Determination of Biogenic Amines from Several Romanian wines

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Biogenic amines (BA) are formed by the decarboxilation of the corresponding α-amino acids. Their content in human organism has to be monitored due to their physiological role (hormones, neurotransmitters) in balance with their toxic effects. Wines contain different quantities of biogenic amines depending on the α-amino acid content of grape and the climate of the viticulture area, the vinification process and the hygiene kept throughout the processing. The present paperwork presents the analysis of biogenic amine content (histamine, tyramine, 2-phenylethylamine, putrescine, cadaverine, iso-pentyl-amine) from some Romanian wines produced by three vineyards situated in three different regions with distinct microclimates for grape production.

Keywords: biogenic amines, Romanian wines, HPLC analysis, OPA derivatization

Biogenic amines are compounds formed by the corresponding α-amino acids decarboxylation and most of them play an important role in metabolic processes. For instance histamine [2-(4-imidazolyl)ethylamine] discovered in 1910 [1] is generated by histidine decarboxylation. Histamine is a mediator of anaphylactic reactions [2] and an important hormone and neurotransmitter. The excess of histamine has many consequences, like: headaches, diarrhea, hypotension, arrhythmia, urticaria, as well as other side effects [3]. Small quantities of histamine are necessary due to their physiological importance in cell growth and differentiation, circadian rhythm, learning process, gastric acid secretion [4]. Other amines involved in important biological processes are polyamines like: putrescine, cadaverine, spermine and spermidine which are regulators of cell growth in plants and animals [5]. Equilibrium between the biosynthesis and the biodegradation has to be established for all these amines.

Since the concentration of biogenic amines can change during food processing and storage and their formation has also been proposed as an indicator of food quality [6,7]. High levels of these amines are associated with low quality products or with improper practices, indicating poor hygienic conditions during processing [8]. The toxicity of these amines makes them responsible for food poisoning in certain foods, especially fish and sea-fruits based foods. Limits for their contents are proposed and also used to assess the freshness of these foods [9]. For wines, as for other foods, only some countries have recommended levels of histamine content due to its enhanced toxicity in the presence of alcohol. The following maximum values have been proposed: 2 mg/L in Germany, 5-6 mg/L in Belgium, 8 mg/L in France and 12 mg/L in Switzerland [10].

In such context the study of biogenic amines content in Romanian wines, produced in different Romanian vineyards, seems of interest. Thus, three different vineyards from three regions of Romania, namely Murfatlar, Recaş and Miniş have been chosen. Experimental results showed a link between the biogenic amines content and the region where the grapes are cultivated, as well as the wine making process and the ageing of wines [11,12].

Experimental part

Materials and methods

Reagents used in the experimentation, as well as solvents and standards were purchased from Sigma-Aldrich, Fluka and Merck, as follows:

- Sigma-Aldrich: histamine, tyramine, 2-phenylethylamine, putrescine, cadaverine;
- Fluka: isopentyl-amine, 1-hexylamine, *o*-phthalaldehyde (OPA), potassium carbonate, potassium dihydrogenophosphate, di-sodium hydrogenophosphate;
- Merck: ethanol, acetonitrile.

The 23 selected wines are commercially available and have been purchased from shops for the wines produced in Murfatlar vineyard, from the retail shop of the vineyard for the wines produced in Recas vineyard and directly from the vineyard for the wines produced in Minis vineyard. After purchase, wines were kept in cold storage (4-5°C) and in the absence of light until analyzed, in order to avoid any further transformation.

The analyses were carried out on a HP 1090 HPLC instrument, with fluorometer with excitation (λ_{ex} = 330 nm) and emission (λ_{em} = 450 nm). The separation was performed using Licrocart 250-4 Licrosphere 100 Rp-18 columns, thermostated at 23^{0}C. A solution of potassium dihydrogenophosphate 2.268 g/L and di-sodium hydrogenophosphate 5.933 g/L in millipore water, adjusted at a pH of 7.2 was used as eluent A and acetonitrile was used as eluent B. The sample elution was performed in stages, with a flow rate of 1 mL/min, and a solvent gradient elution as presented in table 1.

As internal standard, 1-hexylamine was used, in a concentration of 0.210 g/L and calibration curves were made for each of the analyzed biogenic amine.

The method involved pre-column derivatization of the samples with OPA and fluorimetric detection of the formed complex.

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Table 1
GRADIENT OF THE A AND B ELUENTS OVER TIME

Time (minutes)	Eluent A (%Vol)	Eluent B (%Vol)
0	60	40
10	30	70
30	30	70
32	60	40

Sample preparation

Wine samples were left the necessary time (2 h) to heat at room temperature and then filtered on filtering paper (10 µm). A 3 mL sample from the filtered wine was collected and introduced in a centrifugation vial; then 0.050 ml of internal standard (1-hexylamine, 0.210 g/L), 1 mL of ethanol and 3 g of K_2CO_3 were added. The vial was vigorously shaken, followed by a 10 min centrifugation at 4000 rpm. After centrifugation the ethanol solution containing the biogenic amines was siphoned into a second vial while in the centrifugation vial another 1 mL of ethanol is added, followed by a strong shake of the vial and another 10 min of centrifugation. The ethanol solution is once more siphoned into the second vial. By this procedure the biogenic amines have been extracted in the ethanol solution, the extract being used in the quantitative determination.

The centrifugation vials are presented in figure 1: Vial A contains the sample after homogenization and prior to centrifugation, while Vial B contains the sample after centrifugation.

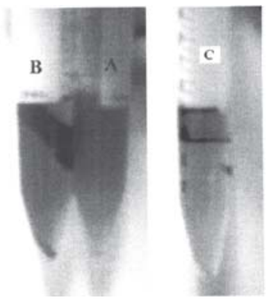

Fig.1. Wine samples: A - Prior to centrifugation, B - After centrifugation, C – After separation

After the centrifugation the ethanol is visibly separated from the aqueous part of the sample due to the high salt content of the water part. In figure 1C a vial is presented for illustrating the clear separation of the ethanol.

From the extract a 1 mL sample solution was collected and introduced in an HPLC vial.

The HPLC pre-column derivatization was performed automatically by the instrument, via the auto-sampler when 3 µL of ethanol solution sample is mixed with 4 µL of OPA and 4 µL of borate buffer and the 11 µL sample mix is injected.

Results and discussions

A number of 23 commercially available wines considered to be representative for the three Romanian vineyards (Minis, Recas, Murfatlar), have been analyzed. The vineyards chosen have a long tradition in producing wines and along with the tradition come the advantages and disadvantages associated. As advantages one can name the care and love for the wines and the cultivation of grapes and the existence of established techniques as well as enological practices. As disadvantage, one can mention the traditionalism and the resistance to new techniques and innovation. Due to the geographical positioning of Romania, 44-48° Nordic latitude, not far from the northern limit of grape-wine cultivation, grape wine needs a special care for cultivation. Meanwhile, the climate generates a great variety of conditions for producing wines. In table 2 are presented the wines chosen for biogenic amine determination, grouped in accordance with the vineyard of origin.

Romanian grape cultivation being one of microclimates, the enological factors are different from one region to another, this resulting in a wider range of wines. Thus southern vineyards like those from the Dobrogea hills, which have a high solar exposure and high temperatures climate offer great conditions for producing high quality red wines, while the northern Transylvanian vineyards with a lower solar exposure and lower temperatures climate offer optimal conditions for producing white wines.

Table 2
WINES TYPE SELECTED FOR EXPERIMENTATION

Sample Code	Vineyard / sample name	Wine type	Year
	Minis Vineyard		
Mi-CP5371-R	Mara	Red, liquorish	2002
Mi-CP5372-R	Cabernet sauvignon	Red, dry	2003
Mi-CP5373-R	Cadarca	Red, dry	2003
Mi-CP5374-R	Burgund	Red, dry	2004
Mi-CP5375-R	Merlot	Red, dry	2003
Mi-CP5376-W	Feteasca regala	White, dry	2004
	Recas Vineyard		
Re-CP5737-R	Pinot noir	Red, dry	2005
Re-CP5738-R	Cabernet sauvignon	Red, dry	2007
Re-CP5739-R	Merlot	Red, dry	2006
Re-CP5740-Ro	Merlot rose	Rose, dry	2005
Re-CP5741-W	Riesling	White, dry	2005
Re-CP5742-W	Feteasca	White, dry	2002
Re-CP5743-W	Sauvignon blanc	White, dry	2003
Re-CP5744-W	Pinot grigio	White, dry	2005
	Murfatlar Vineyard		
Mu-CP6021-W	Pinot gris	White, demi-sweet	2007
Mu-CP6022-W	Premiat 2006	White, demi-dry	2006
Mu-CP6023-W	Rai de Murfatlar	White, sweet	2006
Mu-CP6024-W	Sauvignon blanc	White, demi-dry	2006
Mu-CP6025-W	Chardonnay	White, demi-sweet	2007
Mu-CP6026-R	Rai de Murfatlar	Red, sweet	2007
Mu-CP6027-R	Merlot	Red, demi-dry	2007
Mu-CP6028-R	Zestrea Murfatlar	Red, demi-sweet	2007
Mu-CP6029-R	Cabernet sauvignon	Red, demi-dry	2007

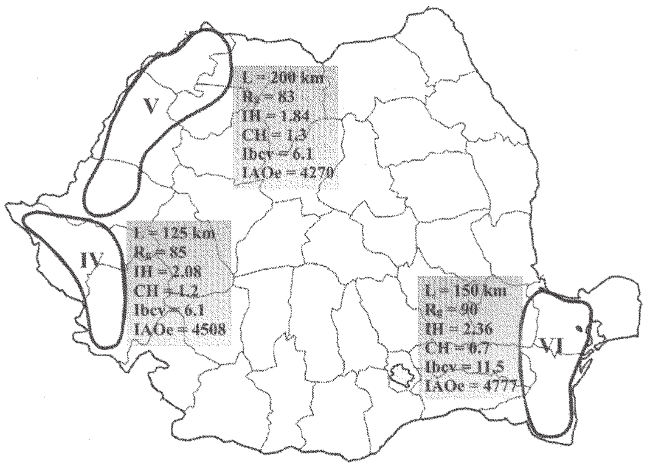

Fig.2. Romanian grapevine regions. General characterization (12).
L – latitude (km), R_g – Global radiation (kcal/cm^2), IH – heliothermal index, CH – hydrothermal index, Ibcv – grapevine bioclimatic index, IAOe – enological aptitude index

Table 3
ECOCLIMATE CHARACTERISTICS OF THE SELECTED ROMANIAN GRAPEVINE REGIONS

Vineyard region	Altitude, m	Yearly rain, mm	Global radiation Kcal/m^2 (r_g)	Year interval 1 april – 30 september: Solar hours	Active thermal intake (t_a)	Bioclimatic index Ibcv	Enological aptitude index IAOe
IV. Banat Hills	146	681	85	1424	3218	6.5	4508
V. Crişana and Maramureş hills	222	635	83	1412	2960	6.1	4270
VI. Dobrogea hills	101	420	90	1606	3164	11.5	4777

Table 4
BIOGENIC AMINES CONTENT OF THE ANALYZED ROMANIAN WINES

Sample code	Biogenic amines content (mg/L): Histamine	Tyramine	Putrescine	2-phenyletyl-amine	Cadaverine	Isopentyl-amine	Total amines
Mi-CP5371-R	0.789	0.600	3.971	0.400	0.195	0.259	6.212
Mi-CP5372-R	1.500	1.107	6.034	0.249	0.198	0.210	9.299
Mi-CP5373-R	1.383	0.459	5.440	0.128	0.166	0.089	7.664
Mi-CP5374-R	1.001	0.208	8.408	0.288	0.181	0.237	10.322
Mi-CP5375-R	1.308	0.653	7.827	0.218	0.230	0.169	10.404
Mi-CP5376-W	0.210	0.166	0.303	0.075	-	0.030	0.784
Re-CP5737-R	-	0.094	2.872	3.244	0.166	2.434	8.809
Re-CP5738-R	0.800	0.118	2.972	0.956	0.218	0.381	5.446
Re-CP5739-R	2.389	0.977	7.012	1.659	0.189	0.674	12.900
Re-CP5740-Ro	-	0.032	2.397	1.230	0.070	2.712	6.441
Re-CP5741-W	-	-	2.763	1.394	0.182	3.865	8.205
Re-CP5742-W	-	-	3.295	0.056	0.096	0.027	3.474
Re-CP5743-W	-	-	5.411	0.036	0.080	-	5.528
Re-CP5744-W	-	-	2.000	0.695	0.117	2.209	5.020
Mu-CP6021-W	0.588	0.067	1.856	1.158	0.124	4.304	8.098
Mu-CP6022-W	0.547	3.132	2.708	1.975	0.100	5.365	13.827
Mu-CP6023-W	0.555	0.110	2.066	2.224	0.127	7.909	12.991
Mu-CP6024-W	0.683	-	1.728	1.888	0.981	4.775	10.054
Mu-CP6025-W	-	-	1.685	1.519	0.107	4.836	8.147
Mu-CP6026-R	2.998	3.083	15.872	0.485	0.405	0.708	23.549
Mu-CP6027-R	1.194	0.077	3.207	0.343	0.174	0.417	5.413
Mu-CP6028-R	1.278	0.280	4.645	0.955	0.230	1.589	8.978
Mu-CP6029-R	0.996	0.235	2.870	1.069	0.228	1.977	7.375

In figure 2 the Romanian grapevine regions are presented, with the associated vineyards [13].

The analyses of the biogenic amine content for wines produced in the regions IV - Banat Hills (Recas vineyard), V – Crişana and Maramureş hills (Minis vineyard) and VI - Dobrogea hills (Murfatlar vineyard) have been performed. The main eco climate characteristics of the vineyards selected from the chosen grapevine regions are presented in table 3 [13].

The results obtained by the HPLC analyses are presented in table 4.

From the experimental data it is very clear that most of the wines have a high content in putrescine, regardless of the wine type (red or white) this being usually due to the ageing of the wines in oak barrels along with a poor cleaning of these barrels from one batch to another. This can also occur if the yeast and malolactic bacteria have survived during the ageing of the wines [14].

Wines from Minis vineyard have a total content in the analyzed biogenic amines below 10 mg/L or a slightly higher than 10 mg/L but below 11 mg/L. Except for content in putrescine, biogenic amines content of the Minis wines are low, which is a very good thing taking into consideration that the vineyard did not use any selected yeasts for the alcoholic fermentation and also did not use selected malolactic bacteria, all fermentation processes resulting due to the microorganisms naturally present on the grape skin. The white wine from the Minis vineyard has the lowest content in biogenic amines from all the wines that have been studied, cadaverine being below the detection limit. These findings are in accordance with the fact that the vineyard has optimal climate conditions for the obtaining of white wines and the applied technology is safe enough.

The wines from the Recas vineyard also have a low content in biogenic amines, all but one of the wines having below 10 mg/L total content in biogenic amines, Merlot being the only wine that has a content above 10 mg/L. It is notable the fact that the overall content in histamine of the wines coming from Recas vineyard is the lowest compared with the wines coming from the other vineyards. This can be attributed also to the fact that the vineyard uses *Saccharomyces oviformis* for the alcoholic fermentation and *Oenococcus oeni* bacteria for the malolactic fermentation [14-17]. The small content in putrescine and cadaverine indicated a clean technology used in this vineyard for wine making.

The wines from the Murfatlar vineyard have the highest content in the studied biogenic amines, four of the nine wines selected for the experimentation having above 10 mg/L total content in biogenic amines and the sweet red wine Rai de Murfatlar from 2007 having the highest content in total biogenic amines from all the 23 wines selected for the experimentation. The high content in biogenic amines of the wines from this vineyard can be attributed to the very hot climate, when the fermentation processes usually have spoilage processes that take place in parallel. Problems may appear also due to an improper processing of the grapes and a longer maceration periods for color extraction for red wines, as well as to high *p*H levels [11,14].

Conclusions

From the experimental data the following statements resulted:

- in most of the Romanian wines the total content of biogenic amines is below 10 mg/L or around this value>

- histamine content is in most cases below the 2 mg/L value, which is the low limit recommended by Germany except two red wines having a slightly higher value, however below 3 mg/L;

- the red wines have a higher content of biogenic amines, compared with the white wine, due to the differences of production method; red wines require fermentation with maceration so that the colors are extracted from the grape skin and also have a malolactic fermentation step in for reducing the acidity of the wine;

- the white wine Feteasca regala 2004 from Minis vineyard has the smallest content in total biogenic amines;

- the sweet red wine Rai de Murfatlar from Murfatlar vineyard has the highest content of total biogenic amines;

- from the experimental data a certain relation between the climate, fermentation technology and the biogenic amine content has been established. Hot climate suitable for microorganism development, high *p*H values and application of an improper technology may generate higher amount of biogenic amines. However, further detailed studies need for establishing a correlation between the above factors and biogenic amines content in wines.

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