapes present characteristic flavors and are highly appreciated by some consumers, surpassing the production of products from European grapes [1, 2]. Consumption of beverages from hybrid grapes has been correlated to the increase of longevity in some Brazilian regions [3]. However, there are few reports of the juices and wines elaborated from hybrid grapes that demonstrate the functional quality [2].

Bioactive compounds, as biogenic amines (BAs) and phenolic compounds, are being investigated due to their influence on quality, security and nutraceutical characteristics of foods [1, 4, 5], as well as, antioxidant properties. Some studies indicate, for example, that wines elaborated from 'Isabel' and 'BRS Violeta' hybrid grapes present good functional properties that are attributed to antioxidant activity and higher content of bioactive compounds [2, 3]. Putrescine (put), spermidine (spd) and spermine (spm) may exert antioxidant protection by reducing lipoxygenase activity and consequently preventing the free radicals overproduction and inhibiting transbilayer movement of phospholipids [6]. Despite BAs being essential for many physiological functions in humans, high concentrations of some specific amines can result in collateral effects [7]. The histamine (his) content is frequently associated to impaired sensorial quality of wine, as well as, to induce toxic symptoms in humans, including pseudo-allergies, headache and others [8]. In addition to his, tyramine (tyr) is common in wine and the content of both BAs are related to climate and agricultural techniques [4]. Several BAs are consumed in a typical meal (meat, cereals, vegetables, processed

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foods, wine, juices, etc.) and the total amount of these compounds should not be considered individually. In addition, amines as put, tyr, agmatine and cadaverine (cad) may interact with intestinal amine oxidases and impair the human histamine detoxification system [9].

In Brazil, the cultivation of hybrid grapes, genetically adapted to grown in climatic conditions different from traditional cultivars, has been intensified and new varieties are being developed for the elaboration of juices and wines [1–3]. Hybrid grapes skins have more pectin content than *Vitis vinifera* grapes and the pectin fermentation results in methanol production, which can be toxic for consumers. However, methanol concentrations, in juice or wine, never reaches dangerous levels (LD50 = 350 mg/kg) [10]. Besides the health problems, the monitoring of the amine levels in grape juices and wines can be an important marketing advantage, and allow establishing BA profiles for safety and quality control in these grape beverages. Therefore, the aim of this study was to identify and quantify the levels of BAs and TPC, as well as the antioxidant activity, in juices and red wines elaborated from hybrid grapes developed in Brazil.

Material and Methods

The chemicals are presented as Supplementary Material.

Samples

Grapes were harvested and cooled for 24 h before submission to beverage's production at Embrapa Uva e Vinho (Bento Gonçalves, Rio Grande do Sul, Brazil) (29° 10′ 17" S latitude, 51° 31' 09" W longitude and 691 m altitude) in the 2015/16 crop. The genetic characteristics of the grapes used are described in Table 1 (Supplementary Material). The juices were elaborated from hybrid grapes – 'Bordô', 'Isabel', 'BRS Concord clone 30' ('BRS CC30'), 'BRS Rúbea', 'BRS Cora', 'BRS Carmem', 'BRS Violeta' and 'BRS Magna', using an in-line system [1]. For the wine elaboration we used 'Isabel', 'BRS CC3', 'BRS Rúbea', 'BRS Cora', 'BRS Carmem', 'BRS Violeta', 'BRS Magna', 'Seleção 13′ (Sel13) and 'Seleção 34′ (Sel34). After 90 days packaged at 18 °C, the biochemical analyses were performed in triplicate.

Total Phenolic Compounds (TPC)

The TPC of juices and wines was determined using the Folin-Ciocalteu reagent [11]. It was used a calibration curve with gallic acid (1.54 up to 38.46 mg/L) and the results were expressed in mg gallic acid equivalent *per* liter (mg GAE/L) of grape juice or wine.

Biogenic Amines Profile (BAs)

The BAs were extracted ($n = 3$) and isolated according to the procedure described by Lima et al. [12] and were then analyzed by UHPLC [13]. The identification of amines was performed by comparing the retention time and the UV-spectrum with different commercial standards. The quantification was performed through a calibration curve of the respective commercial standards. The results are expressed in mg/L.

DPPH Assay

The free radical scavenging activity of the wines and grape juices was evaluated using the stable radical (2,2- diphenyl-1-picrylhydrazyl) according to Brand-Williams et al. [14].

FRAP Assay

The antioxidant capacity via FRAP was determined according to Benzie and Strain [15]. FRAP values were obtained at 595 nm after 120 min of reaction comparing the results with a calibration curve. The results were expressed as mmol of reduced iron (Fe^{2+}) *per* liter of sample.

Statistical Analysis

The average and the standard deviation for each sample were calculated. The variance analysis (ANOVA) and multiple comparison test (Tukey test - $p < 0.05$) were performed using STATGRAPHICS Centurion ($n = 3$). The principal component analysis was performed using the XLSTAT software – version 2017 (Addinsoft, France).

Results and Discussion

BAs, TPC and Antioxidant Activity in Hybrid Grape Juice

In the eight analyzed grape juices, several BAs were identified (Table 1), with exception of the spd in juices elaborated from 'BRS Cora', 'BRS Carmem' and 'BRS Violeta'. There was wide variation in the total amine profiles, with values ranging from 3.92 ('BRS Magna') to 26.73 mg/L ('BRS Violeta'). Both spm and put were the majority amines. In contrast, the less-common BAs were his and cad, which are undesirable molecules in foods due to their toxic effects [13, 16].

Aiming to establish a descriptive grouping for the juices that indicate the functions of the analyzed biochemical attributes, we opted for comparing the obtained results using a principal component analysis (PCA). The dispersion of the varieties according to the PC1 and PC2 axis are shown in Fig. 1aj and 1bj. PC1 and PC2 explained 67.55% of the data variance (Fig. 1aj

Table 1 Composition of BAs (mg/L), TPC (mg GAE/L), FRAP (mmol $FeSO_4$/L) and DPPH (%) in hybrid grape juices and wines

BA	'Bordô'	'Isabel'	'BRS CC30'*	'BRS Rúbea'	'BRS Cora'	'BRS Carmem'	'RS Violeta'	'BRS Magna'	'Sel13'*	'Sel34'*
Juices										
put	2.44 ± 0.41a	0.43 ± 0.13b	2.04 ± 0.43ab	1.71 ± 0.76ab	3.31 ± 1.11a	1.67 ± 0.60ab	3.36 ± 0.82a	0.48 ± 0.05b	–	–
cad	0.12 ± 0.03cd	0.24 ± 0.03bc	0.54 ± 0.18ab	0.31 ± 0.17ab	0.49 ± 0.13ab	0.05 ± 0.05d	0.64 ± 0.21a	0.36 ± 0.15ab	–	–
hist	0.57 ± 0.14ab	0.44 ± 0.03ab	0.11 ± 0.02d	0.27 ± 0.14bc	0.11 ± 0.01cd	0.62 ± 0.16a	0.37 ± 0.19ab	0.17 ± 0.07cd	–	–
tir	1.27 ± 0.04a	0.16 ± 0.03d	0.23 ± 0.12d	1.56 ± 0.28a	0.91 ± 0.07bc	1.24 ± 0.08ab	0.81 ± 0.04c	0.14 ± 0.05d	–	–
spd	0.77 ± 0.10ab	1.03 ± 0.18ab	1.13 ± 0.92a	0.53 ± 0.36ab	ND	ND	ND	1.07 ± 0.30a	–	–
dop	0.66 ± 0.02a	0.22 ± 0.07cd	0.29 ± 0.19bc	0.45 ± 0.16ab	0.12 ± 0.01d	0.52 ± 0.08ab	0.35 ± 0.03bc	0.22 ± 0.07cd	–	–
spm	8.58 ± 0.22b	2.69 ± 0.04cd	0.92 ± 0.74d	5.29 ± 2.16c	13.01 ± 0.73a	0.85 ± 0.75d	14.38 ± 1.17a	0.72 ± 0.32d	–	–
serm	0.29 ± 0.01de	0.06 ± 0.02e	0.30 ± 0.00de	2.18 ± 0.16c	5.26 ± 0.38b	0.63 ± 0.08d	6.20 ± 0.06a	0.39 ± 0.03d	–	–
serh	0.53 ± 0.01d	0.13 ± 0.04f	5.49 ± 0.00a	0.21 ± 0.01g	1.01 ± 0.06b	0.40 ± 0.03e	0.62 ± 0.03c	0.37 ± 0.01e	–	–
Total BAs	15.23	5.40	11.05	12.51	24.22	5.98	26.73	3.92	–	–
TPC	281.19 ± 17.44b	145.14 ± 1.79d	152.60 ± 4.98d	141.57 ± 18.62d	166.36 ± 0.41d	232.08 ± 11.03c	472.88 ± 6.80a	277.63 ± 8.43b	–	–
FRAP	35.22 ± 3.50bc	12.31 ± 1.79d	27.10 ± 2.71c	28.96 ± 4.69c	38.22 ± 1.43b	18.53 ± 3.82d	47.27 ± 2.08a	34.96 ± 1.55bc	–	–
DPPH	43.04 ± 8.73a	13.87 ± 0.27c	18.99 ± 4.65bc	25.24 ± 5.91bc	21.73 ± 9.76bc	19.40 ± 3.15bc	34.52 ± 3.18ab	26.31 ± 6.22abc	–	–
Wines										
put	3.55 ± 0.15g	9.86 ± 1.06ef	13.59 ± 0.00e	3.75 ± 1.61g	37.65 ± 2.43b	22.10 ± 2.15d	4.26 ± 0.21g	9.29 ± 1.45f	32.22 ± 0.45c	44.00 ± 0.48a
cad	0.05 ± 0.01b	0.47 ± 0.11b	0.34 ± 0.00b	0.14 ± 0.05b	0.02 ± 0.03b	0.26 ± 0.04b	0.05 ± 0.08b	2.20 ± 0.30a	2.43 ± 0.09a	2.54 ± 0.55a
hist	0.33 ± 0.00c	0.02 ± 0.01c	0.05 ± 0.01c	0.49 ± 0.35c	0.52 ± 0.30c	3.55 ± 0.32a	0.63 ± 0.55c	1.48 ± 0.33b	0.53 ± 0.16c	ND
tir	0.64 ± 0.03bc	0.19 ± 0.13cd	1.15 ± 0.00ab	0.87 ± 0.10bc	0.46 ± 0.05bc	1.80 ± 0.24a	0.63 ± 0.55bcd	0.07 ± 0.02d	0.97 ± 0.14b	0.44 ± 0.39bc
spd	1.20 ± 0.09c	2.12 ± 0.02c	3.52 ± 0.09b	0.34 ± 0.59d	ND	7.74 ± 0.55a	1.50 ± 0.22c	0.44 ± 0.23d	ND	ND
dop	0.10 ± 0.00c	1.25 ± 0.07c	0.89 ± 0.00c	0.14 ± 0.05c	ND	11.04 ± 1.50a	5.17 ± 3.39b	0.57 ± 0.13c	3.19 ± 0.12bc	0.03 ± 0.00c
spm	1.12 ± 0.10d	9.16 ± 0.88c	0.66 ± 0.00d	21.68 ± 0.82b	20.58 ± 2.74b	22.47 ± 2.69b	21.15 ± 0.91b	0.16 ± 0.15d	1.32 ± 0.31d	34.08 ± 1.70a
serm	0.20 ± 0.02fg	0.02 ± 0.00g	0.52 ± 0.00fg	9.31 ± 0.17c	8.87 ± 0.47cd	8.67 ± 0.14d	23.63 ± 0.10a	0.68 ± 0.01f	4.71 ± 0.12e	22.51 ± 0.30b
serh	1.64 ± 0.06d	0.03 ± 0.00g	0.25 ± 0.03g	0.20 ± 0.01g	2.21 ± 0.33c	1.12 ± 0.01e	3.04 ± 0.05b	0.26 ± 0.01g	3.40 ± 0.12a	0.64 ± 0.04f
Total BAs	8.83	23.12	20.97	36.92	70.31	78.75	60.06	15.15	48.77	104.24
TPC	421.64 ± 12.96d	150.89 ± 9.81h	195.97 ± 25.00g	271.89 ± 3.56f	181.73 ± 8.96g	344.84 ± 27.68e	827.05 ± 3.56a	296.80 ± 26.87e	519.81 ± 8.96c	570.82 ± 10.68b
FRAP	70.48 ± 0.77b	14.46 ± 1.30e	25.24 ± 6.96de	34.31 ± 6.26d	25.05 ± 1.83de	28.39 ± 2.86d	77.24 ± 6.56a	29.03 ± 1.32d	42.50 ± 1.80c	50.43 ± 1.15c
DPPH	64.44 ± 1.34bc	45.00 ± 0.32d	46.15 ± 1.27cd	21.12 ± 1.89e	51.14 ± 4.19cd	24.76 ± 1.42e	66.34 ± 5.31ab	57.35 ± 5.96bc	71.42 ± 4.95a	48.26 ± 9.99cd

Total BAs: total biogenic amines, put: putrescine, cad: cadaverine, his: histamine, tir: tyramine, spd: spermidine, dop: dopamine, spm: spermine, serm: mono-hydrate serotonin, serh: serotonin hydrochloride. Values represent the means of three repetitions ± standard deviation. Different letters in the same line indicate a significant difference according to Tukey test ($p < 0.05$). (*) BRS Concord clone 30, Sel13 – Seleção 13, Sel34 – Seleção 34. TPC: total phenolic compounds. ND: not detected. – not analyzed

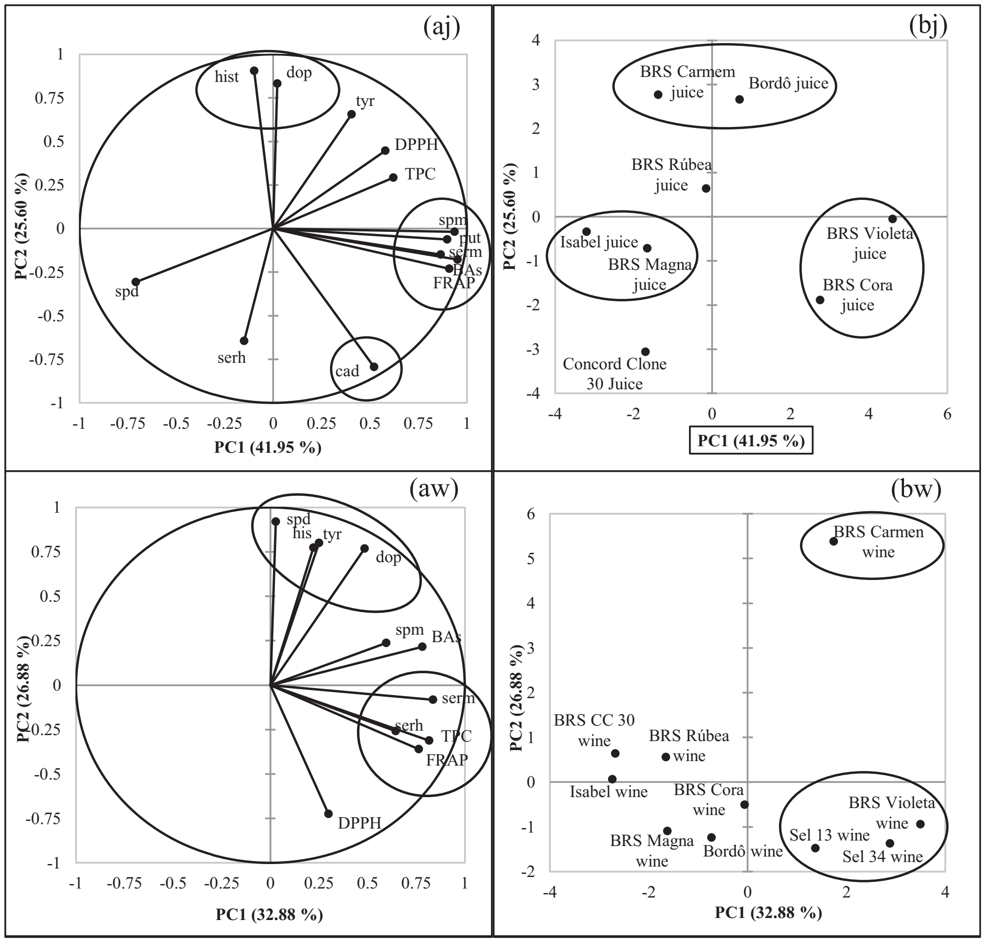

Fig. 1 Two-dimensional projection (a) and scores (b) of biochemical attributes, BAs, TPC and antioxidant activity in the first two principal components in the analyzed juices (j) and wines (w). Not defined abbreviations are found in the bottom of Table 1.

and 1bj). The PC1 axis corresponds to 41.95% of the total variance of the data and presents strong positive correlation (PC1+, > 0.9) with spm, mono-hydrated serotonin (serm), total BAs and with antioxidant activity (FRAP method). These variables were positively correlated to the juices elaborated from 'BRS Violeta' and 'BRS Cora', suggesting that they are sources of these compounds and present high antioxidant activity.

The juices elaborated from 'BRS Violeta' and 'BRS Cora', grapes developed in Brazil, stood out due to the high spm values (above 50% of the total BAs) and the highest contents of serm (20% above the total). In the same way, the juice of 'BRS Concord Clone 30' stood out due to the serotonin hydrochloride (serh) content (PC1- and PC2-), constituting 50% of the total BAs in this beverage. Due to the importance of serotonin as neurotransmitter, these juices may exert relevant physiological effects in the control of satiety, appetite and behavioral parameters [17]. The BAs ($r = 0.77$, $p < 0.05$), mainly spm ($r = 0.74$, $p < 0.05$) and serm ($r = 0.71$, $p < 0.05$), were the mainly responsible for the antioxidant activity in the grape juices, even compared with the TPC ($r = 0.67$, $p < 0.05$). Polyamines (spm and spd) present in foods are strong antioxidant compounds with higher antioxidant properties than natural or well accepted synthetics compounds such as α-tocopherol, octyl gallate and palmitoyl-ascorbic acid [7].

His and dopamine (dop) are positively correlated with the second principal component (PC2+), which is responsible for grouping the grape juices elaborated from 'BRS Carmem' and 'Bordô'. The dop and his are responsible for many physiological functions in plants, such as cell division, regulation, growth, flowering, fruit development, response to stress and to senescence [6, 9]. In human health, dop has been described as harboring an antioxidant potential higher than food additives (butylated hydroxyanisole – BHA, and butylated hydroxytoluene – BHT), flavonoids and is similar to strong antioxidants, such as gallocatechin gallate and ascorbic acid [18]. In addition, dop has been related to a decrease in Parkinson's disease symptoms in humans [19], which makes these beverages sources of molecules with important beneficial properties for human health. However, his is a strong allergenic and can produce hypotensive effects, besides mediating primary and immediate symptoms in allergenic responses. Thus, some countries have established limits for the content of his in beverages made from grapes [4]. Levels between 2 to 10 mg/L can cause headache when large quantities of wines are consumed [5]. It is worth pointing out that, despite the presence of higher contents of his in juices of 'Carmem' and 'Bordô' grapes (0.62 and 0.57 mg/L, respectively), the levels are significantly below of what is considered harmful to humans. The juices elaborated from the cv. BRS Magna and Isabel grapes presented the lowest total BA content, despite standing out regarding spd content (PC1- and PC2-). Juices elaborated from cv. Isabel, the main grape used in the juice industry in Brazil, presented the lowest BA levels, containing mainly spm. Similar values were measured in juices of early ripening grape cv. Isabel, and in 'BRS Cora' for put, cad, and spd, but 'BRS Cora' was lower in spm [20].

The concentration of TPC showed significant differences in the juices ($p < 0.05$), with values between 145.14 ('Isabel' juice) and 472.88 mg/L ('BRS Violeta' juice) (Table 1). The highest contents were detected in juice from the 'BRS Violeta' and 'BRS Magna' (same genetic origin), followed by the juice from 'Bordô' grapes. The juice from the 'Isabel' grape showed the lowest level of total phenols, with only 30% in relation to the juice from 'BRS Violeta'. However, most of the samples (hybrid grapes) presented levels of TPC higher than the ones elaborated with wine grapes (7.60–157.48 mg/L) [21]. The TPC also presented positive and significant correlation with the antioxidant activity (FRAP: $r = 0.67$; DPPH: $r = 0.67$, $p < 0.05$). The 'BRS Violeta' juice presented the highest antioxidant activity by the FRAP method (47.27 mmol $FeSO_4$/L). 'Bordô' (43.04%) and 'Magna' (26.31%) juices (measured *per* DPPH) also presented interesting results in relation to the antioxidant properties of these beverages. 'Isabel' juice presented only 26% of the antioxidant activity compared to the 'BRS Violeta'. The antioxidant activity results are similar to dates demonstrating the potential of the hybrid grapes for the elaboration of juices with high biological activity [1]. Due to the TPC content, the juices present clear functional differences in function of the grape cultivar. This implies, in particular, an advantage for facilitating a blend to compensate good functional characteristics in juices that have low contents of phenolic compounds but are elaborated with highly productive grapes.

BAs, TPC and Antioxidant Activity in Red Wines

Despite of the genetic similarity of some grape cultivars used for the elaboration of wines in this study, high variability in the BAs profile ($p < 0.05$) was observed (Table 1). PCA analyses of wines (Fig. 1aw and 1bw) show that PC1 and PC2 explained almost 60% of the data variance. The PC1 is positively correlated to the amines his, tyr, spd and dop, explaining 32.88% of the data variance. The wine elaborated from 'BRS Carmem' presented the highest total BA content, standing out mainly due to the content of spd, his, tyr and dop (PC2+ and PC1+). The wines from 'BRS Magna' and 'Bordô' grapes had the lowest total BA content (PC1- and PC2-). It is worth noting that the wines with higher concentrations of BAs were elaborated with 'Sel34' grapes, with content 11 times higher than 'Bordô' wine, which presented the lowest concentration of these compounds.

Considering all wines evaluated (Table 1), put and spm occurred in higher concentration. From the nine BAs analyzed, only his was not identified in wines elaborated with 'Sel34' grapes. In this wine and in wines from 'BRS Cora' and 'Sel13' grapes, we did not detect spd. Some results of this study are higher than the values described in the literature about Brazilian wines elaborated from *V. vinifera* regarding serotonin (0.60 mg/L) [22]. However, the wines from hybrid grapes analyzed in our study presented similar results compared to those described by Agustini et al. [23] for spd (4.6 mg/L), put (9.5 mg/L), and revealed inferior results regarding his (11.8 mg/L) and tyr (10.4 mg/L). Our results also show similarity in relation to the levels of dop found in white wines (0.3 mg/L) [24] and inferior values in comparison to the spm and cad levels compared to those in high quality wines (spm - 0,27 and cad - 3.27 mg/L) [25].

The wines elaborated from 'BRS Violeta', 'Sel13' and 'Sel34' grapes grouped (PC1+ and PC2-), mainly due to the high TPC content (Fig. 1bw). These compounds were positively correlated to the first principal component, which explained 32.88% of the data variance. The average value of TPC of 'BRS Violeta' wine was 4.48 times superior compared to 'Isabel' grape wine, which contained the lowest content (Table 1). In addition, there was a strong positive correlation of serm also with the first principal component and the 'BRS Violeta' and 'Sel34' wines that stood out in these functional compounds.

The antioxidant activity varied widely according to the analyzed wine and to the method used (Table 1). The highest antioxidant activity was found in 'BRS Violeta' wine by the FRAP method (77.24 mmol $FeSO_4$/L). 'Sel13' wine showed the highest correlation with antioxidant activity among the analyzed wines (FRAP: $r = 0.97$, $p < 0.05$). Serh and serm were responsible for the antioxidant activity ($r = 0.73$ and $r = 0.59$, respectively). The consumption of some wines such as the ones elaborated with 'BRS Violeta' and 'Sel34', represent an interesting source of bioactive compounds and demonstrate the potential of these grapes as a functional food.

The total BA content was approximately three times higher in wines than in juices (Table 1). This result may be derived from the influence of bacteria and yeast during the alcoholic fermentation process [26–28], that is not present in juices. Generally, malolactic fermentation is also considered to be a determining factor for the production of BAs, and studies have shown that, in this phase, the main BAs produced are put, his and tyr [29]. There are reports that some BAs (put and cad) are indicators of poor sanitary conditions during the winemaking process or grape production. However, other studies indicate that certain amines such as put can appear during the winemaking process as consequence of yeast and bacteria metabolism [29]. With few exceptions, the levels of BAs, TPC and antioxidant activity in wines were higher than in juices produced with the same grape cultivar, reinforcing that the winemaking process is associated to increased bioactivity in wines.

Conclusions

The beverages produced from hybrid grapes showed an interesting potential for human consumption, considering that juices and wine analyzed had a high concentration of TPC and beneficial amines. Furthermore, these beverages presented good antioxidant activity, low levels of undesirable BAs, and are safe for human consumption, even for individuals with allergic susceptibility. The beverages elaborated from 'BRS Violeta' grapes show the highest antioxidant activity. The values are notably superior compared to beverages elaborated from 'Isabel' grape, which is currently the most frequently used by the juice industry in Brazil. The antioxidant activity found in juices and wines can be attributed both to the TPC and to the BAs, mainly serotonin, which present strong positive correlation with antioxidant activity, regardless of the type of beverage analyzed. In addition, the juice from 'BRS Cora' also stands out, considering the amines and antioxidant activity content. Wines elaborated from 'BRS Carmem', 'Sel13' and 'Sel34' show very promising results, due to the high content of the BAs and phenolic compounds. Results found in the current study highlight the phytochemical potential of these grapes cultivars for beverage production in regions not traditionally producing grapes.

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Compliance with Ethical Standards

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Conflict of Interest Authors declare that they have no conflict of interest.

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