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# Composition of amino acids and bioactive amines in common wines of Brazil

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**ABSTRACT.** Since most consumed wines in Brazil are common wines and since their representativeness is not accounted for in scientific research, current study quantifies bioactive amines and their precursors in Brazilian sweet and dry common wines, correlates the formation of amines with physical and chemical parameters and clusters studied areas by their amine and amino acid contents. Forty-seven wine samples varying in type, color and origin were analyzed simultaneously for seventeen amino acids, ammonium ion and five bioactive amines by reversed-phase high performance liquid chromatography and ultraviolet detection after the derivation phase. Physical and chemical analyses comprised titratable acidity, pH, organic acids, sugar and alcohol contents. Sweet wines had lower concentrations of amino acids and bioactive amines. Dry white wines had higher amino acid contents when compared to those in dry red wines. Since multivariate data analysis confirmed similarities between the studied regions, their unity as potential viniculture area was reinforced. Amine levels in Brazilian common wines were reported for the first time and results reinforced the importance of bioactive amines quantification and the use of suitable vinification practices to reduce their formation.

**Keywords:** biogenic amine, food safety, liquid chromatography.

## Composição de aminoácidos e aminas bioativas em vinhos comuns brasileiros

**RESUMO.** Considerando que os vinhos mais consumidos no Brasil são vinhos comuns e que sua representatividade não é demonstrada na pesquisa científica, o presente estudo tem por objetivo quantificar as aminas bioativas e seus precursores em vinhos comuns brasileiros suaves e secos, correlacionar a formação das aminas com parâmetros físico-químicos e agrupar as áreas estudadas por meio do seu conteúdo em aminas e aminoácidos. Foram analisadas 47 amostras de vinhos que variam quanto ao tipo, cor e origem; foram analisados, simultaneamente, 17 aminoácidos, íon amônio e cinco aminas bioativas por cromatografia líquida de fase reversa depois de uma etapa de derivatização. Análises físico-químicas incluíram acidez total, pH, ácidos orgânicos, açúcar e teor alcoólico. Os vinhos doces tiveram menor concentração de aminoácidos e aminas bioativas. Os vinhos brancos secos apresentaram maior conteúdo em aminoácidos quando comparados com os apresentados nos vinhos tintos secos. Uma vez que a análise multivariada confirmou similaridades entre as regiões estudadas, sua unidade como uma área potencial para a vinicultura foi reforçada. Os níveis de aminas em vinhos comuns brasileiros foram reportados pela primeira vez e os resultados reforçam a importância da quantificação de aminas bioativas e o uso de práticas apropriadas de vinificação para reduzir a sua formação.

**Palavras-chave:** aminas biogênicas, segurança alimentar, cromatografia líquida.

## Introduction

Eighty percent of wines consumed in Brazil comprise common wines produced by American and hybrid grape cultivars (BARNABÉ et al., 2007). The 'Terci' cultivar is one of the most cultivated grapes in the southern state of Paraná, Brazil, and its cultivation has increased substantially in south Brazil during the last decades (SCHUCK et al., 2012). Due to the large extension of Brazilian territory and its peculiar regionalism, the above cultivar is also called 'Bordo (Ives)' in the state of Rio Grande do Sul and 'Folha de Figo' in the state of Minas Gerais (CAMARGO, 2003). According to Brazilian viticulture statistics, the state of Paraná is the fourth major grape producer, featuring more than a hundred thousand tons of grapes in 2010 (MELLO, 2013). To date, most studies on the *Vitis labrusca* species concentrated on its physical and chemical characterization (RIZZON; MIELE, 2005, 2006; RIZZON et al., 2000; TECCHIO et al., 2007), with few researches on the discrimination of Brazilian viticulture regions (MIELE et al., 2010). The state of

Paraná is characterized by small wine-producing properties and family labor, which are factors that satisfy current consumers demand for a regional identity of the products. Since the product's unique characteristics must be underscored to differentiate a wine-producing region, the profiles of amino acids and bioactive amines, related to wine quality, may contribute towards initial analyses of a viticulture region.

Amino acids, proteins, peptides and ammonium ion are the main components of the nitrogen fraction of musts and wines. Their presence in wine is due to grape composition, to the excretion by living yeasts at the end of fermentation, to proteolysis during their autolysis and to enzymatic degradation of grape proteins (SOUFLEROS et al., 2003). Total free amino nitrogen (the sum of total amino acids but proline) is usually used as a quality indicator of musts since low rates imply fermentation problems due to insufficient nutritional reserves of yeasts which may promote sluggish/protracted fermentation (HERBERT et al., 2000). The above occurs because amino acids are used as nitrogen source for growth for yeasts and lactic acid bacteria (SOUFLEROS et al., 2003). Besides the nutritional role, amino acids have also the important function in assuring an appropriate bouquet to wine (GARDE-CERDÁN; ANCÍN-AZPILICUETA, 2008).

Nevertheless, amino acids may act as precursors of a class of compounds, named bioactive amines, which are not so benefic to wine quality. These substances might be extant in all food containing proteins or free amino acids and submitted to conditions that allowed microbial or biochemical activity (SILLA-SANTOS, 1996). They may have been caused by enzymatic decarboxylation of amino acids through the metabolism of yeasts during alcohol fermentation and/or lactic acid bacteria during malolactic fermentation (LONVAUD-FUNEL, 2001). The presence of decarboxylase encoding gene do not guarantee enzyme activity and, consequently, amine formation. An adequate ambient for enzyme functionality is also necessary since physical and chemical parameters such as pH, alcohol content, sulfur dioxide and temperature influence this activity (SMIT et al., 2008; SOUZA et al., 2005).

Similar to amino acids concentration, bioactive amine contents also vary largely due to differences in viticulture and vinification techniques and to grape variety, geographic origin and vintage (BARRADO et al., 2009; DEL PRETE et al., 2009; GARCÍA-VILLAR et al., 2007; MARQUES et al., 2008; SOUFLEROS et al., 2003).

The importance of analyzing biogenic amines arises from their pharmacological properties and toxicological aspects and also because they may be indicators of food quality since their occurrence is normally associated with inadequate sanitary conditions during vinification (SILLA-SANTOS, 1996; PEREIRA et al., 2008). Although no information in Brazilian legislation on the safe levels of bioactive amines ingestion is available, it is already known that the consumption of great amounts of histamine, tyramine and phenylethylamine may have toxic effects on humans. Although their amounts in wine are quite low when compared with those in other fermented food products, the presence of other compounds in wine composition, such as ethanol, acetaldehyde and other biogenic amines (putrescine and cadaverine, for instance) could enhance their side effects (ANCÍN-AZPILICUETA et al., 2008; SOUFLEROS et al., 2007). The intoxication symptoms associated with histamine are headaches, heart palpitations, nausea, rushes, low blood pressure and digestive problems, whereas tyramine and phenylethylamine are related to migraine and hypertension (SILLA-SANTOS, 1996).

Furthermore, amine levels are, eventually, set as a manner for assuring the wine's origin and also for attending commercial interests. Although the regulatory limits of biogenic amines have not yet been established by the Organisation Internationale de la Vigne et du Vin (OIV), some countries have established maximum limits for histamine content in wine (from 2 mg $L^{-1}$ in Germany to 10 mg $L^{-1}$ in Switzerland) (MARQUES et al., 2008). Therefore, the above permitted levels must be observed when import and export aspects are implied.

Considering the large market associated with *Vitis labrusca* grapes in Brazil, the lack of literature on its wine composition and on the relevant topic of food safety, the objectives of the present research were (1) to identify the amino acid and bioactive amines profile of Brazilian common wines and to correlate amine levels to physical and chemical parameters; (2) to classify wine samples according to their geographical origin through amino acid and bioactive amine contents.

## Material and methods

### Wine samples and standards

Current research comprised forty-seven common wines (*Vitis labrusca*), 32 red and 15 white, produced, respectively, from Terci and White Niagara grapes, 2009 vintage, in the municipalities of Colombo and Almirante Tamandaré, Paraná State, Brazil. Samples labeled with the letter A

belong to Colombo and those labeled with the letter B belong to Almirante Tamandaré. Regarding their sugar content, twenty-five were dry (< 5 g $L^{-1}$) and twenty-two were sweet (> 20 g $L^{-1}$).

Ammonium ion and seventeen free amino acids, specifically, alanine (Ala), arginine (Arg), aspartate (Asp), cysteine (Cys), glutamate (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The same company also supplied five bioactive amine standards: putrescine (PUT), spermidine (SPD), histamine (HIM), tyramine (TYM) and tryptamine (TRM). Solutions of these standards were prepared with HCl 0.1 M.

### Determination of the physicochemical characteristics

Physical and chemical characteristics were determined according to methods described by the Normative Instruction 24 of the Brazilian government (BRASIL, 2005). The pH was determined by a digital pH meter (model 330i, WTW, Germany) and titratable acidity was determined by titration with NaOH 0.1 M. The alcohol degree was measured by the densimetry method while reducing sugars were quantified with Fehling's solution. Lactic and malic acids were determined by methodology described by Zotou, Loukou and Karava (ZOTOU et al., 2004) for organic acids determination in wines.

### Reaction of derivatization and hplc analysis

Samples were derivatizated and analysed for biogenic amines according to the RP-HPLC procedure proposed by Gómez-Alonso et al. (2007). For the derivatization step, 1.75 mL of borate buffer 1 M (pH 9), 750 $\mu$L of methanol, 1 mL of sample without any pre-treatment, 20 $\mu$L of internal standard (L-2-aminoadipic acid, 1 g $L^{-1}$) and 30 $\mu$L of diethyl ethoxymethylenemalonate (DEEMM) reacted in a screw-cap test tube for 30 min. in an ultrasound bath. Heating the solution at 70°C for 2 hours was sufficient to degrade the DEEM excess and reaction byproducts.

The analysis were performed on a Varian ProStar HPLC (Varian Inc., Walnut Creek, CA, USA) comprising a ProStar 240 ternary pump, a ProStar 410 autosampler and a ProStar 330 photodiode array detector. Chromatographic separation was performed in an ACE HPLC column (5 C18-HL; 5 $\mu$m; 250 x 4.6 mm) through a binary gradient using 25 mM acetate buffer pH 5.8 with 0.02 g $L^{-1}$ sodium azide as phase A and a mixture of acetonitrile and methanol 80:20, with a flow rate 0.9 mL $min.^{-1}$ as phase B. The linear gradient for solvent A comprised: 0 min., 90%; 20 min., 90%; 30.5 min., 83%; 33.5 min., 83%; 65 min., 60%; 73 min., 28%; 78 min., 18%; 82 min., 0%; 85 min., 0%.

Detection was performed at 280 nm while quantification was based on the internal standard method. Target compounds were identified according to retention times and UV-vis spectral characteristics of the derivatives of the corresponding standards and quantified by interpolation in analytical curves (between 0.5 and 60.0 mg $L^{-1}$ for bioactive amines and between 0.10 and 128.0 mg $L^{-1}$ for amino acids). Samples were diluted when compliance with the working range was required. Detection limits were calculated as three times the baseline noise.

### Statistical analyses

Normality analyses were evaluated by Kolmogorov test. Data were submitted to analysis of variance (ANOVA) and the means were compared by Tukey's test at 99 and 95% confidence level. Pearson correlation was used to investigate the correlation between the physical and chemical characteristics and bioactive amines. All data were evaluated with Microsoft Excel 2010, supplemented by PHStat2 version 3.04.

The Principal Component Analysis (PCA) was used to provide information on the possibility of grouping wine samples according to viticultural region. Chemometrics were performed with Matlab 7.0.1.

## Results and discussion

### Levels of amino acids and bioactive amines content in the wines

The first noteworthy aspect in the analyzed wines was the great variation observed by a wide range in amino acids and amine contents. This fact probably reflected differences on viticulture and enological practices among the participating rural wineries. Table 1 shows results of dry and sweet wines.

As a rule, the wines presented the same profile for prevalent amino acids, with the predominance of proline, followed by alanine. Prevalence may be explained by the fact that proline is not consumed under anaerobic conditions and it accumulates in wines due to the metabolism of arginine (CEJUDO-BASTANTE et al., 2010). According to total amino acid contents, dry red wines registered 251.5 mg $L^{-1}$, which was lower than the concentration of 447.8 mg $L^{-1}$ in dry white wines. (GOMEZ-ALONSO et al., 2007).

**Table 1.** Amino acids and amine contents of wines (mg $L^{-1}$).

| | Dry wines (n = 25) | | | | Sweet wines (n = 22) | | | |
|---|---|---|---|---|---|---|---|---|
| | Red wines (n = 19) | | White wines (n = 6) | | Red wine (n = 13) | | White wines (n = 9) | |
| Compound | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| Asparagine | 13.6 | 3.3-41.7 | 14.3 | 1.2-28.7 | 7.8 | nd-28.0 | 7.1 | nd-13.4 |
| Glutamic acid | 17.6 | nd-64.2 | 31.6 | 2.6-50.5 | 10.5 | nd-40.1 | 12.4 | nd-35.3 |
| Serine | 8.3 | 1.4-23.9 | 17.8 | 4.2-30.4 | 9.0 | 0.8-23.3 | 8.3 | 2.0-15.4 |
| Histidine | 4.0 | 0.8-9.3 | 4.0 | 0.7-6.0 | 5.0 | 0.8-13.9 | 3.1 | 1.1-6.7 |
| Glycine | 9.5 | 1.3-36.6 | 12.0 | 3.7-19.0 | 9.9 | 1.6-30.5 | 7.7 | 1.0-16.8 |
| Threonine | 8.5 | 1.9-23.0 | 11.8 | 1.9-17.1 | 7.8 | 1.6-19.3 | 5.1 | 0.8-12.4 |
| Arginine | 7.7 | 1.4-36.0 | 15.3 | 1.7-40.9 | 7.3 | 0.8-21.8 | 12.1 | 0.9-50.1 |
| Alanine | 19.9 | 1.3-95.4 | 85.2 | 5.4-208.7 | 19.6 | 1.8-59.3 | 28.5 | 0.6-73.5 |
| Proline | 85.7 | 14.3-212.2 | 129.7 | 51.5-192.9 | 46.1 | 19.8-103.2 | 39.2 | 5.7-153.0 |
| Tyrosine | 9.3 | 3.3-29.5 | 11.5 | 2.0-19.6 | 8.7 | 1.3-21.9 | 6.9 | 0.6-16.5 |
| Ammonium ion | 23.4 | 2.1-138.7 | 33.6 | 3.8-68.1 | 9.4 | 1.7-24.1 | 11.2 | 1.0-28.7 |
| Valine | 6.8 | 1.4-17.0 | 11.5 | 1.9-17.9 | 7.3 | 1.4-15.2 | 5.0 | 0.9-12.0 |
| Methionine | 5.0 | 1.4-11.2 | 5.8 | 1.2-9.7 | 6.2 | 1.9-14.0 | 2.5 | 0.5-8.7 |
| Cysteine | 2.2 | 0.4-5.4 | 1.5 | 0.3-2.4 | 2.9 | 0.6-7.9 | 1.4 | nd-2.5 |
| Isoleucine | 4.3 | 0.7-10.9 | 6.8 | 1.1-12.2 | 4.3 | 0.3-12.3 | 3.1 | 0.4-8.8 |
| Leucine | 7.9 | 0.7-20.4 | 15.2 | 3.4-31.7 | 7.2 | 0.7-14.7 | 7.4 | 0.7-24.6 |
| Phenylalanine | 7.7 | nd-20.6 | 19.3 | 2.0-38.3 | 2.6 | 0.0-11.3 | 1.5 | 0.3-5.0 |
| Lysine | 10.1 | 0.2-36.1 | 20.7 | 4.1-38.0 | 8.5 | 0.3-21.3 | 11.2 | 0.1-35.0 |
| Histamine | 3.5 | nd-11.8 | 2.27 | 0.52-5.36 | 2.4 | 0.8-4.0 | 1.0 | 0.3-2.4 |
| Spermidine | 1.0 | nd-4.6 | 1.70 | 0.54-3.10 | 1.6 | nd-4.1 | 1.1 | nd-3.9 |
| Tyramine | 2.5 | nd-10.4 | 4.37 | 0.98-12.82 | 2.1 | nd-10.0 | 1.0 | nd-5.2 |
| Putrescine | 9.5 | nd-45.4 | 7.20 | 0.83-37.03 | 6.4 | nd-28.1 | 1.3 | nd-2.6 |
| Tryptamine | 0.6 | nd-7.9 | nd | Nd | 0.1 | nd-1.2 | 0.02 | nd-0.2 |
| Total amines | 17.2 | - | 15.5 | - | 12.6 | - | 4.4 | - |
| Total amino acid | 251.5 | - | 447.8 | - | 180.2 | - | 173.8 | - |

nd – non-detected levels.

Results agreed with amino acids rates determined for other grape varieties in Greece where the preponderance of white wines for this parameter was 488.0 mg $L^{-1}$, whereas that of red wines presented 229.0 mg $L^{-1}$ (SOUFLEROS et al., 2007). Nevertheless, wines from the Spanish region of Castilla-La Mancha demonstrated the highest total amino acid contents for red wines.

Results for sweet wines, either red or white, revealed lower amino acid contents when compared to those of the dry ones. This result contradicted Soufleros et al. (2003) who showed that Greek sweet white wines had a higher concentration of amino acids than that of dry wines due to the early fermentation stopping comprising the former´s winemaking technology. On the other hand, Brazilian sweet wines were permitted to have an addition of sucrose originated essentially from sugar cane to correct the low sugar concentration from a bad vintage and/or to reach a final sugar concentration above 20 g $L^{-1}$. In current study, the sweet wines actually had a stage of sucrose addition. Since this compound is an agent ordinarily used to cause osmotic dehydration of fruits and since the process enhances the transference of anthocyanin pigments to the sugar solution (OSORIO et al., 2007), it is reasonable to presume that such addition could reduce maceration time in winemaking. Thus, an improving extraction of pigments would lead to a premature achievement of the desired color in wine and to a shorter skin contact. Consequently, it allowed a small fraction of amino acids to be extracted to the must, justifying lower amino acid contents in Brazilian red sweet wines. Moreover, amino acids consumption by the yeast might be altered during alcohol fermentation, depending on temperature, oxygen concentration and sugar content. According to Agenbach (1977), since the sugar content increased and more nitrogen was required, the minor concentration of amino acids in sweet wines, especially white sweet ones, could also be a consequence of greater consumption of these nutrients.

Regarding bioactive amine contents, sweet wines also presented a lower quantity when compared with dry wines. Since amines were mainly produced by amino acid decarboxylation, the lower contents of bioactive amines could be the result of a previous minor level of precursors. Although the establishment of a relationship between the formation of amines and the consumption of their precursors amino acids was difficult, studies have already proven the existence of this relationship (HERBERT et al., 2005; MARTÍN-ÁLVAREZ et al., 2006).

The greatest content of bioactive amines was encountered in dry wines, with red wines featuring higher concentrations than the white wines. Since most white wines do not undergo malolactic fermentation and since they do not have longer periods of skin contact, the anime contents in white wines are frequently lower than those in red ones

(GARCÍA-MARINO et al., 2010; GOMEZ-ALONSO et al., 2007). Moreover, it is thought that red wine has a great diversity of amine-producing microorganisms owing to fermentation technology (ARENA; MANCA DE NADRA, 2001).

Among the five amines investigated, putrescine was prevalent in all types of wines. Since some lactic acid bacteria have the ability of converting, enzymatically, arginine into putrescine via the arginine-deiminase (ADI) pathway, this prevalence might be reasonably justified (ANCÍN-AZPILICUETA et al., 2008).

Figure 1 shows the individual contribution of the amines under analysis for samples with the highest contents of total bioactive amines.

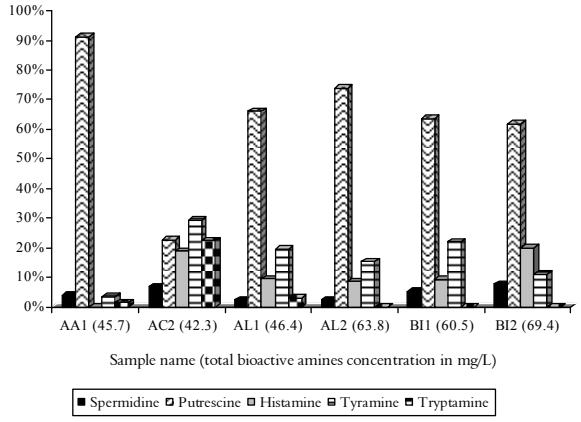


**Figure 1.** Individual amine contribution (%) on wines with the highest levels of total bioactive amines (mg $L^{-1}$). Rates are shown between parentheses. Samples labeled A belong to the municipality of Colombo and those labeled B belong to the municipality of Almirante Tamandaré.

As commented above, putrescine was a prevalent amine, with the exception of the sample AC2 in which tyramine contributed with 29.4%. In absolute numbers, it represented 10.4 mg $L^{-1}$ below 25.0 mg $L^{-1}$ considered to be the toxic level for tyramine (SOUFLEROS et al., 2007). Furthermore, all analyzed wines revealed concentrations below 13 mg $L^{-1}$. Together with tyramine, histamine is one of the most important amines in the toxicological field and thus the maintenance of their concentrations at low levels is highly important. Since the Swiss histamine limit is 10 mg $L^{-1}$, 98% of the wines complied with the limit. In fact, concentrations were below or equal to 8.8 mg $L^{-1}$. The remaining 2% corresponded to sample BI2 with 11.8 mg $L^{-1}$ histamine. Results reinforced the necessity of routine detection and quantification of bioactive amines in detecting and avoiding stages of the process that might contribute to the above-mentioned formation of molecules. In fact, malolactic starters and shorter grape skin maceration have been associated with a reduction on amine production, while wine storage on lees increased their formation (MARQUES et al., 2008; MARTÍN-ÁLVAREZ et al., 2006). Arrieta and Prats-Moya (2012) detected histamine levels above 10 mg $L^{-1}$ in aged Spanish wines and underscored that a moderate intake of these wines did not represent any risk for healthy people. Nevertheless, histamine-intolerant people ingesting MAOI drugs must be careful. Spermidine contributed least to total levels ($\leq$ 6.8 mg $L^{-1}$), whereas tryptamine was detected in only three of the six samples shown in this figure. In fact, it was the amine with the lowest incidence among the table wines analyzed.

### Physical and chemical characteristics and their correlation with amine formation

Table 2 shows physical and chemical results. Wine acidity may be evaluated by titratable acidity, organic acids concentration and pH rates. Analyzed wines had high titratable acidity rates ranging between 86.39 and 115.53 mEq $L^{-1}$. Significant differences were detected between the red dry wines and white wines, both sweet and dry. Another Brazilian study employing the same variety of red grape, cultivated in Flores da Cunha, Rio Grande do Sul State, Brazil, described the wines as having a pronounced acidity, with 91 mEq $L^{-1}$ as mean titratable acidity (TECCHIO et al., 2007). Regarding pH rates, means ranged between 3.24 and 3.38, with red wines showing the lowest rates and the white wines the highest. The statistically difference noticed between titratable acidity rates of dry red wines and sweet white wines, also revealed their significant difference in pH values. In the above-mentioned study, Tecchio et al. (2007) found similar pH rates, ranging between 3.04 and 3.48. Korean wines elaborated with *Vitis labrusca* grapes also presented low pH rates, with mean 3.20 (LEE et al., 2006). In contrast, Brazilian fine wines revealed higher results, between 3.80 and 4.07 (SOUZA et al., 2005). Hence, high total acidity rates and low pH rates seemed to be a peculiarity in *Vitis labrusca* varieties. When taking into consideration sugar concentration, ethanol contents and the previously mentioned physical and chemical parameters, every sample analyzed was in compliance with the limits established by current Brazilian regulations (BRASIL, 1988). High performance liquid chromatography for the determination of malic and lactic acid concentrations assured that malolactic fermentation had been initiated in all samples but its completion was restricted only to a few wines (data not shown).

**Table 2.** Physical and chemical results and concentration of organic acid in wines.

| | Dry wines (n=25) | | Sweet wines (n=22) | |
|---|---|---|---|---|
| | Red wine | White wine | Red wine | White wine |
| | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| Titratable acidity§ | 115.53 ± 15.86 [a] | 87.34 ± 18.09[b] | 108.48 ±17.12 [a] | 86.29 ± 8.48[b] |
| pH | 3.24 ± 0.14 [a] | 3.34 ± 0.14[a, b] | 3.24 ± 0.11[a, b] | 3.38 ± 0.12 [b] |
| Sugar† | 2.67 ± 1.21 [a] | 1.40 ± 0.34 [a] | 104.16 ± 57.94[b] | 142.76 ± 41.41 [b] |
| Ethyl Alcohol‡ | 12.02 ± 2.17[a] | 11.09 ± 2.65[a] | 12.26 ± 2.07[a] | 11.34 ±1.30[a] |
| Malic acid† | 0.91 ± 1.01[a] | 0.66 ± 0.72[a] | 0.87 ± 0.76[a] | 0.72 ± 0.74[a] |
| Lactic acid† | 2.33 ± 1.36[a] | 2.96 ± 1.99[a] | 2.19 ± 1.27[a] | 2.62 ± 1.44[a] |

Only results on the same line were compared. Different letters on the same line indicate statistically different rates (tukey's test, $p < 0.05$). Same letters on the same line indicate rates not statistically different. § mEq/L; † g/L; ‡ % v/v 20°C.

Besides many other fermentation substances and products, the wine's physical and chemical factors may influence the concentration and diversity of microorganisms in the wine and may also affect decarboxylase enzyme activity (SMIT et al., 2008; SOUZA et al., 2005). Therefore, the formation of bioactive amines was correlated with the results of pH, sugar and alcohol content, titratable acidity, malic and lactic acid concentrations. As in other studies (SOUFLEROS et al., 2007; SOUZA et al., 2005), since individual amines showed significant correlations between each other, the influence of common factors on their formation might be suggested. The highest coefficient correlation rate occurred between putrescine and tyramine ($R = 0.6541$, $p \leq 0.01$). Another remarkable result was the negative correlation between histamine and sugar concentrations ($R = -0.3522$, $p \leq 0.05$). As sugar concentration increased, histamine levels decreased significantly. This might be related to the above explanation on the minor content of amino acids in sweet wines, which could have led to lower contents of bioactive amines. Moreover, positive correlations were found between pH rates and three different amines, or rather, tyramine ($R = 0.4759$, $p \leq 0.01$), spermidine ($R = 0.3228$, $p \leq 0.05$) and putrescine ($R = 0.3481$, $p \leq 0.05$). These correlations could be explained by the fact that higher pH rates seemed to favor the proliferation of bacteria strains, responsible for the formation of bioactive amines (ANCÍN-AZPILICUETA et al., 2008). Since putrescine levels increased simultaneously with lactic acid concentrations ($R = 0.3975$, $p \leq 0.05$), lactic acid bacteria during malolactic fermentation not only converted malic acid into lactic acid but also contributed to amine production. The implications of lactic acid bacteria in amine formation during spontaneous malolactic fermentation were described previously (LONVAUD-FUNEL, 2001; IZQUIERDO-CAÑAS et al., 2008).

### Region discrimination by amino acids and bioactive amines contents with chemometrics

Chemometrics is a useful tool to apply statistical and mathematical methods to chemical results. The application of multivariate data analysis techniques, such as the principal component analysis (PCA), reduces data dimensionality to a small number of components to unravel and interpret the samples' properties, allowing possible clustering according to their geographical origin (COZZOLINO et al., 2011). PCA was therefore carried out with amino acid and amine concentrations as analytical data to segregate the viticulture regions of the municipalities of Colombo and Almirante Tamandaré involved in current research. The dimensions of the corresponding matrix were 47 samples x 5 amines plus 17 aminoacids and ammonium ion. Data were autoscaled to provide similar weights for all variables in the PCA model. Hence, 73.37% of the total variance of the samples was explained by three principal components (PC). PC1 accounted for 57.52% of the variation, whereas PC2 and PC3 respectively explained 8.23 and 7.62% of total variance. The interpretation of the results was mainly based on PC1 and PC2 scores and loading biplot (Figure 2).

Although the other plots of scores and loadings involving other combinations of PC were also examined (graphs not shown), they did not provide any new information on the wines' characterization. Investigation on samples distribution according to the producing regions did not show relevant patterns and merely reflected the absence of sufficient differences between wines from the neighboring municipalities of Colombo and Almirante Tamandaré. Since the absence of clusters could be justified by the loading distribution with variables completely situated in PC2+, the parameters contributed only slightly in the effective discrimination of wines analyzed. The bioactive amine contents of Spanish wines from six different origins were also insufficient to establish relevant differentiation patterns (GARCÍA-VILLAR et al., 2007). Nevertheless, Greek regions had their wines successfully separated by employing amino acid contents as data for discriminant analysis and principal component analysis (SOUFLEROS et al., 2003).

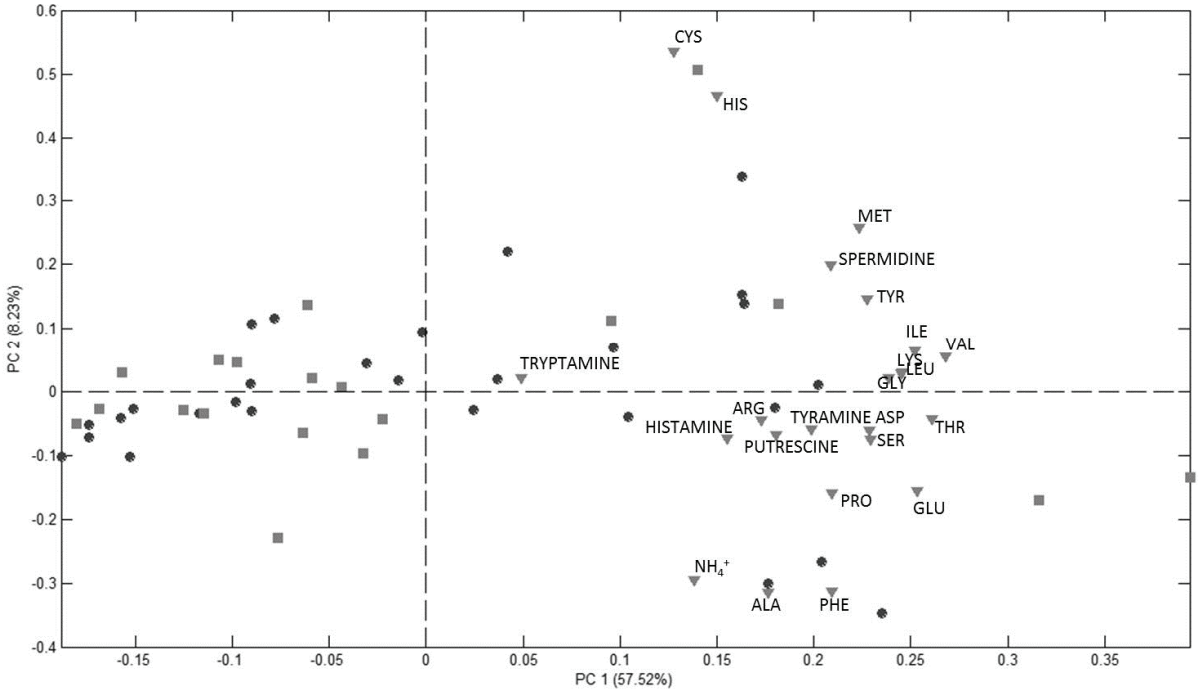


**Figure 2.** PCA score and loading biplot of wines employing amino acids and amines contents as variables. Symbols: gray squares (wines from Almirante Tamandaré); black circles (wines from Colombo); gray triangle (variables).

The absence of clusters, shown in current study, demonstrated that the two analyzed regions were not sufficiently distant geographically from each other to provide enough edaphoclimatic changes to alter the grape composition cultivated in both areas and, consequently, wine composition (referring specifically to amino acid and amine content).

## Conclusion

As far as it is known, current essay is the first report on amino acids and bioactive amines in Brazilian common wines. Results from bioactive amines quantification have reinforced the importance of adequate vinification practices to reduce their formation to acceptable levels.

Since the municipality of Colombo has a hundred-year-old tradition in winemaking with a constant demand for its products, the ever-growing enthusiasm among consumers for quality and safety food with a clear regional identity motivates a potential expansion of the viniculture area, along with that of Almirante Tamandaré, once their similarities have been pointed out.

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