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Changes of the content of biogenic amines during winemaking of Sauvignon wines

Karin Kovačević Ganić*, L. Gracin, Draženka Komes, Natka Ćurko, T. Lovrić

University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

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Summary

The aim of the present work was to study the changes of the content of biogenic amines during winemaking and maturation processes of wines made from *Vitis vinifera* cv Sauvignon grapes from Slavonia region (vintage 2008). Biogenic amines were quantified using a reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection after pre-column derivatization with *o*-phthalaldehyde (OPA). Samples used in this study were obtained during production of Sauvignon wines in three different ways in order to produce quality wine (QW), quality press wine (QPW) and macerated wines (MW). The QPW corresponds to the first fraction obtained by direct pressing pomace, while the QW is the free run wine. The MW was obtained by grape mash cryomaceration. Putrescine and tryptamine were the most prevalent amines, followed by histamine, cadaverine and tyramine. The macerated wine posses higher content of biogenic amines than press and free run wines. In all analysed wines the content of biogenic amines tends to increase during winemaking and maturation and the main increase was detected during the malolactic fermentation. The detected content of histamine and tyramine were below the content considered to have an adverse effect on human health.

Keywords: biogenic amines, fluorescence, OPA, white wines, wine production

Introduction

The presence of biogenic amines in wine is becoming increasingly important to consumers and producers alike, due to the potential threats of toxicity to humans and consequent by trade implications. In the scientific field, biogenic amines have the potential to be applied as indicators of food spoilage and/or authenticity.

Biogenic amines in wine can originate from the plant (grape berries) itself or be produced during the fermentation processes, ageing or storage when wine is exposed to the activity of decarboxylase positive microorganisms. Biogenic amines can be formed from their respective amino acid precursors by various microorganisms present in wine, at any stage of production, ageing or storage (Smit et al., 2008).

Some researchers have found that biogenic amines are formed by yeasts and increased during the alcoholic fermentation (Caruso et al., 2002). Several studies support a view that biogenic amines are mainly formed in winemaking during the malolactic fermentation, by the action of lactic bacteria (Landete et al., 2007; Constantini et al., 2006; Moreno-Arribas et al., 2003; Lonvaud-Funel, 1999).

Several studies, suggested that biogenic amines are indicators of a lack of hygiene condition during winemaking process (Leitao et al., 2005; Shalaby et al., 1996; ten Brink et al., 1990; Zee et al., 1983).

The main biogenic amines associated with wine are putrescine, histamine, tyramine and cadaverine, followed by phenylethylamine, spermidine, spermine, agmatine and tryptamine (Smit et al., 2008). Some amines, such as putrescine and other polyamines, may already be present in grape berries (Bover-Cid et al., 2006). Biogenic amines are produced by grape vines in response to stress factors like heat, salt, water deficiency etc.

The non-volatile biogenic amines (histamine, putrescine, cadaverine, spermine, spermidine, agmatine, tyramine, tryptamine) and phenylethylamine (a volatile amine) are formed mainly by microbial decarboxylation of corresponding amino acids (Halasz et al., 1994; ten Brink et al., 1990). Volatile amines are believed to be formed by the reductive amination or transamination of the corresponding aldehyde or ketone (Ouch et al., 1981; Smith, 1980).

High content of biogenic amines can cause undesirable physiological effects in sensitive humans, especially when alcohol and acetaldehyde are present. Histamine is known to cause headaches, low blood pressure, heart palpitations, edema and other symptoms. Tyramine and phenylethylamine can produce hypertension through the release of noradrenaline and norephedrine, respectively, which are vasoconstrictors. Putrescine and cadaverine, although non-toxic themselves, aggravate the adverse

*Corresponding author; University of Zagreb, Faculty of Food Technology and Biotechnology, Department of process engineering, Pierottijeva 6, 10000 Zagreb, Croatia; kkova@pbf.hr

effects of histamine, tyramine and phenylethylamine, as they interfere with the enzymes that metabolize them (Shalaby, 1996; Silla, 1996). Putrescine is also potentially dangerous, because it can react with nitrites to form carcinogenic nitrosamine (Halasz et al., 1994). For this reason, some countries have established regulations regarding either their intake content in various kinds of food or their maximum limit requirements. In spite of the toxicological implications, no legal limit has been defined for biogenic amines in wines. Some countries have established rough guidelines concerning maximum recommended content for histamine, which are quantitatively much lower than in other food due to the presence of alcohol (Lehtonen, 1996).

Generally the toxic dose in alcoholic beverages is considered to be between 8 and 20 mg/L for histamine, 25 and 40 mg/L for tyramine, while as little as 3 mg/L of phenylethylamine can cause negative physiological effect (Soufleros et al., 1998).

Beside toxic effect, some biogenic amines also have other negative consequences, particularly regarding sensory characteristics of wine and economic implications. A study by Rohn et al., (2005) suggested that histamine can be identified at the high content in commercial wines by well trained assessors. The study employed mouthfeel descriptors such as ″irritation in deep throat″, and ″crawling of the tongue″. No specific taste could be attributed to histamine. In contrast, Wantke et al., (2008) determined that sensory wine quality is unrelated to histamine levels.

The aim of the present work was to study the content of biogenic amines during the winemaking process of wines made from *Vitis vinifera* cv Sauvignon grapes from Slavonia region, in order to improve the knowledge about the origin and evaluation of biogenic amines in wines.

Materials and methods

Samples and vinification

V. vinifera cv Sauvignon grapes were processed in the 2008 vintage by wine cellar from the Slavonia region. The QPW corresponds to the first fraction obtained by direct pressing pomace and QW is the free run wine. The MW was obtained by grape mash cryomaceration at 7 ºC during 10 h. The technical production procedure of wine consisted of the following steps: free run must, must after pressing and macerated must were placed in stainless steel tanks; then, 20 g/hL of potassium-metabisulphite was added followed by sedimentation at 12 °C for 48 hours. Pure must was decanted. Alcoholic fermentation was conducted without yeast inoculation. The fermentation temperature was kept at 17 °C. After fermentation (sugar content below 2.5 g/L) the spontaneous malolactic fermentation took place. After alcoholic and malolactic fermentation the wine was decanted. The wine was kept under these conditions and regularly controlled for levels of free SO_2. Corrections were made if necessary. Following, the wine was decanted again. After that, wine was kept for a month in oak barrels. In March 2009, wine clarification was carried out with 100 g/hL bentonite (Fortbenton-Ever, Italy). After that, the wine was filtered through a filter (Strassburger, Germany) followed by microfiltration (∅ 0.65 μm). Before bottling, the wine was sulfited with SO_2, so that the portion of free SO_2 amounted to 20 mg/L. The wines were bottled and stored at the temperature of 16 °C, corresponding to the temperature of wine cellar.

Samples were taken during winemaking and maturation process and they are summarized in Table 1. Samples were taken in triplicate.

Table 1. Samples taken during the production of quality wine, quality press wine and macerated wines

Step	Samples
1	Grape must
2	End of alcoholic fermentation
3	Before malolactic fermentation
4	End of malolactic fermentation
5	First racking
6	One month in oak barrels
7	Before bottling
8	Two month in bottles

Chemicals

Tryptamine (Trp) - purity ≥ 99 %, hydroxytyramine chlorhydrate (Htyr) - purity ≥ 98 %, phenylethylamine (Pea) - purity ≥ 99 %, putrescine (Put) - purity ≥ 98 %, cadaverine (Cad) - purity ≥ 99 %, serotonine 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.

Enological parameters

Determination of enological parameters are described by the Ordinance on physical and chemical methods of analysis of must, wines other products made of grapes and wine and fruit wines (Croatian Official Gazette 106/2004).

HPLC determination of biogenic amines

The biogenic amines content was determined by HPLC according to method of 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. 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, 5μm 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 (peak area vs. content). 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. All analyses were repeated three times, and the results were expressed as mean values in milligrams per liter of wine ± SD.

Method validation

The reliability of the chromatographic method was studied in terms of repeatability, sensitivity, linearity and recovery (Table 2).

The sample analytes were identified by comparison with the retention times of biogenic amines standard solution. For the determination of the retention times, the reference standards were injected both individually and as a mixture. Quantification was performed by the external standard method based on peak areas of the eluated biogenic amines derivatives. The linearity was evaluated by the construction of calibrated curves, using the chromatographic peaks areas of the fluorescence response from triplicate injections of standards at six increasing concentrations for all biogenic amines.

The repeatability of the method was estimated by the relative standard deviation (RSD) of the areas for five consecutive injections of the same standard solution during a day.

Detection limits were estimated from the area corresponding to three times the system noise level.

Recovery has been estimated as (amount found in the spiked sample - the amount found in the sample) 100/amount added.

Table 2. Analytical characteristic of method for the determination of biogenic amines

Biogenic amines	Linearity (R^2)	Detection limit[a] (mg/L)	Repeatability (RSD%)	Recovery (%)
Tryptamine	0.996	0.025	3.22	87
Phenylethylamine,	0.997	0.051	2.52	84
Putrescine	0.999	0.034	4.11	91
Cadaverine	0.997	0.061	1.70	105
Histamine	0.993	0.020	3.80	88
Tyramine	0.991	0.015	2.53	89
Serotonine	0.995	0.036	1.50	102
Spermine	0.999	0.009	2.51	92
Spermidine	0.998	0.015	1.75	95
Hydroxytyramine	0.990	0.085	5.20	98

R^2, coefficient of determination; [a] three times the noise level; Recovery (amount found in the spiked sample-the amount found in the sample)·100/amount added

Statistical analysis

The data was statistically analyzed using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). ANOVA was performed to determine the effect of wine making procedure and maturation on the content of biogenic amines.

Results and Discussion

Results of basic enological parameters of Sauvignon wines are shown in Table 3. The parameters such as pH, temperature, SO_2 and a variety of substrates and products of fermentation have influence on the concentration and diversity of microorganisms in the wine, but can also affect decarboxylase enzyme activity and gene expression. Hence, at higher pH, higher content of biogenic amines are produced in most cases (Martin-Alvarez et al., 2006; Landete et al., 2005; Gardini et al., 2005). The analyzed wines did not differ significantly according to the basic enological parameters, except small variation in the content of total extracts.

Table 3. Enological parameters of wines

	QW	QPW	MW
Alcohol content (v/v %)	12.58	12.01	12.95
Total extract (g/L)	28.80	28.03	28.95
Specific gravity (20/20 ºC)	0.9957	0.9960	0.9961
pH	3.45	3.48	3.49
Total acidity (tartaric acid) (g/L)	6.13	6.10	5.95
Reducing sugar (g/L)	1.60	1.51	1.58
Volatile acidity (acetic acid) (g/L)	0.44	0.32	0.51
Total SO_2 (mg/L)	45.3	47.1	50.3

QW-quality wine; QPW-quality press wine; MW-macerated wine

The average values of biogenic amines in the analysed samples are shown in Table 4, 5 and 6. Ten biogenic amines were detected: tryptamine, hydroxytyramine, phenylethylamine, putrescine, cadaverine, histamine, tyramine, serotonine, spermine and spermidine.

Table 4. Changes of the content of biogenic amines during quality wine production and maturation (mg/L±standard deviation)

Biogenic amines	1	2	3	4	5	6	7	8
Trp	0.14±0.01	0.59±0.16	0.75±0.12	1.32±0.11	1.15±0.22	1.38±0.19	1.62±0.30	1.71±0.11
Pea	0.05±0.02	0.24±0.07	0.35±0.09	0.55±0.12	0.42±0.14	0.47±0.11	0.57±0.21	0.62±0.20
Put	0.51±0.05	0.92±0.21	1.15±0.12	2.01±0.15	1.98±0.22	1.76±0.15	2.15±0.17	2.22±0.25
Cad	0.10±0.02	0.14±0.09	0.20±0.05	0.34±0.10	0.32±0.12	0.37±0.14	0.42±0.05	0.59±0.22
Hist	0.09±0.03	0.10±0.05	0.20±0.09	0.95±0.15	0.98±0.33	1.22±0.20	1.30±0.25	1.50±0.23
Tyr	-	0.09±0.01	0.15±0.02	0.18±0.05	0.21±0.11	0.17±0.09	0.18±0.05	0.22±0.06
Ser	-	0.01±0.01	0.06±0.01	0.07±0.05	0.05±0.02	0.06±0.01	0.07±0.02	0.09±0.01
Spm	-	0.05±0.01	0.09±0.04	0.12±0.01	0.13±0.09	0.10±0.05	0.09±0.03	0.12±0.02
Spd	-	0.20±0.04	0.19±0.05	0.24±0.09	0.15±0.08	0.12±0.02	0.14±0.05	0.17±0.09
Htyr	-	0.05±0.01	0.07±0.02	0.07±0.02	-	0.08±0.05	0.11±0.02	0.08±0.01

Trp: tryptamine, Pea: phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine: Tyr: Tyramine, Ser: serotonine: Spm: spermine, Spd: spermidine, Htyr: hydroxytyramine. Steps: 1: grape must, 2: end of alcoholic fermentation, 3: before malolactic fermentation, 4: end of malolactic fermentation, 5: first racking, 6: one month in oak barrels, 7: before bottling, 8: two month in bottles.

Table 5. Changes of the content of biogenic amines during quality press wine production and maturation (mg/L±standard deviation)

Biogenic amines	1	2	3	4	5	6	7	8
Trp	0.15±0.05	0.45±0.12	0.52±0.19	1.25±0.16	1.12±0.62	1.55±0.36	2.14±0.52	2.21±0.65
Pea	0.07±0.02	0.15±0.09	0.21±0.11	0.51±0.17	0.77±0.22	0.70±0.32	0.65±0.10	0.60±0.31
Put	0.48±0.11	0.72±0.15	0.84±0.17	1.98±0.42	2.23±0.52	2.55±0.44	3.01±0.12	3.10±0.22
Cad	0.08±0.02	0.22±0.02	0.20±0.06	0.17±0.05	0.33±0.05	0.42±0.11	0.50±0.15	0.57±0.18
Hist	0.07±0.03	0.42±0.09	0.34±0.11	0.79±0.25	1.01±0.32	1.15±0.32	1.23±0.30	1.31±0.31
Tyr	-	0.15±0.02	0.09±0.01	0.14±0.05	0.20±0.11	0.21±0.11	0.22±0.12	0.20±0.11
Ser	-	0.04±0.01	0.03±0.01	0.08±0.01	0.10±0.02	0.09±0.01	0.11±0.02	0.09±0.05
Spm	-	0.05±0.01	0.17±0.02	0.18±0.06	0.20±0.11	0.17±0.02	0.19±0.03	0.12±0.05
Spd	-	0.14±0.03	0.11±0.02	0.19±0.05	0.22±0.06	0.18±0.05	0.17±0.11	0.20±0.09
Htyr	-	0.04±0.01	0.08±0.01	0.09±0.01	0.10±0.05	0.14±0.02	0.13±0.04	0.11±0.06

Trp: tryptamine, Pea: phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine: Tyr: Tyramine, Ser: serotonine: Spm: spermine, Spd: spermidine, Htyr: hydroxytyramine. Steps: 1: grape must, 2: end of alcoholic fermentation, 3: before malolactic fermentation, 4: end of malolactic fermentation, 5: first racking, 6: one month in oak barrels, 7: before bottling, 8: two month in bottles.

Table 6. Changes of the content of biogenic amines during macerated wine production and maturation (mg/L±standard deviation)

Biogenic amines	1	2	3	4	5	6	7	8
Trp	0.10±0.05	0.42±0.11	0.52±0.11	1.12±0.22	1.23±0.10	2.11±0.15	2.42±0.11	2.50±0.44
Pea	0.09±0.02	0.19±0.06	0.22±0.03	0.56±0.12	0.65±0.03	0.78±0.19	0.70±0.23	0.69±0.51
Put	0.83±0.11	1.12±0.62	1.56±0.14	2.53±0.32	2.34±0.10	2.72±0.42	3.01±0.38	3.31±0.39
Cad	0.18±0.02	0.17±0.05	0.27±0.09	0.59±0.25	0.61±0.11	0.77±0.33	0.80±0.21	0.87±0.20
Hist	0.25±0.03	0.85±0.19	0.78±0.13	0.99±0.31	0.66±0.11	1.42±0.16	1.55±0.16	1.65±0.52
Tyr	-	0.36±0.10	0.45±0.12	0.53±0.12	0.41±0.19	0.40±0.05	0.48±0.09	0.52±0.10
Ser	-	0.05±0.02	0.05±0.01	0.09±0.05	0.03±0.01	0.08±0.05	0.08±0.08	0.14±0.01
Spm	-	0.15±0.06	0.18±0.04	0.22±0.06	0.29±0.11	0.25±0.11	0.20±0.05	0.18±0.06
Spd	-	0.11±0.04	0.14±0.09	0.20±0.11	0.21±0.09	0.28±0.09	0.18±0.09	0.20±0.11
Htyr	-	0.07±0.01	0.05±0.01	0.04±0.01	0.09±0.02	0.12±0.14	0.09±0.01	0.10±0.02

Trp: tryptamine, Pea: phenylethylamine, Put: putrescine, Cad: cadaverine, His: histamine: Tyr: Tyramine, Ser: serotonine: Spm: spermine, Spd: spermidine, Htyr: hydroxytyramine. Steps: 1: grape must, 2: end of alcoholic fermentation, 3: before malolactic fermentation, 4: end of malolactic fermentation, 5: first racking, 6: one month in oak barrels, 7: before bottling, 8: two month in bottles.

In all analysed wines the content of biogenic amines tends to increase during winemaking and maturation (Fig. 1.). The main increase of biogenic amines in wine is related to malolactic fermentation (step 2) and maturation process in oak barrels (step 6). Commercial *O.oeni* strains are selected for their oenological parameters, including the absence of amino acids decarboxylases. According to *in vitro* studies conducted by Moreno-Arribas et al. (2003), none of four commercial malolactic starter cultures examined could produce histamine, tyramine or putrescine. Inoculation with *O.oeni* starter cultures that are unable to produce biogenic amines is a viable option for the control of these compounds in wine (Martin-Alvarez et al., 2006). It seems that co-inoculation of *O.oeni* starter cultures together with alcoholic fermentation has the potential to curb biogenic amine formation even more than conventional inoculation for malolactic fermentation after the completion of alcoholic fermentation (Van der Merwe, 2007).

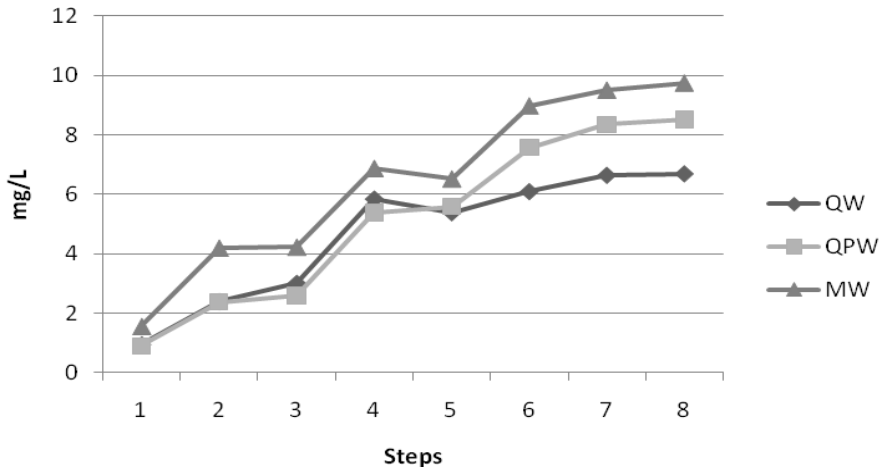

Fig. 1. Changes of the content of total biogenic amines in quality wine, quality press wine and macerated wine during winemaking and maturation process (Table 1)

Soufleros et al., (1998) determined low content of biogenic amines (histamine, tyramine and putrescine) after alcoholic fermentation and their increase during and after spontenous malolactic fermentation, with a corresponding decrease in the content of precursors amino acids.

The reason for increase of the content of biogenic amines during maturation could be that SO_2 added to the wine after malolactic fermentation did not completely stop all biochemical reactions and enzyme activities. Gerbaux and Monamy (2000) found that the content of histamine increased between four and eight months after malolactic fermentation in Pinot noir and Chardonnay.

Cryomacerated wines have (MW) higher content of biogenic amines then press wine (QPW) or free run wine (QW). Grape skin maceration promotes extraction of grapes components such as phenolic compounds, proteins, amino acids and polysaccharides. During cold maceration, grape must wasleft in contact with grape skins at the lower temperature prior to alcoholic fermentation. Soleas et al., (1999) found no correlation between length of skin contact and content of biogenic amines. The researchers Martin-Alvarez et al. (2006) and Bauza et al. (1995) found that duration of skin maceration is very important variable which affects the content of biogenic amine in wine, and that longer maceration time could favour increased production of biogenic amines.

Putrescine was the predominant amine in all of the analyzed wines, with the maximum of 3.31 mg/L detected in MW two months after bottling. The contents of serotonine, spermine and hydroxytyramine were detected in the lowest content.

A possible explanation for the production of high levels of putrescine in wine has been proposed by Mangani et al., (2005), who proved that putrescine could be produced by *O.oeni* from ornithine as well as from arginine at wine pH 3.2.

This research provides a further insight into evaluation of biogenic amines in wine and showed the influence of the winemaking and maturation conditions on their content.

Conclusions

Putrescine and tryptamine were the most prevalent amines, followed by histamine, cadaverine and tyramine. Wine obtained by grape mash cryomaceration showed the highest content of biogenic amines. The main increase in the content of biogenic amines in wine was detected after malolactic fermentation. The detected content of histamine and tyramine were below the levels considered to have an adverse effect on human health.

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