Determination of bioactive amines in tropical wines produced at Brazilian's Northeast

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ABSTRACT

The wine production in the region of the Sub-middle São Francisco river Valley has started there are few years ago.The region has a climate variability that allows to obtain two or three harvests per year, which influences significantly the composition of the grapes. This work aimed to evaluate the amount of amines present in white and red tropical wines produced in the Northeast of Brazil. The winemaking was done by the traditional method. After stabilizing the wines were bottled and then analyzed for the determination of the amines putrescine, spermidine and spermine, by thin layer chromatography (TLC). The harvests were in June (harvest I) and November (harvest II) 2009. The results showed that the edaphoclimatic conditions of the two harvests had significant influence ($p > 0.05$) on bioactive amines concentration, with high values for red wines. In addition, tropical wines from Sub-middle São Francisco river Valley showed adequate correlation between bioactive amines as compared to other wines in the world.

Keywords: *Vitis vinifera* L., oenological potential, semiarid climate, tropical wines, regional identity.

RESUMO

A produção de vinhos na região do Submédio do Vale do São Francisco começou a poucos anos. A região apresenta uma variabilidade climática que permite a obtenção de duas a três safras anuais, o que influencia de forma significativa a composição das uvas. Este trabalho teve como objetivo avaliar os teores de aminas presentes em vinhos brancos e tintos elaborados no Nordeste do Brasil. A vinificação foi realizada através do método tradicional. Após a estabilização os vinhos foram engarrafados e posteriormente analisados para a determinação das aminas putrescina, espermidina e espermina, através de cromatografia de camada delgada (CCD). As colheitas foram realizadas em junho (safra I) e novembro (safra II) de 2009. Os resultados monstraram que as condições edafoclimáticas da safras I e II apresentaram influência significativa ($p > 0,05$) em relação aos teores de aminas bioativas, que foram maiores para os vinhos tintos. Além disso, os vinhos tropicais do Vale do Submédio São Francisco apresentaram correlação adequada entre as aminas bioativas se comparados a outros vinhos do mundo.

Palavras-chave: *Vitis vinífera* L., potencial enológico; clima semiárido, identidade regional.

INTRODUCTION

The Sub-middle of the São Francisco river Valley is a region located in Northeast of Brazil, presenting high temperatures throughout the year (annual average temperature is 26,5ºC), high luminosity and available water for irrigation, being possible to have two or three harvests per year, depending on the cycle of each cultivar. In this way, the physico-chemical composition and wine quality can vary greatly depending of the harvest date and winamaking process, according to the intra-annual climate variability (TONIETTO; CARBONNEAU, 1999; TONIETTO; TEIXEIRA, 2004).

Bioactive amines are nitrogenous compounds of low molecular weight in which one, two or three hydrogen atoms of ammonia are replaced by alkyl or aryl groups. Amines are formed during normal metabolic processes in all living organisms and thus are present in foods and are essential for cell division and growth (BARDÓCZ, 1995).

The polyamine spermidine is usually abundant in the pericarp of grapes, followed by binding its precursor, putrescine. Other amines, such as spermine, agmatine, cadaverine, histidine, tyramine and phenylethylamine are also found in smaller amounts. The seeds of the grapes also contain spermine, putrescine and cadaverine in high concentrations (SHIOZAKI *et al*. 2000; KISS *et al.*, 2006).

Due to the lack of studies related to the presence of bioactive amines in brazilian tropical wines, this work aimed to evaluate the influence of the two harvests, in June and November 2009, on the concentrations of the amines putrescine, spermidine and spermine in white and red wines produced in the semiarid tropical climate conditions.

MATERIAL AND METHODS

The work was performed using Verdejo, Sauvignon blanc, Viognier, Petit Verdot, Tempranillo and Syrah grape cultivars. The grapes were harvested from plants located in a commercial vineyard, conducted in trellis system, spaced 2.5 x 1.5 m, grafted on the rootstock IAC-766, using drip irrigation. The wines were elaborated by the traditional methods (PEYNAUD, 1997). The clusters were destemmed and lightly crushed. The must was placed in 200 L steel tank, adding SO2 at 40 $mg.L^{-1}$ and active dry yeast (*Saccharomyces cerevisiae*) at 0.2 $g.L^{-1}$. The white wine fermentation occurred at 18 °C. For red wines clusters were destemmed and lightly crushed, the maceration time was five days with two daily pumping. Alcoholic fermentation was conducted at 25 °C, and malolactic fermentation occurred at 18 °C. At the end of the fermentation, the wines were stabilized in a cold chamber at 0 ºC for 30 days, then wines were bottled and analyzed.

The determination of bioactive amines putrescine, spermidine and spermine was performed through thin layer chromatography method (Flores; Galston, 1982) optimized (Lima *et al.*, 2006). The quantification was performed using standards of putrescine, spermidine and spermine (Sigma-Aldrish) applied with the samples on glass plates (20 x 20 cm) coated with silica gel 60G (0.25 micron) (Merck) and expressed in $mg.L^{-1}$. All tests were performed in triplicate and submitted to analysis of variance, using Tukey test at 5% significance level, with the statistical program version 7.5 beta Assistat (2008).

RESULTS AND DISCUSSION

Influence of the harvest date on the concentrations of putrescine, spermidine and spermine

The Tab. 1 shows the results for evaluation of average levels of putrescine, spermidine and spermine in Verdejo, Sauvignon blanc, Viognier, Petit Verdot, Tempranillo and Syrah wines, for the first (harvest I) and second harvest (harvest II) of 2009. Significant differences ($p> 0.05$), were found in the harvest I for spermidine, 17 times higher than the levels of the harvest II. For spermine, results showed great similarities between the harvests.

The results may be explained by the different climate conditions found in each season. For wines elaborated from grapes harvested in June (harvest I), the rainfall was lower than November (harvest II), although the temperatures of this period were higher. For both harvest seasons of 2009 the sum of the levels of polyamines spermidine and spermine was higher than the levels of putrescine, showing thus a positive relationship between the concentrations of these amines. High concentrations of putrescine in wine may be undesirable to contribute with a putrid aroma, it's a precursor required for synthesis of polyamines and its presence in wines is crucial, since the sum of total polyamines (spermidine and spermine) is greater than the content of putrescine, providing a flavor balance and antioxidant effects (FLORES *et al.* 1989; BARDÓCZ, 1995; WALTERS, 2003; GLORIA, 2005; Garcia-Villar *et al.,* 2007). Soufleros *et al.* (2007) found significant correlation between the levels of the amines putrescine and spermidine present in wines. According to Martin-Alvarez *et al.* (2006), the different climate conditions can affect the levels of free amino acids and microflora of the grape, which can influence the formation of the amines.
The results suggest that the climate conditions of the Sub-middle São Francisco river Valley allowed the production of grapes from the varieties evaluated, with higher concentrations of total polyamines (the sum of the levels of spermidine and spermine) and lower content of putrescine, showing a positive correlation between the concentrations of these amines.

Tab. 1 Effect of the harvest date (harvest I in June and harvest II in November2009) on putrescine (PUT), spermidine (EPD) and spermine (ESM) concentrations in brazilian tropical wines

2009	Concentration (mg.L -1) *		
	Putrescine	Spermidine	Spermine
Harvest I (June)	0.467 ± 0.44 aa	0.446 ± 0.07 ab	0.135 ± 0.09 BC
Harvest II (November)	0.086 ± 0.01 bB	0.026 ± 0.01 bc	0.102 ± 0.04 ba

* Means (± SD) followed by same letter do not differ (lowercase for columns and uppercase letters for rows), by Tukey test at 5% probability.

Influence of the grape varieties on the levels of bioactive amines
Tab. 2 presents the mean levels of putrescine, spermidine and spermine of the harvests I and II of 2009, determined in white wines (Verdejo, Viognier and Sauvignon blanc) and red wines (Petit Verdot, Tempranillo and Syrah). Verdejo wines had lower levels of putrescine as compared to other white and red wines. It was observed that spermidine was present in higher concentrations, suggesting a correlation between the amines, if the sum of the polyamines spermidine and spermine is higher than those of putrescine.
According to Drolet *et al.* (1986) and Bardócz (1995), spermine and spermidine, as well as the diamines putrescine and cadaverine, are effective sequestrants of free radical in many chemical enzyme systems in vitro. They can inhibit lipid peroxidation and preventing senescence, are important to stability and permeability of cell membranes and reduce mucosal permeability to macromolecules and allergenic proteins, preventing food allergies (DROLET *et al.* 1986; BARDÓCZ, 1995; LOSER, 2000).
The three red wines evaluated showed high levels of putrescine. However, Petit Verdot and Tempranillo wines showed adequate correlation between the concentrations of putrescine and spermidine and spermine, the sum of these two being greater than the concentration of putrescine. Syrah wines had higher levels of putrescine (0.676 $mg.L^{-1}$) as compared to white and other red wines, and the sum of the contents of spermidine and spermine polyamines were lower than those of putrescine, contributing negatively for the aroma, in this case. Mo Dugo *et al.* (2006) showed the levels obtained for Petit Verdot, Tempranillo and Syrah wines for putrescine and the levels were 0.7 mgL^{-1}, 0.1 mgL^{-1} and 0.4 mgL^{-1}, respectively. The amines spermidine and spermine were not determined.

According to Bover-Cid *et al.* (2006), for red winemaking process is usually performed in the presence of the skins and pulp, so the putrescine level can be increased. This fact could explain higher levels of this amine in red wines. Petit Verdot wines presented the best results in this study, showing correlation between the total amines spermidine and spermine and putrescine content, providing more balance in flavor and aroma.

The polyamines have antioxidant effects, protecting cells, membranes, nucleic acids and polyunsaturated fatty acids against oxidative damage. In this way the presence of polyamines in wine is desirable in appropriate concentrations (LOVAAS, 1997). The values obtained in this study to the types and levels of bioactive amines have shown that red wines produced in a tropical condition presented adequate correlation between the amines as compared to other wines in the world, only Syrah wines were disbalanced according to the spermidine and spermine concentration, that were lower than putrescine.

Tab. 2 Concentration of putrescine (PUT), spermidine (EPD) and spermine (ESM) in brazilian tropical red and white wines elaborated in 2009.

Varieties	Concentration ($mg.L^{-1}$)*		
	Putrescine	Spermidine	Spermine
Verdejo	0,076 ± 0,00 cF	0,206 ± 0,27 aC	0,136 ± 0,08 bB
Sauvignon blanc	0,096 ± 0,00 bE	0,200 ± 0,25 aC	0,106 ± 0,04 bC
Viognier	0,165 ± 0,13 bD	0,236 ± 0,27 aB	0,130 ± 0,04 cB
Petit Verdot	0,306 ± 0,32 aC	0,215 ± 0,26 bC	0,200 ± 0,14 bA
Tempranillo	0,340 ± 0,34 aB	0,280 ± 0,37 bA	0,071± 0,02 cD
Syrah	0,676 ± 0,83 aA	0,280 ± 0,36 bA	0,068 ± 0,02 cD

* Means (± SD) followed by same letter do not differ (lowercase for columns and uppercase letters for rows), by Tukey test at 5% probability.

CONCLUSION

The tropical wines produced in Northeast of Brazil showed adequate correlation between bioactive amines as compared to other wines, because it showed higher levels of total polyamines (sum of the contents of spermidine and spermine) and lower content of putrescine.

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