Biogenic Amine Production during Spontaneous and Inoculated MLF of Zweigelt Wines

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Summary

Biogenic amines are organic nitrogenous compounds produced in wines mainly during malolactic fermentation (MLF). Its presence is a health risk and it could negatively affect the wine quality. The objective of this work was to determine the biogenic amines content in Croatian Zweigelt wines produced with different MLF inoculation time. Biogenic amines were determined by liquid chromatographic method. Results showed significant influence of MLF on the concentrations of biogenic amines. Control wines had the lowest amount of total biogenic amines while wines in which MLF was conducted after alcoholic fermentation had significantly the highest concentrations of total biogenic amines. Between them histamine was the most abundant amine ranging from 1.14 mg/L in control wines up to 2.94 mg/L in wines that undergo MLF after alcoholic fermentation. It can be concluded that MLF process and time of inoculation can significantly influence the formation of biogenic amines. These results suggest that co-inoculation using *Oenoccocus oeni* commercial strain Uvaferm ß is a worthwhile alternative compared to traditional post AF inoculation for Zweigelt winemaking.

Key words

biogenic amines, coinoculation, histamin, Zweigelt

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Introduction

Biogenic amines (BA) are nitrogenous low molecular weight organic bases that can have an aliphatic (putrescine, cadaverine, spermidine, spermine), aromatic (histamine, tyramine) or heterocyclic (tryptamine) structure. They are widely present in food, especially in fermented food, mostly as a consequence of the decarboxylation of their free precursor amino acids (Vincenzini et al., 2009). These amines are a health risk for sensitive individuals. Symptoms include: nausea, respiratory discomfort, hot flushes, cold sweat, palpitations, headaches and an increase or decrease of blood pressure (Anli and Bayram, 2009). Additionally, wines as matrix increases these adverse effects due to the concomitant intake of ethanol, which reduces/inhibits the activities of monoamine oxidase and diamine oxidase, enzymes responsible of metabolizing different kind of amines. Some amines as putrescine, cadaverine, spermine and spermidine can increase the histamine toxicity (Ancin-Azpilicueta et al., 2008). The levels of biogenic amines produced in wine largely depend on the abundance of amino acid precursors in the medium, whose content can be influenced by vinification methods, grape variety, geographical region and vintage (Moreno-Arribas et al., 2000), but it is generally accepted that the biogenic amines formation is highly dependent on the nature of lactic acid bacteria responsible for malolactic fermentation (Ancin-Azpilicueta et al., 2008). During winemaking process two successive fermentations are involved, an alcoholic fermentation (AF) conduced by yeasts, and malolactic fermentation (MLF) carried out by wine lactic acid bacteria. Both, AF and MLF may occur spontaneously from the activity of yeasts and bacteria naturally present in musts and wines, although in the last years winemakers are starting to recognize the benefits of inoculating with commercial starter cultures of yeast and lactic acid bacteria (Izquierdo et al., 2012). Extensive research has been done to correlate biogenic amine production in wine with species of lactic acid bacteria involved in the winemaking process. In the past *Pediococcus* spp. were held responsible for histamine production in wine (Delfini, 1989) but nowadays it is widely known that *Lactobacillus*, *Leuconostoc* and *Oenococcus* spp. are also implicated in biogenic amine production (Moreno-Arribas et al., 2000). Commercial *O. oeni* strains are selected for their oenological parameters, including the absence of amino acid decarboxylases. Guerrini et al. (2002) found that *O. oeni* ability to produce biogenic amines varies and is strain dependent while MorenoArribas et al. (2003) found out that none of four commercial malolactic starter cultures tested could produce histamine, tyramine or putrescine. Malolactic fermentation (MLF) is an integral step in red winemaking. In addition to deacidifying wine it can also influence the composition of volatile fermentation-derived compounds with concomitant affects on wine sensory properties. Long-established winemaking protocols for MLF induction generally involve inoculation of bacteria starter cultures post alcoholic fermentation, however, more recently there has been a trend to introduce bacteria earlier in the fermentation process (Abrahamse and Bartowsky, 2012). Results from some studies have shown that simultaneous yeast/bacteria inoculation poses important risks, such as the development of undesirable/antagonistic interactions between the two microorganisms, stuck fermentation, production of off-odours and higher concentrations of acetic acid (Alexsandre et al., 2004; Carrete et al., 2002). On the other hand, some authors (Krieger et al., 2007) pointed out better performance of the bacteria added at the beginning of alcoholic fermentation due to the low alcohol concentration and higher nutrient availability present in musts. More recently studies carried out by Zapparoli et al. (2009) have reported a reduction in total fermentation time and better control of the malolactic fermentation, due to the early dominance of the inoculated bacterial strain. This study investigates the effects of inoculating 'Zweigelt' grape must with malolactic bacteria at the beginning of alcoholic fermentation (co-inoculation, with yeast) and post alcoholic fermentation (sequencial inoculation) on the basic chemical composition and concentrations of biogenic amines.

Materials and methods

Samples and fermentation

Grape from 'Zweigelt' produced in Zagreb winegrowing region northwest Croatia was harvested at optimum maturity in 2010. Grape was harvested manually, placed in plastic boxes and transported to the vinery. Grape was destemmed, crushed and then transferred into stainless steel tanks for maceration that lasted eight days at 25°C. The experiment included four treatments: a) control wine made without malalactic fermentation (MLF); b) wines where spontaneous MLF was conducted; c) wines where malolactic bacteria Uvaferm ß (Lallemand) was inoculated 24 h after yeast inoculation (COI); d) wines where malolactic bacteria Uvaferm *Alpha* (Lallemand) was inoculated after completion of alcoholic fermentation (SEQ). Alcoholic fermentation of all treatments (in triplicate) was started with selected wine yeast Lalvin ICV D254. At the end of fermentation all the wines were racked, total sulphur dioxide adjusted to 50 mg/L and left to mature in cellar conditions. Basic chemical analyses of must and wine were done using methods proposed by O.I.V. (2001).

HPLC determination of biogenic amines

The biogenic amines content was determined by HPLC method according to Soleas et al. (1999). The derivatizing reagent comprised 1 g *o*-phthalaldehyde per liter of 0.05 M sodium tetraborate containing 2 % (v/v) methanol and 0.2% mercaptoethanol. Twenty five microliters of OPA reagent was reacted with 25 µL of the sample for 99 s and the mixture was filtered through a 0.45 µm filter (Nylon Membranes, Supelco, Bellefonte, USA) before the HPLC analysis. Twenty microliters of each sample were injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Fluorescence detector Varian ProStar 363 (Varian, Walnut Creek, USA), using a reversed-phase column Pinnacle II C-18 (Restek, USA) (150 x 3.9 mm, 5 µm i.d.). Chromatographic conditions were: solvent (A): 0.05 M sodium acetate buffer adjusted to pH 6.6 / tetrahydrofuran (96:4); solvent (B) 100% methanol at a flow rate of 1.2 mL*min^{-1}. The elution was performed with a gradient starting at 100% B to reach 53% B at 2.5 min, 70% B at 7.5 min and 100% B at 15 min, and becoming isocratic for 10 min. Detection was carried out using 340 nm and 420 nm as excitation and emission wavelengths, respectively. The content of each analyte was obtained by direct interpolation of the peak area in the correspondent linear calibration curve. Certain biogenic amines being in salt form, the weight of the salt was taken into account when determining the true weight of the biogenic amine. The data acquisition and treatment were conducted using the Star Chromatography Workstation Version 5 software.

Statistical analysis

One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) comparison test of SAS (SAS Institute, Cary, NC, USA) were used to interpret statistical differences in means, if any, at the $P < 95\%$ confidence level.

Results and discussion

The success of malolactic fermentation is influenced by several oenological parameters, such as pH, temperature, alcohol content and SO_2 concentration (Henick-Kling 1993). Low pH (<3.0) and high alcohol content can have negative influence on the survival of LAB, and consequently malolactic fermentation. As it can be seen in Table 1 pH value and malic acid concentration in Zweigelt must was not too low for causing deacidification pathway problems. As it can be seen from the data shown in Table 2, there were no pronounced difference in basic chemical composition of tested wines. Significant differences were noted in total acidity, pH and ash content between control wines and wines where malic acid degradation was achieved. Important to notice, there was no significant difference in volatile acidity between wines where coinoculated MLF was achieved and wines where malic acid degradation was done after alcoholic fermentation. A slight degradation in spontaneous malolactic fermentation wines could be seen, even there were no significant differences in citric acid concentrations between tested wines. The concentration of biogenic amines produced in wine largely depend on the abundance of amino acid precursors in the medium since biogenic amines increase with an increase in amino acids. Amino acid concentration may be influenced by vinification methods, grapevine variety, geographical conditions (ecological conditions) and vintage (Soufleros et al., 1998). Free amino nitrogen (FAN) concentration was relatively low in used Zweigelt must (Table 1). The minimum amount of nitrogen necessary for a correct fermentation is usually between 120 and 140 mg N/L of must (Blateyron and Sablayrolles, 2001). However, differences in amino acid uptakes and metabolism of different yeast strains can lead to defective fermentation, even in musts with nitrogen content higher than 140 mg N/L. In our case there was no problem with fermentation kinetics leading in all treatments to the total sugar degradation. In general, putrescine is the major biogenic amine found in wines and putrescine producing capability may be considered widespread among lactic acid bacteria strains of oenological interest (Moreno-Arribas et al., 2003). Putrescine concentrations in Zweigelt wines were relatively low (Table 3) and not in accordance with data

Table 1. Basic chemical composition of 'Zweigelt' grape must, 2010.

Sugar (°Oe)	86
Tartaric acid (g/L)	5.5
Malic acid (g/L)	2.2
Lactic acid (g/L)	–
Citric acid (g/L)	0.3
pH	3.32
FAN (mg/L)	109.58

Table 2. Determined basic chemical characteristics of Zweigelt wines produced in 2010 according to different treatment

Compounds	Control	Spontaneous MLF	Coinoculated MLF (COI)	Inoculaton after AF (SEQ)
Alcohol (vol%)	12.1a	12.2a	12.2a	12.3a
Residual sugar (g/L)	2.6b	2.4b	2.2a	2.0a
Total extract (g/L)	23.1b	21.6a	23.4b	22.5a
Total acidity (g/L)	6.9a	5.8b	5.9b	5.8b
Volatile acidity (g/L)	0.3a	0.4a	0.4a	0.4a
pH	3.40a	3.48b	3.48b	3.48b
Ash (g/L)	2.33a	2.29a	2.43b	2.49b
Tartaric acid (g/L)	3.5a	3.2b	3.2b	3.3b
Malic acid (g/L)	2.1	-	-	-
Lactic acid (g/L)	0.1a	1.6b	1.8b	1.8b
Citric acid (g/L)	0.3a	0.2a	0.3a	0.3a
Succinic acid (g/L)	0.2a	0.2a	0.2a	0.2a
FAN (mg/L)	25.67b	23.12a	22.42a	36.37c
Total phenols (mg/L, galic acid equivalents)	1344.5a	1341.0a	1423.23b	1325.11a
Total antocyanins (mg/L malvidin 3-glycoside equivalents)	381.14a	344.96a	437.61b	356.52a
Flavan-3-ols (mg/L, catechin equivalents)	540.74a	557.67a	581.47b	523.39a

Note: Different letters beside the mean of a compound denote a significant difference among treatments (a, b, c for 5%)

Table 3. Concentration of determined biogenic amines (mg/L) in Zweigelt wines

Biogenic amine	Control	Spontaneous MLF	Coinoculated MLF (COI)	Inoculaton after AF (SEQ)
Histamine	1.14a	2.53b	2.46b	2.94c
Tyramine	0.15a	0.33b	0.21a	0.31b
Putrescine	0.23a	0.33b	0.24a	0.31b
Cadaverine	0.15a	0.32b	0.18a	0.23a
2-Phenylethylamine	0.79a	0.89ab	0.94b	1.13c
Spermidine	0.59a	0.73b	0.79bc	0.87c
Tryptamine	1.41a	1.77c	1.55b	2.10d
Spermine	0.03a	0.04a	0.07b	0.06b
Serotonine	0.05a	0.11b	0.07a	0.12b
Σ	4.54a	7.05c	6.51b	8.07d

Note: Different letters beside the mean of a compound denote a significant difference among treatments (a, b, c for 5%)

showed by Izquierdo et al., (2012) where putrescine concentrations were between 2.92 and 4.66 mg/L. Most abundant biogenic amine in tested wines was histamine ranging from 1.14 mg/L in control wines up to 2.94 mg/L in wines where malolactic fermentation was achieved. As it can be seen coinoculation of lactic acid bacteria had positive impact on biogenic amines showing significantly lower concentrations compared with spontaneous wines and wines where MLF was done after AF. Total biogenic amines concentrations in control wines were the lowest confirming data by Martin-Alvarez et al., (2006) pointing out lactic acid bacteria influence in biogenic amine production. Our results showed no toxic dose in Zweigelt wines tested ranging between 1.14-2.94 mg/L for histamine, 0.15-0.33 mg/L for tyramine and 0.79-1.13 mg/L for phenylethylamine. The toxic dose of these biogenic amines in alcoholic beverages is considered to be between 8 and 20 mg/L for histamine, 25 and 40 mg/L for tyramine, but as little as 3 mg/L phenylethylamine can cause negative physiological effects (Soufleros et al., 1998).

Conclusions

The current study has shown that MLF process and time of inoculation of malolactic bacteria can significantly influence the formation of biogenic amines during red wine production process. In summary, simultaneous inoculation of the lactic bacteria *O. oeni* Uvaferm ß and commercial yeast strain had positive influence on chemical composition of produced Zweigelt wines as a consequence of lower amount of some biogenic amines, e.g. histamine, putrescine, and tyramine. These results suggest that co-inoculation using *Oenoccocus oeni* commercial strain Uvaferm ß is a worthwhile alternative compared to traditional post AF inoculation for Zweigelt winemaking.

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