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REGULAR ARTICLE

BIOGENIC AMINES CONTENT IN SELECTED WINES DURING WINEMAKING

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ABSTRACT

The aim of this study was to describe the development of selected biogenic amines (histamine; tyramine; phenylethylamine; putrescine; agmatine; and cadaverine) during the winemaking in 10 selected species grown in Central Europe in 2008. The analysis was performed using ion-exchange chromatography by the sodium-citrate buffers with the post-column ninhydrin derivatization and photometric detection. A comparison of the content of biogenic amines in red and wine varieties showed that red wines have higher concentrations of biogenic amines.

Keywords: winemaking process; biogenic amines; lactic acid bacteria

INTRODUCTION

The wine production (winemaking) covers transformation of grapes to must and in the end to wine. The white grapes are grand, stemming and consequently also pressing. The red grapes are crushed towards the cracking of grape´s skin and the release of juice. It must not

get to damage of the stems and the grape-stones, because we want to prevent the digestion of substances that would negatively affect the organoleptic properties of the product. It succeeds the several-day fermentation and consequently also pressing. It is possible (under certain law conditions) to regulate the sugar content. It is followed by yeast inoculation by *Saccharomyces cerevisiae* and alcoholic fermentation. The next phase is the malolactic fermentation by starter lactic acid bacteria (red wine) or spontaneously (especially white wine). The other steps cover clearing the wine and filtration of the wine and also his maturing. After the further stabilization and filtration, it is possible to close in bottles **(Grainger and Tattersall, 2005).**

Biogenic amines (BA) are the low molecular nitrogen compounds formed mainly by microbial decarboxylation of free amino acids (histidine – histamine; tyrosine – tyramine; phenylalanine – phenylethylamine; arginine and/or ornithine – putrescine; arginine – agmatine; lysine – cadaverine). Behind the major source BA in wines are considered the lactic acid bacteria (LAB) originated from the starter culture (controlling malolactic fermentation) or from the contaminating microflora (**Ancín-Azpilicueta et al., 2008; Anli and Bayram, 2009; Landete et al., 2007; Lonvaud-Funel, 2001**). The excessive intake of histamine could cause e. g. dilatation of peripheral blood vessels, hypotension, urticaria, flushing and headache. High amount of tyramine in food could also induce headache and hypertension. Putrescine and cadaverine have been identified as potentiators of toxic effect of other amines due to inhibition of detoxifying enzymes. Biogenic amines may also serve as indicators of food spoilage (**Halász et al., 1994; Shalaby, 1996**). There are numerous studies that deal with partial factors affecting the content of BA in the wines – such as filtration effect, starter cultures, species, pectolytic enzymes presence etc. (**Ancín-Azpilicueta et al., 2008; Constantini et al., 2009; Hernández-Orte et al., 2008; Marco & Azpilicueta, 2006; Marcobal et al., 2006; Martín-Álvaréz et al., 2006; Moreno et al., 2003**). However, the work dealing with the description of the content development of biogenic amines during the manufacture of wine and a comparison of this development of different species of wines from Central Europe was not found in the available literature.

The aim of this work was (i) to describe the development of the content of selected BA (histamine; tyramine; phenylethylamine; putrescine; agmatine; and cadaverine) during the winemaking in 10 selected species grown in Central Europe, and (ii) to compare the content development of BA by observed species. The study should contribute to the monitoring of biogenic amines occurrence in Czech foodstuffs and beverages and point out the importance of biogenic amines testing.

MATERIAL AND METHODS

Wine samples

Four white wines (Müller Thurgau, Gruner Veltliner, Pinot Gris and Welschriesling) and six red wines (Blue Portugal, Pinot Noir, Dornfelder, Lemberger, André and mixture Blue Portugal, St. Laurent and Blaufränkisch) were monitored. The grapevines were grown in the wine region of Moravia (Czech Republic) in 2008. The starter yeast (*Saccharomyces cerevisiae*) was used for the inoculation of the must or mash (crushed grapes). Potassium disulphide was applied for sulphitation and silicic acid, bentonite and gelatin for clarification. The final sugar content in white mush of 21 kg . 100 l^{-1} must was reached. The sugar content in the mash was increased to 22 kg . 100 l^{-1} mash.

The samples were taken on the day of pressing (white wine), respectively a day of ground (red wine) – day 1^{st}, then the 60^{th} day (after alcoholic fermentation and malolactic fermentation; decanted), 100^{th} day (after flocculation, filtration), 150^{th} and 200^{th} day (after further flocculation, filtration), 250^{th} and 300^{th} day. Each sampling day were collected from each wine two parallel samples.

Biogenic amines determination

The amount of 15 – 20 ml of wine was exactly weighed in 50 ml plastic tubes and frozen at –80 °C. On the second day, the frozen samples were lyophilized by ALPHA 1 – 4 LSC. Five mL of sodium-citrate buffer (pH 2.2) was added to the lyophilized samples. The mixture had been shaking for one hour at the room temperature and centrifuged (20 000 *g* for 45 minutes at 4 °C). The supernatant was filtered (porosity of 0.45 μm) and injected into the chromatography system. Amino Acid Analyzer AAA 400 (Ingos, Prague, Czech Republic) was used for the determination of biogenic amines (histamine; tyramine; phenylethylamine; putrescine; agmatine; and cadaverine). The analysis was performed using ion-exchange chromatogramy by the sodium-citrate buffer with the post-column ninhydrin derivatization and photometric detection (λ = 570 nm). The composition of buffers and the elution program can be found in **Buňková et al. (2009)**. Each of the simultaneously obtained samples was lyophilized twice and each extract was analyzed three times (n = 12).

The results were statistically compared by non-parametric methods - Kruskal-Wallis and Wilcoxon test – using Unistat software version 5.5.

RESULTS AND DISCUSSION

During the winemaking process, the content of the six biogenic amines (histamine; tyramine; phenylethylamine; putrescine; agmatine; and cadaverine) in the four varieties of white wine and in the six varieties of red wine (including one mixture) was monitored. During the three hundred days observed, phenylethylamine, agmatine or cadaverine were not detected in samples.

Figure 1 shows the development of the content of histamine, tyramine and putrescine in the tested varieties of white wine (Müller Thurgau, Gruner Veltliner, Pinot Gris and Welschriesling). In all four white varieties tested putrescine was found. In two wines also tyramine was recorded (Pinot Gris and Welschriesling). The other white varieties contained also histamine (Müller Thurgau and Gruner Veltliner). The concentration of recorded biogenic amines significantly increased ($P < 0.01$) during the 300-day winemaking process. The concentration of 100 mg . l^{-1} for each biogenic amine was evaluated as critical from a toxicological point of view (**Halász et al., 1994; Shalaby, 1996; Kalač and Krausová, 2005**). The latter mentioned limit was not exceeded in any of monitored white wine. The concentrations of histamine ranged up to 25 mg . l^{-1}. In the variety Welschriesling (Figure 1, pard D), the concentration of tyramine reached 90 mg . l^{-1} on 250^{th} a 300^{th} day, which is near the critical amount. The content of putrescine in the varieties Müller Thurgau and Gruner Veltliner at the end of the experiment ranged to 30 mg . l^{-1}. The lowest concentration of putrescine at 300^{th} day was found in Welschriesling (~10 mg . l^{-1}), while in the variety Pinot Gris was reached the amount 60 mg . l^{-1}.

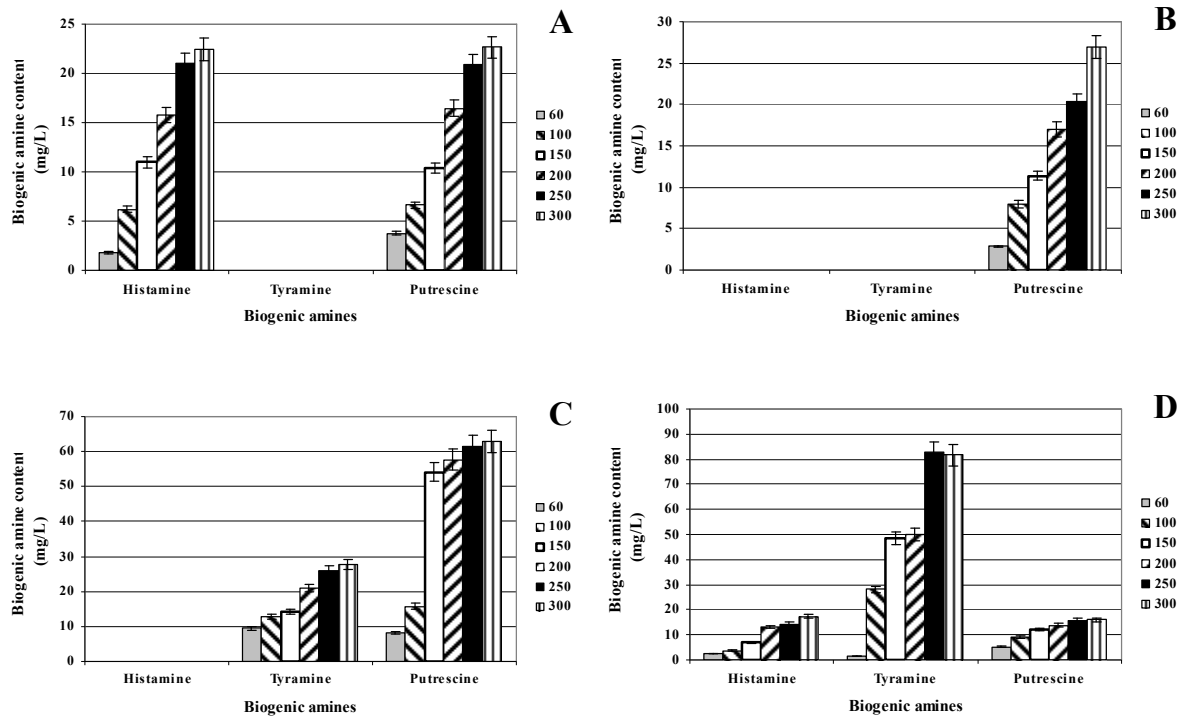

Figure 1 The content of histamine, tyramine and putrescine in red wine tested (Müller Thurgau – part A; Gruner Veltliner – part B; Pinot Gris – part C; and Welschriesling – part D) during 300-day winemaking process

Figure 2 shows the concentration of histamine, tyramine and putrescine found during 300-day monitoring in six varieties of red wine (Blue Portugal, Pinot Noir, Dornfelder, Blaufränkisch, André and the mixture Blue Portugal, St. Laurent and Blaufränkisch). The above mentioned biogenic amines were detected in all six varieties of red wine. The concentrations of the detected biogenic amines in all tested red wines increased during the 300-day experiment ($P < 0.01$). The highest amount of histamine was found at the end of the experiment in varieties Pinot Noir and André (70 – 80 mg . l^{-1}). In the other four varieties of red wines the concentration of histamine was below 50 mg . l^{-1}. The variety Dornfelder showed very high levels of tyramine, which reached during 300-day process 410 – 440 mg.l^{-1}, which exceeds more than four times the critical value from a toxicological point of view. The further variety with high levels of tyramine was the mixture of wines (Figure 2, part F); but the critical value 100 mg . l^{-1} was not exceeded. In the other four varieties (Blue Portugal, Pinot Noir, Lemberger, André), the tyramine concentrations below 30 mg/L were observed.

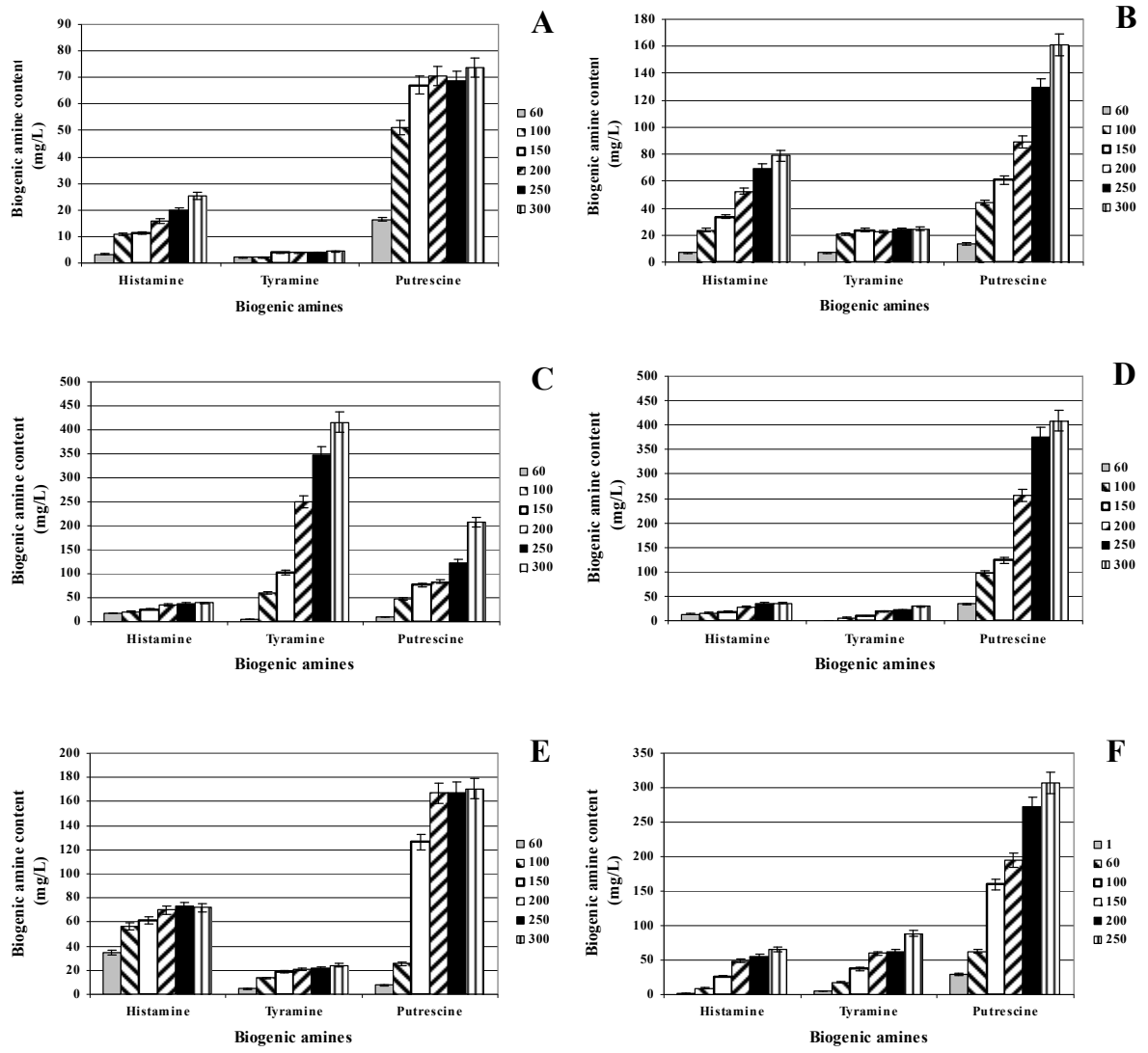

Figure 2 The content of histamine, tyramine and putrescine in white wine tested (Blue Portugal – part A; Pinot Noir – part B; Dornfelder – part C; Lemberger – part D; André – part E; and the mixture of Blue Portugal, St. Laurent a Lemberger – part F) during 300-day winemaking process

Putrescine was the critical biogenic amine in this study. The concentration of putrescine was higher than 100 mg . l^{-1} in most of red wines (with the exception of the variety Blue Portugal; Figure 2). The amount was twice in comparison with the toxicological limit in Pinot Noir, Dornfelder and André varieties. In the varieties Lemberger and the mixture of red wines the concentration of putrescine over 400 mg . l^{-1} and 300 mg . l^{-1} were detected.

A comparison of the content of biogenic amines in red and wine varieties showed that red wines have higher concentrations of biogenic amines. Many authors (e. g. **Ferreira and Pinho, 2006; Marco and Azpilicueta, 2006; Martín-Álvarez et al., 2006**) considered red

wine as a significant source of biogenic amines. Apart from the compounds with significant health benefits (e.g. polyphenols etc.), the red wine should be evaluated as alcoholic beverage with potential risks for consumer health. According to **Ferreira and Pinho (2006), Gardini et al. (2005)**, **Guerrini et al. (2002)**, LAB involved in malolactic fermentation could be considered as a potential source of biogenic amines. Many authors have signed LAB as regularly producers of biogenic amines. Other biogenic amines sources could be spontaneously present and/or contaminanting microorganisms.

CONCLUSION

During 300-day winemaking process, the content of selected biogenic amines in 10 varieties of wine was monitored. The mainly biogenic amines occurring in the tested wines were histamine, tyramine and especially putrescine. The white varieties showed lower levels of biogenic amines in comparison with the red varieties. In many varieties, the content of biogenic amines exceeded the limit of 100 mg . l^{-1}, which could be a risk for health of consumers. Therefore, the further studies dealing with biogenic amines development in wines should be carried out and possibility of reducing of the concentration of biogenic amines in the final product should be found.

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