

Determination of biogenic amine – content of natural wines

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Summary: Nowadays the consumption of natural wines has been increased. In parallel with the higher demand, the study of the parameters of natural wines has also developed. The amines being produced during the decarboxylation procedure of amino acids could be one of the most important features of natural wines. The objective of this study was to measure the biogenic amine content of natural wines from 2000 and 2001. We have determined that the biogenic amine content of natural wines is not different from the biogenic amine composition of normal wines. There was not any considerable difference between the years. In compliance with the average tendency, red wines contain higher histamine-, tiramine- and serotonin values than white wines.

Key words: biogenic amine, Hungarian wines, natural wines

Introduction

In qualifying methods of food interests are concentrated to the examination of biogenic amines because of their physiological effect. Natural wines are prepared with a special method, which differs from the traditional wine making procedures. The significance of natural wines is based on their considerable physiological effect. We have not found figures related to the content of biogenic amines natural wines neither in the domestic oenological literature nor in the special international literature.

Supply of the nitrogen content of the soil cannot be realised but with organic manure, exclusively, if biovine has been intended, so it has special effects on the amino-acid-content of grapes. Furthermore, other treatments during the wine-making method can influence the amino-acid-content and hereby the biogenic amine composition too.

Biogenic amines are degradation-compounds with small molecular weight. They have, more or less, a basic character, they are produced during the metabolic activity of microorganisms and plant- or animal organisms. There are numerous ways for the formation of biogenic amines, in our case the decarboxylation of amino acids is one of the most important ways.

In relation to other fermented foods the biogenic amine-content of wines is low. The reason for their research is that its degradation in the human organism is realized by the enzyme monoamino-oxidase and this enzyme is to a certain degree or completely blocked by medicines and alcohol, hereby amines in wines can accumulate and cause allergic symptoms.

In our earlier work we have studied and stated the amine content of Hungarian wines and champagnes (Kállay et al.,

1984). Table 1 shows the measured values of biogenic amine composition of Hungarian wines. At present, Simonné et al. measure the biogenic amine content of wines, regularly. They widen our knowledge on amine values of domestic wines (Simonné et al., 1999).

Table 1 Biogenic amine composition of Hungarian Wines

Biogenamine	White wine (mg/dm ³)	Red wine (mg/dm ³)
Cadaverin	NQ-0.3	NQ-0.69
Ethyl-amine	NQ-0.4	NQ-0.8
Histamine	0.17-1.25	0.59-2.2
Methyl-amine	0.21-1.30	0.3-0.87
B-phenyl-ethyl-amine	NQ	NQ-0.78
Putrescin	0.31-1.78	0.45-5.49
Serotonin	NQ-0.75	NQ-1.07
Tiramine	0.1-1.1	0.3-0.95
Tryptamine	ND	ND

Concerning the physiological effect, histamine, serotonin and tiramine are the most important biogenic amines.

Histamine can have a poisonous effect in higher doses, its toxic limit is 2-10 mg/l in beverages (Brink et al., 1990).

Tryptamine is significant because of its derivatives. Serotonin is the most important derivative, it has got an essential effect on regulating the blood pressure. Furthermore, it can be a medicine in case of mental depression. According to the literature, serotonin is generally not registered among the examined amines. We are working on the determination of serotonin-concentration of Hungarian wines too, but at present only the serotonin-content of natural wines will be shown.

The toxic range of tiramine is 100–800 mg/kg [3].

According to the special oenological literature, there are works on measuring these compounds in wines but in the case of natural wines, there are not any published measured values yet.

The question arises, what the difference is between natural wines and traditional wines. The vine-growing procedure of biogrape, and the treatments of natural wines are determined by the rules of Biokultúra Egyesület (Association of Bioculture) (Kállay & Sárdy, 2000). The main differences are the following:

- Methods and materials permitted and advised for being used:
 - * For the stabilization of wines, physical methods come to the front such as centrifuging, filtering, cooling, chill proofing.
 - * For clarification, edible gelatine, isinglass, casein and potassium-caseinate, egg white and bentonite with small iron content.
 - * For cleaning and disinfection, citric acid, tartaric acid, hydrogen-peroxide and for barrels sulphur-dioxide can be used in quantities which do not pollute the environment
- Methods and materials prohibited during the natural wine-, grape-juice- and sparkling wine making procedure:
 - * Use of blue fining
 - * Use of pectolytic enzymes
 - * Plastic bottle and cork
 - * Free/total H_2SO_3 in dry wine is maximum 10/50 mg/l, in the case of sweet wine this is 30/100 mg/l [4].

After the EU accession, natural products will appear in the market in higher quantities. In parallel with their spreading, the regulations of Food Safety will be more serious and they will emphasize the importance of the examination of biogenic amines.

According to the above mentioned, the aim of our work was to broaden the knowledge on natural wines and their biogenic amine – content. In this study we have measured the biogenic amine concentration in Hungarian natural wines from 2000 and 2001 because there are no figures to be found neither in the domestic-, nor in the international specific literature.

Experimental goals

During our work, we have searched the answers for the following questions:

- biogenic amine content of natural wines
- especially histamine, tiramine, serotonin content within biogenic amines
- effect of year on biogenic amine content.

Material and method

Wine samples

The samples of natural wines from 2000 and 2001 were given by bioproducers registered in Hungary. The first 12 samples are white natural wines from 2000, the others are red ones. The first 16 samples are white natural wines from 2001, the others are red ones.

Method of measuring

Biogenic amine content of natural wines was determined by High Performance Liquid Chromatography. The chromatographic conditions were the following:

Type of equipment: HPLC typed HP 1050

Column: Nukleosil C-18 200 * 0

Detector: Fluorescent detector, HP 1046 type

Liquid-flow: 10 ml/min

Temperature: 30 °C

λ_{ex} : 340 nm λ_{em} : 440 nm

Composition of the eluent:

A solution: 0,08 M acetic acid

B solution: Acetonitrile HPLC-qualified

The efficiency of the reversed phase chromatographic segregation was enhanced with gradient elution techniques. The gradient composition, and its running in time are shown in Table 2.

Table 2 Gradient – composition

TIME (min.)	A%	B%
3,5	70	30
10	35	65
21	28	72
22	20	80
25	20	80
30	70	30

The identification of the components was realized by standards (SIGMA and FLUKA), the concentration of the components was measured by calibration lines with the standards.

Preparation of wine-sample

Each wine was filtered by membrane filter (0.45 μ m), then reacted with orto-phtaldehyde (OPA) in borate-buffer as see below:

- 0.5 ml of borate-buffer: 0.5 g H_3BO_3 dissolved in 19 ml of distilled water, then setting the pH to 10.4 with KOH -solution concentrated 40 g/100 ml
 - 0.1 ml of OPA reagent (SIGMA)
 - 0.1 ml of wine-sample

20 µl of the prepared wine-sample was injected into HPLC equipment after a reaction time of 2 minutes.

Results

The composition of biogenic amines of natural wines from 2000 is shown in *Table 3*.

The total quantity of biogenic amines varied in the range of 35.6 and 337.4 mg/l. The highest quantity was measured in the case of sample No. 13, the slightest one in the case of sample No. 17 (*Figure 1*).

Looking at histamine (*Figure 2*), the average measured value is 1.7 mg/l. This biogenic amine was found in the highest quantity in sample No.12, low or no measurable content was found in sample No. 8.

The average value of tiramine concentration in wines was 3.7 mg/l (*Figure 3*). The sample No. 14 involved the slightest, the sample No. 13 contained the highest quantity of tiramine.

Concerning serotonin content (*Figure 4*), extremely high value was found in sample No. 13, a very small value was measured in samples 8, 14 and 12.

The composition of biogenic amines in natural wines from 2001 is shown in *Table 4*.

The average total quantity of biogenic amines was 80.7 mg/l. The highest value was measured in sample 15, the smallest one was found in sample numbered 14 (*Figure 5*).

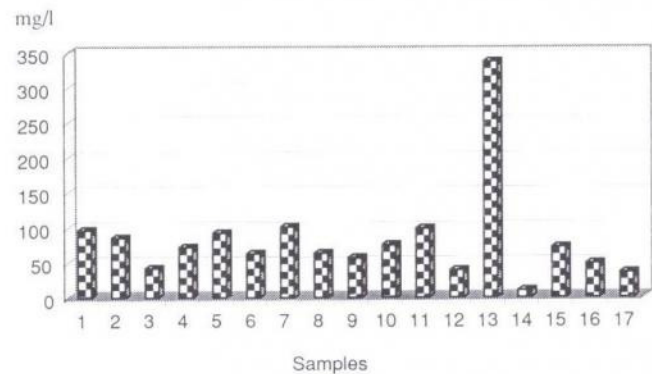


Figure 1 The total biogenic amine-content of natural wines of 2000

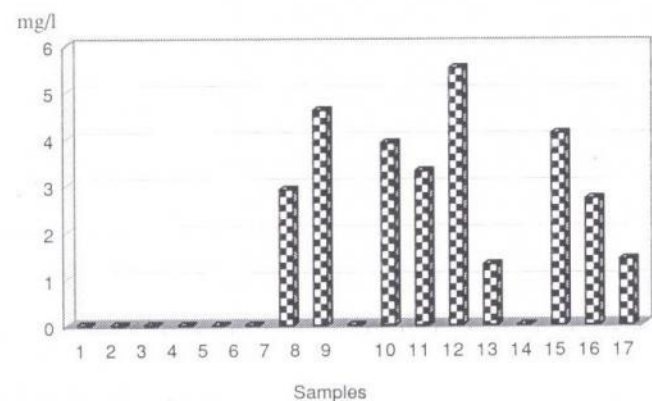


Figure 2 Histamine-content of natural wines of 2000

Table 3 Division of biogenic amines of natural wines of 2000

Samples	Ethylamine	Metilamine	Histamine	Tiarmine	Serotonine	Putrescine	β-phenyl-ethylamine	Cadeverin	Total biogenic amines
1	0	30.1	0	4.8	40.1	14.8	4.8	1,8	96,4
2	6.7	25.1	0	4.6	24.5	15.4	7	2.1	85.4
3	6.8	0	0	4.7	21	3.7	4.1	1.6	41.9
4	0	0	0	4.6	40.6	13.6	10.6	2.5	71.9
5	22.9	37.8	0	5.6	12.8	4.8	6.6	1.8	92.3
6	2.1	3	0	2.1	53.6	1.8	0	0	62.6
7	0	16.7	2.9	6.9	52.2	16.6	3	2.6	100.9
8	26.6	17.7	4.6	2.2	3	4.4	3.5	1.3	63.3
9	16.9	8.1	0	2.3	10.7	13.7	3.1	2	56.8
10	23.8	9.3	3.9	4.2	18.7	7.7	5.5	2.4	75.5
11	22.7	20.8	3.3	3.8	36.1	7.2	2	3	98.9
12	14.3	2.1	5.5	2.3	5.4	2.7	4.6	2.1	39
13	7.2	0	1.3	7.8	286.9	15.7	15.5	3	337.4
14	2.9	1.77	0	0.5	2.7	2.3	0	0	10.17
15	23.5	11	4.1	2.4	9.2	9.6	8.8	3.3	71.9
16	12.8	7.8	2.7	2.1	6	3.2	12.2	1.8	48.6
17	7.3	1.8	1.4	2.3	9.3	3.5	5.9	4.1	35.6
Average	11.6	11.4	1.7	3.7	37.2	8.3	5.7	2.1	81.7

Table 4 Division of biogenic amines of natural wines of 2001

Samples	Ethylamine	Metilamine	Histamine	Tiarmine	Serotonine	Putrescine	β -phenyl-ethylamine	Cadeverin	Total biogenic amines
1	24	0	2.1	4.4	34.2	16	4	1.5	86.2
2	33.7	34	3.4	4.2	27	8.6	3.9	2.4	117.2
3	5.8	15	4.1	2.8	12.3	3.3	2.6	2.1	48
4	11	5.8	0	4.4	32.39	8.6	6.5	10.7	79.4
5	3.3	11.7	2.4	1.8	12	3.2	3	0	37.4
6	3.2	14.8	0	2.7	13.8	3.3	10.1	4.7	52.6
7	5.3	8.7	1.7	1.5	6.4	2.4	4.1	0	30.1
8	9.9	5.8	3	2.5	2.6	1.6	0	2.9	28.3
9	35.7	0	2.8	7.8	82.8	19.1	17.7	20.6	186.5
10	45.2	0	1.6	5.8	76	7.3	17.3	20.9	174.1
11	16.7	19.4	12.6	5.9	45.7	27.8	9.9	2	139.97
12	61.8	16.3	5	5	34.7	20.8	5.6	1.9	151.1
13	24.8	4.4	2.5	5.3	71.4	23.1	13.2	3.3	148
14	4.9	3.1	0	2.3	4.9	0	0	0	15.2
15	44	37.7	23.7	5.4	66.5	30	9.4	1.6	218.3
16	8.7	15.5	3.6	2.1	2.8	3.5	11.5	3.3	51
17	10.1	12.6	0	2.5	16.8	2.6	4.4	2.4	51.4
18	10.6	5.7	1.8	1.8	5.3	3.8	3	2.8	34.8
19	5.7	0	2.6	2.3	14.7	1	2.3	2.4	31
20	5.6	0	2.6	2.7	14.6	5	2.4	4	36.9
21	14	0	2.1	2.4	5.7	2.4	6	1	33.6
22	13	0	2.4	2.1	2	1.5	1.9	1.6	24.5
Average	18	9.6	3.6	3.5	26.6	8.8	6.3	4.2	80.7

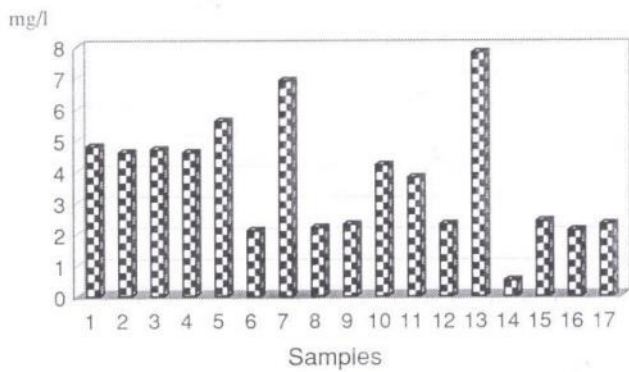


Figure 3 Tiramine-concentration of natural wines of 2000

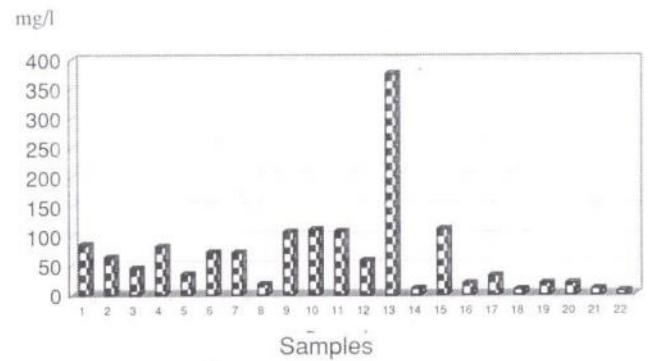


Figure 5 The total biogenic amine-content of natural wines of 2001

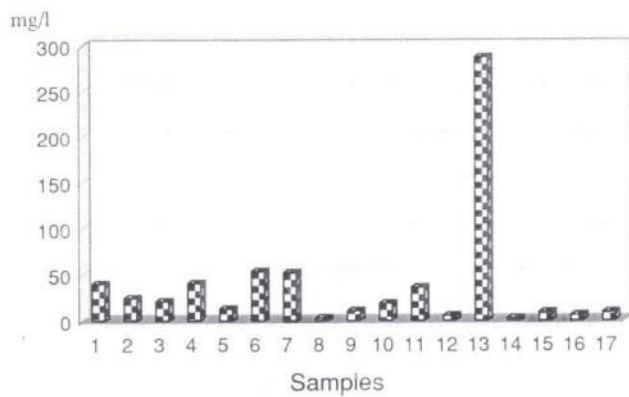


Figure 4 Serotonine-content of natural wines of 2000

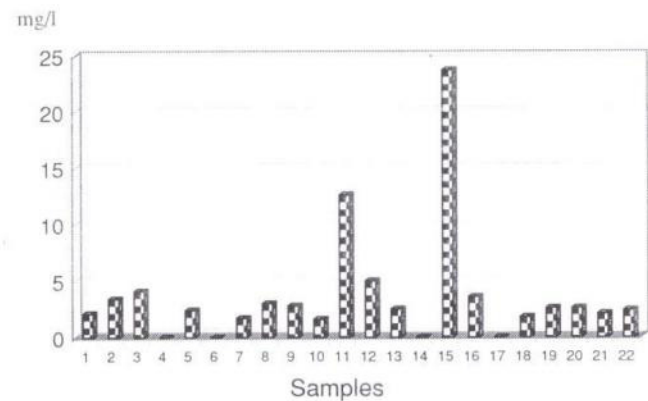


Figure 6 Histamine-content of natural wines of 2001

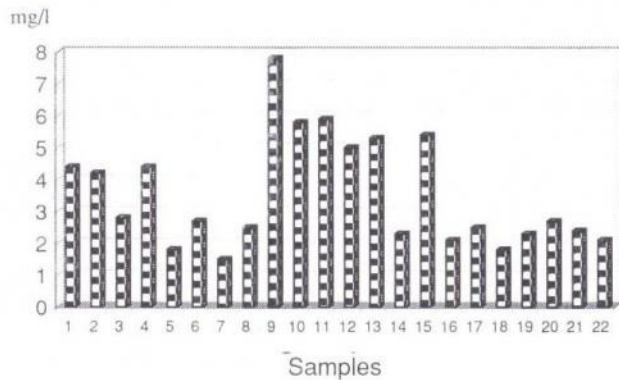


Figure 7 Tiramine-concentration of natural wines of 2001

There were only 3 samples which did not contain histamine at all (samples 4, 14 and 17). The maximum value was 23.3 mg/l (Figure 6).

In the case of tiramine, content the measured average value was 3.5 mg/l and it varied in the range of 1.5–7.8 mg/l (Figure 7).

Looking at serotonin, the slightest value was measured in sample 22, the highest one was found in sample No. 15 (Figure 8).

Comparing the year of 2001 with the year of 2000, we can declare that there are not any considerable differences between the years in the case of total biogenic amine content of natural wines. The measured values of natural wines are convenient to the biogenic amine composition of normal wines found in the specific literature (domestic and international as well.) There are no considerable differences. Concerning red wines, a higher histamine- and tiramine content can be measured than in white wine because of the malolactic fermentation.

Conclusions

Natural wines are in the focus of interest from the standpoint of physiological effect. In parallel with their increased supply, the examinations on natural wines are spread out. The knowledge on the biogenic amine content and the composition of natural wines have to be broadened because we have not found any figures on these compounds yet, although these components play an important role in the human organism.

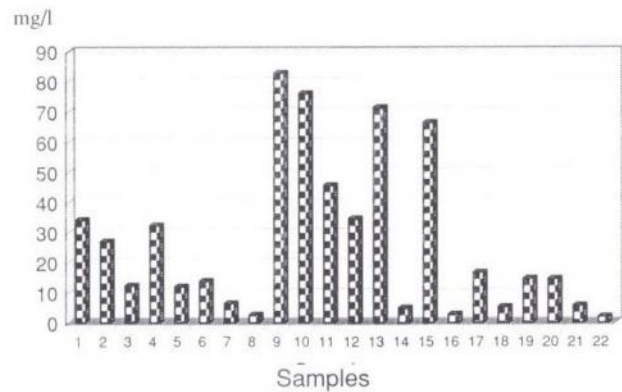


Figure 8 Serotonin-content of natural wines of 2001

We have determined during our work that the biogenic amine content of natural wines does not differ from the biogenic amine content of normal wines, it is varied in the range of 15.2 and 337.4. There is no difference between the two years (average total biogenic amine content is 81.7 in 2000; 80.7 in 2001). Red wines contain higher histamine, tiramine and serotonin then white wines.

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