

# Concentration of Biogenic Amines in 'Pinot Noir' Wines Produced in Croatia

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Ana JEROMEL<sup>1</sup> (✉)

Karin KOVAČEVIĆ GANIĆ<sup>2</sup>

Stanka HERJAVEC<sup>1</sup>

Marin MIHALJEVIĆ<sup>1</sup>

Ana Marija JAGATIĆ KORENIKA<sup>1</sup>

Ivana RENDULIĆ<sup>3</sup>

Marijana ČOLIĆ<sup>4</sup>

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## Summary

The origins of biogenic amines are sound grapes, alcoholic fermentations, malolactic fermentation and microbial activities during wine storage. These biologically produced amines are essential at low concentrations for optimal metabolic and physiological functions in animals, plants and micro-organisms. During alcoholic fermentation the degree of maceration is the first factor that affects the extraction of compounds present in the grape skin, among them aminoacids, precursors of biogenic amines. The aim of the present work was to study the changes of the concentration of biogenic amines in wines made from *Vitis vinifera* 'Pinot noir' from Plešivica (vintage 2009) produced with classical maceration, cold maceration and use of sur lie method. Biogenic amines were quantified using a reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection after pre-column derivatization with *o*-phthalaldehyde (OPA). In 'Pinot noir' wines tested, histamine was the most abundant biogenic amine followed by tryptamine and 2-Phenylethylamine. Total amount of biogenic amines ranged from 8.72 mg/L in wines made with classical maceration up to 9.34 mg/L in sur lie wines. In summary, from the results obtained in this study, it can be concluded that sur lie technology can influence the formation of biogenic acids since the release of amino acids is probably more pronounced in wines aged with lees and stirred weekly. No significant differences were found in the concentration of biogenic amines in relation to the used maceration process.

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## Key words

biogenic amines, 'Pinot noir', maceration, sur lie

<sup>1</sup> University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb

✉ e-mail: [amajdak@agr.hr](mailto:amajdak@agr.hr)

<sup>2</sup> University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

<sup>3</sup> Zagreb County, Ulica grada Vukovara 72, 10000 Zagreb, Croatia

<sup>4</sup> student, University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia

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## Introduction

Biogenic amines (BA) are nitrogenous low molecular weight organic bases that can have an aliphatic, aromatic or heterocyclic 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). The amines histamine, tyramine, putrescine, cadaverine, 2-phenethylamine, agmatine and tryptamine originate from the precursor amino acids histidine, tyrosine, ornithine, lysine, phenylalanine, arginine and tryptophane (Bauza et al., 1995). Musts from grape usually contain low concentration of BA, almost entirely represented by spermidine and putrescine. This diamine is reported to be synthesized by the vine in response to stress conditions (Halasz et al. 1994). Wines are usually characterized by a significantly higher content of BA, red wines compared to white being generally characterized by higher BA content. Soufleros et al. (2007) reported up to 2.11 mg/L histamine, 3.65 mg/L tyramine and 5.23 mg/L putrescine in red wines from Greece, while Marcobal et al. (2005) found in red Spanish wines 3.62 mg/L histamine, 1.40 mg/L tyramine and 7.06 mg/L putrescine. Generally, the toxic dose in alcoholic beverages is considered to be between 8 and 20 mg/L for histamine, 25 to 40 mg/L for tyramine, but as little as 3 mg/L phenylethylamine can cause negative physiological effects (Soufleros et al., 1998). A wide variety of viticultural and oenological factors may have an impact on the levels of biogenic amines produced in wine. While some factors can increase the precursor amino acid concentration in the grape and wine (grape variety, geographical region, vintage, grape skin maceration, ageing practices), other factors influence diversity, growth, decarboxylase activity that can potentially produce the biogenic amines. Some amines, such as putrescine and other polyamines, may be already present in grape berries (Bover-Cid et al., 2006). 'Cabernet sauvignon' was found to have high concentration of putrescine, cadaverine and spermidine in the pericarp of the berries (Glória et al., 1998). Putrescine, cadaverine and spermidine have been also found in high concentration in the seeds of grape berries (Kiss et al., 2006). Therefore, putrescine concentration in wine may be influenced more by geographical region and grape variety than by winemaking practices. Biogenic amines are also dependent on grape variety and vine nutrition, which will determine the concentration and composition of precursor amino acids in grape must (Soufleros et al., 1998). Among the factors that have been suggested as favouring the abundance of amines in wine, some winemaking practices, such as nutrient addition and duration of wine contact with both grape skins and yeast lees, seem to play a major role because they can directly affect the content of the precursor amino acids of BA (Vincenzini et al., 2009). Grape skin maceration promotes extraction of grape components such as phenolic compounds, proteins, amino acids and polysaccharides. Soleas et al. (1999) found no correlation between length of skin contact and concentration of biogenic amines, while Martín-Alvarez et al. (2006) found that the duration of skin maceration significantly affects biogenic amine content in wine.

During ageing of the wine with lees, amino acids are released by yeast and bacterial autolysis. According to Alcaide-Hidalgo et al. (2007) this release of amino acids is more pronounced in wines aged with lees and stirred weekly. Therefore, precaution

should be taken when wines are aged with lees, since this increases the risk of the formation of biogenic amines by residual decarboxylase activities of the lactic acid bacteria responsible for malolactic fermentation.

The aim of the present work was to study the changes of the content of biogenic amines in wines made from grapevine variety 'Pinot noir' (*Vitis vinifera* L.) from Plešivica in vintage 2009 and produced with classic maceration, cold maceration and use of sur lie method.

## Materials and methods

### Samples and fermentation

Grape from 'Pinot noir' produced in northwest Croatia, Plešivica winegrowing district was harvested at optimum maturity in 2009. Grape was harvested manually, placed in plastic boxes and transported to the winery. Grape was destemmed, crushed and then transferred into stainless steel tanks for maceration. The cold-maceration was carried out controlling the skin contact time for five days at temperature below of 15°C by using dry ice. After the cold-maceration period was completed, the temperature of the tanks was left to rise up to 25°C to allow starting the alcoholic fermentation. Classic fermentation was carried out on controlling the skin contact for eight days at 25°C. After this, the mash was drawn off to remove the skins and other solid parts, and the free-run musts were transfer to 225 L barrique barrels and left to finish the fermentation under the same conditions. Alcoholic fermentation was carried out using of Uvaferm 229 starter culture (Lallemand). At the end of fermentation all wines were inoculated with commercial pure strain culture Uvaferm *Alpha* (Lallemand) for conduction of malolactic fermentation. After that, the wine was racked, total sulphur dioxide adjusted to 50 mg/L and left to mature in cellar conditions. Sur lie wines were aged by weekly stirring of lees during six months period, when all the wines (from five repetition) were taken for analysis. 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). Tryptamine (Trp) - purity ≥ 99%, hydroxytyramine chlorhydrate (Htyr) - purity ≥ 98%, phenylethylamine (Pea) - purity ≥ 99%, putrescine (Put) - purity ≥ 98%, cadaverine (Cad) - purity ≥ 99%, serotonin chlorhydrate (Ser) - purity ≥ 98%, spermine (Spm) - purity ≥ 97%, spermidine (Spd) - purity ≥ 99%, sodium tetraborate - purity ≥ 99%, methanol - purity ≥ 98%, tetrahydrofuran - purity ≥ 99%, and mercaptoethanol - purity ≥ 99%, were obtained from Sigma-Aldrich, Steinheim, Germany. Histamine (Hist) - purity ≥ 99%, tyramine (Tyr) - purity ≥ 99%, sodium acetate - purity ≥ 99%, and *o*-phthalaldehyde (OPA) application for fluorescence - purity ≥ 99%, were purchased from Merck, Darmstadt, Germany. The derivatizing reagent comprised 1g *o*-phthalaldehyde per liter of 0.05 M sodium tetraborate containing 2% (v/v) methanol and 0.2% mercaptoethanol. 25 µL 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 column (Restek, USA) (150 x 3.9 mm, 5 $\mu$ m 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. 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. Because certain biogenic amines are 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

As it can be seen from the data shown in Table 1 there was no pronounced difference in basic chemical composition of tested wines. Significant difference was noted in ash, total phenols and in flavan-3-ols concentration mainly due to used fermentation technique. The importance of wine lees in this context comes from the fact that lees can adsorb phenolic compounds (Mazauric and Salmon, 2005) and release to wine both phenolic compounds (Somers et al., 1987) and enzymes (after autolysis) that can modify the phenolic fraction concentration (Ibern-Gómez et al., 2000).

The concentration of biogenic amines produced in wine largely depends 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). According to our results vinification method significantly influenced the concentration of some biogenic amines (Table 2). Wines aged on the lees had significantly higher concentration of histamine, 2-phenylethylamine and tryptamine while there was no difference between wines produced with classical maceration and cold maceration. Martin-Álvarez et al. (2006) left their wines in contact with lees for two months after alcoholic fermentation and noticed increase in methylamine and putrescine while tyramine concentration was lower. Our results are not in agreement with that statement, probably because wines were analyzed after longer ageing period. Various research groups (Gerbaux and Monamy 2000, Landete et al., 2005) noticed that after the initial increase in biogenic amines during storage, a general decrease or stabilization in concentration could be observed. But Coton et al. (1998) found out that even when no more culturable cells were detectable, histidine decarboxylase could still be

**Table 1.** Determined chemical characteristics of wines 'Pinot noir' produced in 2009 according to different treatment

Compounds	Classic maceration	Cold maceration	Sur lie	LSD5%
Alcohol (vol%)	13.68	13.71	13.62	n.s.
Residual sugar (g/L)	2.5	2.9	2.2	n.s.
Total extract (g/L)	26.54	26.38	27.94	n.s.
Total acidity (g/L)*	6.9	6.8	6.5	n.s.
Volatile acidity (g/L)**	0.62	0.60	0.69	n.s.
pH	3.62	3.69	3.71	n.s.
Ash (g/L)	2.36a	2.28a	2.49b	0,08
Total phenols (mg/L, galic acid equivalents)	2047.11a	2098.23b	2157.51c	19.95
Flavan-3-ols (mg/L, catechin equivalents)	145.56a	147.23a	195.11b	8.45

Different letters beside the mean of a compound denote a significant difference among treatments; n.s.: not significant; \*as tartaric acid; \*\*as acetic acid

**Table 2.** Concentration of determined biogenic amines in 'Pinot noir' wines. Means and standard errors are presented (mg/L $\pm$ s.e.)

Biogenic amine	Classic maceration	Cold maceration	Sur lie	LSD 5%
Histamine	3.26 $\pm$ 0.06	3.36 $\pm$ 0.09	3.49 $\pm$ 0.06	0.12
Tyramine	0.17 $\pm$ 0.01	0.20 $\pm$ 0.01	0.22 $\pm$ 0.02	n.s.
Putrescine	0.39 $\pm$ 0.09	0.40 $\pm$ 0.08	0.46 $\pm$ 0.07	n.s.
Cadaverine	0.32 $\pm$ 0.05	0.34 $\pm$ 0.06	0.40 $\pm$ 0.05	n.s.
2-Phenylethylamine	1.35 $\pm$ 0.13	1.47 $\pm$ 0.15	1.69 $\pm$ 0.19	0.11
Spermidine	0.52 $\pm$ 0.11	0.59 $\pm$ 0.09	0.61 $\pm$ 0.09	n.s.
Tryptamine	2.55 $\pm$ 0.10	2.74 $\pm$ 0.12	2.89 $\pm$ 0.17	0.09
Spermine	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.08 $\pm$ 0.02	n.s.
Serotonine	0.08 $\pm$ 0.05	0.11 $\pm$ 0.04	0.10 $\pm$ 0.04	n.s.
$\Sigma$	8.72 <sup>a</sup>	8.86 <sup>a</sup>	9.45 <sup>b</sup>	0.19

active-thus biogenic amines can be produced during aging. In 'Pinot noir' wines tested, histamine was the most abundant biogenic amine followed by tryptamine and 2-Phenylethylamine. 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). According to Alcaide-Hidalgo et al. (2007) putrescine content of the wines aged with lees increases during ageing, although it remains stable in those aged without lees. As shown in Table 2 putrescine concentration in 'Pinot noir' was relatively similar and low probably because malolactic fermentation was conducted by commercial pure strain culture *Uvaferm Alpha*. In summary, from the results obtained in this study, it can be concluded that sur lie technology can influence the formation of biogenic acids since the release of amino acids is probably more pronounced in wines aged with lees and stirred weekly. No significant differences were found in the concentration of biogenic amines in relation to the used maceration process. Our results showed no toxic dose in 'Pinot noir' wines tested ranging between 3.26-3.49 mg/L for histamine, 0.17-0.22 mg/L for tyramine, and 1.35 -1.69 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).

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