# Influence of the immobilized yeast cells technology on the presence of biogenic amines in wine

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## Summary

Biogenic amines are basic nitrogenous low molecular weight compounds with biological activity. Biogenic amines are important because they contain a health risk for sensitive humans. Biogenic amines in the wine can be formed from their precursors by various microorganisms present in the wine, at any stage of production. The aim of the present work was to study the changes of the content of biogenic amines in wines made from grape variety *Frankovka* and *Pinot noir* (*Vitis vinifera* L.) from Kutjevo vineyards, located in the east part of continental Croatia, vintage 2012, produced with cold maceration and use of fermentation method with immobilized yeast cells. Biogenic amines were quantified using a reversed-phase high performance liquid chromatography (RP-HPLC). Histamine was the most abundant biogenic amine followed by 2-Phenylethylamine. Total amount of biogenic amines ranged from 8.81 mg/L in wines produced with immobilized yeast cells up to 9.91 mg/L in wines made in classical fermentation process. From the results obtained in this study, it can be concluded that immobilized yeast cells technology can influence on the formation of biogenic amines.

*Keywords:* biogenic amines, immobilized yeast cells

## Introduction

Red wine is a fickle mistress, we swirl, we sniff, we sip, but, unfortunately for our heads, most of us don't spit. Main suspect behind the red wine headache are biogenic amines. Consequently the presence of biogenic amines in wine is becoming increasingly important to consumers and producers. Biogenic amines are important because they contain a health risk for sensitive humans. Beside headaches, symptoms include nausea, respiratory discomfort, hot flushes, cold sweat, palpitations, red-rash, high or low blood pressure.

Biogenic amines (BA) are nitrogenous low molecular weight organic bases that can have an aliphatic, aromatic or heterocyclic structure. They are widely present in fermented beverages, mostly as a consequence of the decarboxylation of their free precursor amino acids (Vincenzini et al., 2009). Until now, different analytical methods have been developed to determine BA in foodstuffs samples. Concerning wine analysis, most analytical methods used for the determination of BA are based on chromatographic methods. HPLC technology has been the most popular analytical approach for the analysis of wines. A number of different detection systems have been used for this purpose, most often assays employ fluorescence detection or UV detection with precolumn or postcolumn derivatization techniques with a wide range of derivatizing agents. The most frequently used derivatizing agents, which reacts with either primary, or secondary amino groups, or even tertiary amines and in extreme reaction conditions, providing very stable derivatives are: dansyl-chloride; fluorescein isothiocianate; o-phthaldehyde and fluorescamine (Busto et al., 1997; Mafra et al., 1999). Regarding their detection, the most frequently found BA in wine are histamine, cadaverine, putrescine, 2-phenylethylamine and tyramine (Košmerl et al., 2013, Čuš et al., 2013). Wines are usually characterized by content of BA, red wines compared to white being generally characterized by higher BA content (Soufleros et al., 2007, Marcobal et al., 2005). 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 3.62 mg/L histamine, 1.40 mg/L tyramine and 7.06 mg/L putrescine in red Spanish wines. European Union (EU) has not established regulations for the wine industry, but only suggested the *„Safety threshold values"*. 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 (Karovičova

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and Kohajdova, 2005). A wide variety of viticultural and oenological factors may have an impact on the levels of biogenic amines in wine. Some amines may be already present in grape berries (Bover-Cid et al., 2006, Kiss et al., 2006). While some factors can increase the precursor amino acid concentration in the grape and wine, other factors can potentially decrease production of the biogenic amines. Among the factors that have been suggested as favouring the abundance of amines in wine, some winemaking practices seem to play a major role because they can directly affect the content of the precursor amino acids of BA (Martin-Alvarez et al., 2006, Alcaide-Hidalgo et al., 2007, Vincenzini et al., 2009). Therefore, precaution should be taken in production process. The aim of the present work was to study the changes of the content of biogenic amines in wines made from grapevine variety *Frankovka* and *Pinot noir* (*Vitis vinifera* L.) from Kutjevo vineyards, located in the east part of continental Croatia, vintage 2012, produced by cold maceration and use of fermentation method with immobilized yeast cells.

## Materials and methods

*Wine production*

The wines was produced from the grapes varieties: *Frankovka* and *Pinot noir* (*Vitis vinifera* L.). The cold-maceration was carried out controlling the skin contact time for 4 days at temperature below of 15 °C. After the cold-maceration period was completed mash was drawn off to remove the skins and other solid parts, and left to finish the fermentation.

Sample nb. (1 - 2) of wines were produced using classical technological fermentation procedure; with selected yeast *Feromol-Bouqet 125,* and controlled thermal regime, lead trough outer chilling of fermentors with running water, with the aim of keeping the average temperature in intervals of 16 - 22 °C. The average duration of the fermentation of all grape varieties under these conditions was 40 days.

Sample nb. (1*- 2*) were produced using technological procedure of fermentation as shown in Fig. 1: Fermentation with immobilized yeast cells /selected yeast *Feromol-Bouqet 125*, immobilized in Ca-alginate gel (Gaserod, 1998, Poncelet et al., 2001) / in internal loop gas-lift fermentor with alginate beads as yeast cariers and controlled thermal regime using outer refrigeration of fermentors with running water, with the aim of keeping the average temperature in intervals of 16 - 22 °C. The average duration of fermentation under these conditions was 14 day for each set.

The samples of young wine were exempted at the end of fermentation and before filtration so the wine was insufficiently clear, slightly dull.

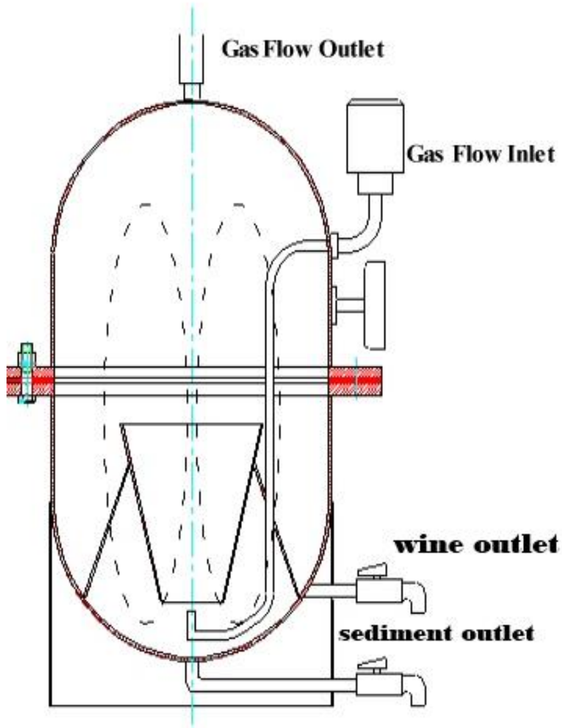


**Fig. 1.** Reactor for fermentation with immobilized yeast cells

*Chemical analysis of wine*

For the evaluation of the quality of wine fundamental analytical techniques were applied. In industrial control laboratories these techniques represent the basis for the determination of quality parameters, defined by O.I.V. (2001), Anonymous (1996) and AOAC (1995).
Chemical analysis of wine included specific mass, alcohol, total extract, total sugar, total acidity, total and free $SO_2$, total nitrogen analysis and the analysis of ash.

*HPLC analysis of biogenic amines in wine*

The biogenic amines content was determined by HPLC method according to Paris Soleas et al. (1999). Biogenic amines were separated using a liquid chromatograph HP 1100 (Agilent Technologies, Waldbronn, Germany), with an auto-sampler and UV/VIS detector with variable wavelength, and a fluorescence detector. The separation after *o*-phthaldialdehyde (OPA) derivatisation was performed on a reversed-phase column Zorbax Eclipse XDB C8 (150 mm × 4.6 mm, particle size 5 μm) equipped with a guard column Meta Guard Inertsil C18. The biogenic amines standard were obtained from Sigma-Aldrich, Steinheim, Germany and OPA application for fluorescence detector were purchased from Merck, Darmstadt, Germany.

*Statistical analysis*

One-way analysis of variance (ANOVA), and LSD comparison test, with a confidence interval of P < 95 %, was run to evaluate statistical differences on the measured chemical and physical parameters.

## Results and discussion

The results obtained in the chemical analysis of wine reported in Table 1 show chemical and physico-chemical properties for samples of new unclarified wine. The obtained results showed that all wine samples produced using technological procedure as shown in Fig. 1 (fermentation with immobilized yeast cells) had a slightly raised amount of alcohol, ranging from 12.83 – 13.47 %, in relation to the amount of 12.76 – 13.22 % in wines which were produced using classical technological procedure. The quantity of alcohol corresponds to the requirements of Regulations of wine (Anonymous, 1996). It is important to stress that the immobilized cells gave wines with lover contents of total extract (19.30 – 22.10 g/L). The amount of total extract in wines produced using classical technological procedure was within the recommended values, from 19.60 – 24.62 g/L. The differences in the amount of total extract in wine sample are in conformity with the characteristics of quality wines obtained from examined grape varieties (Ribereau-Gayon et al., 1998, O.I.V., 2001). In wine samples produced with immobilized cells, slightly lower amount of total acids was noted (5.10 - 5.88 g/L), than it was in wines which were produced using classical technological procedure (5.30 – 6.08 g/L), which correspondents to Yajima and Yokotsuka (2001). The amount of total sugars (2.75 - 3.40 g/L) was significantly higher in all-new wines produced using classical technological procedure in relation to the value in wine samples produced using immobilized yeast cells (2.30 – 2.53 g/L). Markedly high amount of total sugars in the wine results thereby with smaller content of ethanol in this new wine (Delfini et al., 2001). The presence of free $SO_2$ in all wine samples, ranging from 5.90 - 7.74 mg/L, corresponds to results of Antonelli et al. (1999). By the analysis of obtained wines, it has been found that there were significant differences among the determined properties.

**Table 1.** Results of chemical analysis of wine

| Determinate characteristics | Pinot noir | Pinot noir* | Frankovka | Frankovka* |
|---|---|---|---|---|
| **Specific mass (20/20 °C) (g/mL)** | 0.9918 ± 0.10 | 0.9914 ± 0.30 | 0.9940 ± 0.20 | 0.9930 ± 0.25 |
| **Alcohol (%vol.)** | 13.22 ± 0.15 | 13.47 ± 0.25 | 12.76 ± 0.20 | 12.83 ± 0.30 |
| **Total extract (g/L)** | 19.60 ± 0.05 | 19.30 ± 0.25 | 24.62 ± 0.42 | 22.10 ± 0.40 |
| **Total sugar (g/L)** | 2.75 ± 0.10 | 2.53 ± 0.30 | 3.40 ± 0.32 | 2.30 ± 0.35 |
| **Total acidity (g/L)** | 5.30 ± 0.08 | 5.10 ± 0.35 | 6.08 ± 0.35 | 5.88 ± 0.40 |
| **Ash (g/L)** | 1.64 ± 0.10 | 1.74 ± 0.18 | 2.10 ± 0.40 | 1.80 ± 0.25 |
| **Free $SO_2$ (mg/L)** | 7.24 ± 0.18 | 7.74 ± 0.25 | 5.90 ± 0.18 | 6.60 ± 0.10 |
| **Total $SO_2$ (mg/L)** | 118.55 ± 0.20 | 119.40 ± 0.20 | 115.60 ± 0.10 | 116.33 ± 0.18 |
| **Total nitrogen (mg/L)** | 260.50 ± 0.20 | 250.00 ± 0.10 | 240.50 ± 0.10 | 220.00 ± 0.10 |

*immobilized yeast cells

According to obtained results vinification method significantly influenced the concentration of some biogenic amines (Table 2). Total amount of biogenic amines ranged from 8.81 mg/L in wines made with immobilized yeast cells up to 9.91 mg/L in wines made in classical fermentation process. In general, histamine was the major biogenic amine found in wines (3.21 - 3.49 mg/L) and histamine producing capability may be considered widespread among various oenological factors (Halasz et al., 1994, Bauza et al., 1995, Gerbaux and Monamy, 2000, Alcaide-Hidalgo et al., 2007). As shown in Table 2 putrescine concentration was relatively similar in *Frankovka* and *Pinot noir* (0.39 - 0.41 mg/L), and low (Gerbaux and Monamy, 2000) probably because fermentation was conducted by commercial pure strain culture *Feromol-Bouqet 125*. In tested wines histamine was the most abundant biogenic amine followed by tryptamine and 2-phenylethylamine (Gloria et al., 1998). In summary, from the results obtained in this study, it can be concluded that technology with immobilized yeast cells can influence the formation of biogenic acids.

**Table 2.** Results of HPLC analysis of biogenic amines in wine

| **Biogenic amine (mg/L)** | **Pinot noir** | **Pinot noir*** | **Frankovka** | **Frankovka*** |
|---|---|---|---|---|
| **Putrescine** | 0.41 ± 0.19 | 0.39 ± 0.08 | 0.41 ± 0.06 | 0.40 ± 0.04 |
| **Cadaverine** | 0.35 ± 0.05 | 0.34 ± 0.05 | 0.42 ± 0.05 | 0.40 ± 0.05 |
| **2-Phenylethylamine** | 2.37 ± 0.15 | 2.33 ± 0.15 | 2.64 ± 0.18 | 2.30 ± 0.18 |
| **Spermidine** | 0.58 ± 0.11 | 0.53 ± 0.09 | 0.65 ± 0.09 | 0.61 ± 0.09 |
| **Tryptamine** | 1.74 ± 0.10 | 1.63 ± 0.12 | 1.89 ± 0.17 | 1.69 ± 0.17 |
| **Serotonine** | 0.18 ± 0.05 | 0.15 ± 0.04 | 0.16 ± 0.05 | 0.13 ± 0.05 |
| **Tyramine** | 0.23 ± 0.01 | 0.19 ± 0.01 | 0.25 ± 0.02 | 0.20 ± 0.02 |
| **Histamine** | 3.36 ± 0.06 | 3.25 ± 0.09 | 3.49 ± 0.06 | 3.21 ± 0.06 |
| **Σ Biogenic amines** | **9.22** | **8.81** | **9.91** | **8.94** |

*immobilized yeast cells

## Conclusions

The obtained results showed that fermentation with immobilized yeast cells had significant influence on presence of biogenic amines in wine. Moreover, the compounds that mostly contribute to the typical biogenic amines profile of wine, such as histamine, cadaverine, putrescine, 2-phenylethylamine and tyramine are affected by the fermentation process with immobilized yeast cells.

It seems that the fermentation with immobilized yeast cells is a promising approach in the wine-making process, with a reduced content of biogenic amines in wine.

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