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Comparison of the biogenic amine content of traditional and bio-wines

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Summary: There has been a growing tendency in organic farming in recent years, in which organic grape growing and the production of bio-wine are also significant. The literature on bio-wine is rather limited, that is why we consider it really important to contribute to the data. As a result of the special conditions in organic farming we can expect bio-wines to have a different composition of nitrogen compounds from that of traditional wines. Biogenic amines could thus specially characterize bio-wines. In our research we compared the biogenic amine content of wines produced using organic methods and of those where traditional procedures were used. Our measurements have proved that it is not possible to clearly distinguish bio-wines from wines produced with traditional methods on the basis of amine content.

Key words: biogenic amines, HPLC, bio-wines, organic growing

Introduction

There has been a growing attention turned towards the biogenic amine content of foods, because of its effects on human health/body. Biogenic amines are natural components of food products, especially of fermented ones, such as cheese, wines, champagne (*Sarkadi*, et. al.,1990). They can certainly be found in other food products as well, so there is also some research being done on pork (*Szerdahelyi*, 1994).

Biogenic amines belong to the nitrogenous compounds of must and wines. They are decomposition products of small molecular weight, which also used to be called rotting by-products – a false assumption, however, as these substances are not always produced in the process of rotting (*Kállay*, 1994).

The term "biogenic amine" refers to many different groups of compounds. As regards chemical structure, they can be aliphatic, aromatic or heterocyclic, including aliphatic diamines putrescine and cadaverine, aliphatic polyamines spermidine, spermine, agmatine, aromatic amines tiramine, adrenaline, β-phenyl-ethylamine, noradrenaline, dopamine, heterocyclic monoamines histamine, triptamine and serotonin (*Lasztity*, 1981).

According to distillability two major groupsave distinguished: volatile amines, including primary, secondary and terciary aliphatic amines, and the aromatic amines benzyl amine and phenyl-ethylamine. Cadaverine, putrescine, tiramine, triptamine and histamine are non-volatile amines. (*Kállay* et al., 1997)

There are various ways to produce amines: amination of aldehydes, ketones, ketonic acids; methylation of nitrogenous compounds; hydrolysis of nitrogenous parts of compounds of big molecular weight; or enzymatic decarboxylation of amino acids. Biogenic amines in food and wines indicate the presence of microorganisms, as they are generally products of metabolic processes of microorganisms (*Dukes* et al., 1998).

Amines in must and wines are also usually produced through decarboxylation of amino acids. *Figure 1.* demonstrates the possible ways leading to the formation of biogenic amines, with the precursor compounds. Cadaverine, agmatine and tiramine are synthesised one way, while in case of other amines, such as histamine, putrescine, spermidine there are several ways (*Bardócz*, 1993).

Biogenic amines have significant physiological effects, that is why they are in the centre of scientific attention.

The effects of histamine are the best-known. It is famed for its allergenic and inflammatory effects, in bigger amounts it may cause a state of shock, and it has toxic effects in different doses. Other unpleasant effects of bigger amounts of histamine include rashes due to the expansion of capillaries. It also has a direct heart effect, causing either a decrease or an increase of the heart rate. In case of asthmatic patients it may result in serious symptoms because of the restricting effects on the bronchioles and alveoli (*Falus*, 1994).

Many scientists have examined the toxic effects of tiramine, who have concluded that the so-called cheese poisoning is connected to tiramine. Tiramine liberates noradrenaline in the sympathetic nervous system, which makes the heart contract and increases blood pressure.

Cadaverine, putrescine and spermidine are found in both animals and plants. They play an important role in cell division and growth, as well as in the growth of tumours (*Bardócz* et al., 1993).

Serotonin is another biogenic amine of great significance. In recent years it has been used in human medicine as antidepressant. Among fruits it it bananas that have an especially high content of serotonin. Serotonin has been detected not only in plants, but also in animal tissues, for example in fish, wasps and the poison of scorpions and toads (*Bauza* et al., 1995).

As for bio-wines, there are no data recorded concerning their biogenic amine content. As in case of organic grapes the method to supply nitrogen is different from traditional procedures, we can expect to see some differences in the nitrogenous compounds of bio-wines.

Below you find a short summary of the characteristics of organic farming and the rules of making bio-wine.

The main characteristics of growing organic grapes:

"Ecological agriculture aims to create sustainable, variable, well-balanced, environmentally friendly, profitable agricultural systems, which produce quality food." Its main principles are the following:

- Avoiding polluting technologies.
- Maintaining and improving the natural productivity of soil.
- Decreasing the consumption of non-renewable resources, while increasing the use of renewable ones.
- Satisfying the needs of special species (*Solti*, 2000).

Supplementation of nutrients and manuring can be summerized as follows:

- Chemical fertilizers are forbidden, green manuring is done in accordance with previous measurements.
- Supplementation of nutrients to the soil is only allowed using organic materials.
- Emphasis on soil-ecology (*Sárközy* & *Szőnyi*, 2000).

Main rules of bio-wine production:

Recommended procedures:

- Separation and filtration with the appropriate devices
- Heating: in case of problematic grapes, must pasteurization, heating the fruit and the must to 30°C and 60°C respectively, short-term heating to high temperatures, especially when making preserved must and grape juice.
- Cold stabilization
- Addition of extra air to must (hyperoxidation)

Forbidden procedures:

- Use of yellow salt
- Use of copper sulphate
- Use of ascorbic acid (vitamin C) and sorbinic acid (potassium sorbate)

Based on the above, our aim was to examine the biogenic amine composition of bio-wines, where we also compared the biogenic amine content of bio-wines with that of their traditional equivalents. We were looking to find an answer to the question whether there is a significant difference between bio- and traditional wines regarding their biogenic amine content.

Material and method

Wine samples:

We collected bio-wines of year 2002 from Hungary's bio-wine cellars and their traditional equivalents of the same grapes grown in the same area.

Researsch methods:

Preparation of the samples:

Membrane filtration of must and wine through a 0.45ìm membrane was followed by reacting with OPA (ortho-phthalaldehyde) in the presence of borate buffer.

Chromatographic circumstances were as follows:

Device: HPLC, type HP

Column: nucleosil 100 C-18 (250x4mm)

Detection: HP 1046 A fluorescent detector

Flow: 1ml/min

Temperature: 30°C

λ 340nm λ 440nm

Eluent composition: solution A: 0.08 M acetic acid

solution B: HPLC quality acetonitrile

Table 1. Gradient composition:

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

Identification of the components was done with the help of standards, their concentration determined based on the calculated calibration lines. Identification of the various compounds by the calibration lines was done on the basis of elution times. All compounds are defined in histamine, except for serotonin.

Calibration lines were as follows:

Histamine: X= 0.014Y+0.0897

Serotonin: X= 0.051Y-2.113

Results

The composition of biogenic amines is summarized in *Table 2 and 3*.

The ethylamine content of white wines is absolutely irregular, in some samples no ethylamine was detectable (Látrány Királyleánnyka bio and control; Badacsony Hárslevelű bio and control; Etyek Riesling bio and control). In the other samples the control wines contained bigger amounts of ethylamine than the bio-wines.

As for ethylamine content of red wines, there is no significant difference between organic and non-organic

Table 2. Distribution of biogenic amines in control and natural white wines

Cultivar	ethylamine		methylamine		histamine		tyramine		serotonine		putrescine		2-pheny lethylamine		cadaverine	
	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio
Látrányi Irsai Olivér	4,5	0	0	0	0	0	0	0	9,9	0	2,9	0	0	0	0	0
Látrányi Királyleányka	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Badacsonyi Sárga Muskotály	41,2	20,9	11,3	0	0	0	0	6,6	30,8	26,6	7,3	0	0	5,8	0	0
Badacsonyi Szürkebarát	24,6	14,3	60,8	15,2	0	0	13,9	28,2	26,5	28,2	5,5	23,7	0	32,54	4,4	0
Badacsonyi Olaszrizling	15,34	15,69	39,48	11,6	0	0	10,55	4,2	36,7	24,2	0	16,6	0	6,2	0	0
Badacsonyi Hárslevelű	0	0	16,46	6,9	0	0	4,83	5,5	33,77	5,5	0	3,6	4,07	0	0	0
Etyeki Chardonnay	0	8,2	0	0	0	0	0	0	7,9	11,45	3,3	3,3	2,4	0	0	0
Etyeki Olaszrizling	0	0	0	0	0	0	0	3,3	0	3,3	0	0	0	0	0	0
Mean:	**10,705**	**7,38625**	**16,005**	**4,2125**	**0**	**0**	**3,66**	**5,975**	**18,19625**	**12,40625**	**2,375**	**5,9**	**0**	**0**	**0**	**0**

Table 3. Distribution of biogenic amines in control and natural red wines

Cultivar	ethylamine		methylamine		histamine		tyramine		2-pheny serotonine		putrescine		lethylamine		cadaverine	
	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio	control	bio
Villányi Cabernet Sauvignon	4,21	2,79	2,3	0	0	2,1	7,8	2,1	17,6	0	4,25	0	0	0	0	0
Látrányi Merlot	0	0	12,1	32	0	0	15,3	7,2	12,5	8,9	6,2	0	0	0	0	0
Látrányi Cabernet Sauv.	2,5	6,3	7,3	0	0	0	0	1,6	4,4	13,9	3,6	0	4	2,6	0	0
Látrányi Zweigelt	4,6	0	23,9	0	0	0	3,9	0	4,7	11,65	0	0	0	0	1,9	0
Látrányi Kékfrankos	7,8	2,64	10,8	0	0	0	3,2	0	3,6	3,65	4,9	0	1,8	0	0	0
Mean:	**3,8223**	**2,346**	**11,28**	**0**	**0**	**0**	**6,04**	**0**	**8,56**	**7,62**	**3,79**	**0**	**0**	**0**	**0**	**0**

samples here, either. Ethylamine content was measured to be between 4.2–7.8mg/l in case of the control samples, and between 2.6–6.3mg/l in bio-wines.

It was always the control items that contained a bigger amount of methylamine in case of white wines. However, in several pairs of samples no methylamine was detectable at all. Thus, it is impossible to clearly differentiate bio- and traditional wines based on these data.

In case of red wines, it was also the traditional wines in which bigger amounts of methylamine were detected (2.3–23.9 mg/l).

No histamine was detectable in any of the white samples, which also supports the idea based on the above results, i.e. it is not possible to distinguish bio-wines from the control wines. I was only able to detect histamine in one of the red wine samples, 2.1 mg/l in organic Villány Cabernet Sauvignon. This amount is, nevertheless, too small to indicate any hygienic problems.

Tiramine content does not help to tell organic and traditional samples apart, either. Even where there is a difference between their data, there is no tendency as to whether bio-wines or traditional ones contain more tiramine. The control Badacsony Szürkebarát had 13.9 mg/l, the bio Szürkebarát had 28.2 mg/l; whereas the red Látrány Merlot measured 15.3 mg/l with the bio Merlot containing 7.2 mg/l of tiramine.

Serotonin was measured in both the control and the bio samples. Serotonin content of white control wines was between 7.9–126.5 mg/l, that of bio-wines between 5.6–176.1 mg/l. The differences are sometimes significant, but in other cases they are not. Moreover, there is no tendency as to whether it is bio-wines or traditional ones that more serotonin.

The results were the same with red wines. Látrány Cabernet Sauvignon and Zweigelt have a serotonin concentration three times that of the control samples.

The presence of putrescine is also rather variable in white wines. It is not possible to distinguish bio and traditional samples based on putrescine. As for red wines, it was only in the control group that putrescine was detectable, 3.6–6.2 mg/l.

β-phenyl ethylamine was detected in very few samples. In case of white wines, the amounts were bigger in bio-wines than in the control wines. In the red wines, putrescine was found in both control and bio Látrány Cabernet Sauvignon, 4mg/l and 2.6mg/l, respectively.

Cadaverine was detected in two samples, 4.4mg/l in Badacsony Yellow Muscadelle and 1.9mg/l in Látrány Zweigelt.

Based on the above results we cannot clearly differentiate between control and bio pairs of samples. That is why we regarded the wines as a set of data and compared their averages using a t-probe. As a result of the t-probe we can state with a probability of 95% that there is no significant difference between white bio and control wines regarding ethylamine, histamine, tiramine, serotonin, β-phenyl ethylamine and cadaverine content. There is significant difference, however, in methylamine and putrescine content. The concentration of methylamine was much higher in control wines, whereas there was a bigger amount of putrescine in bio-wines *(Figure 1)*.

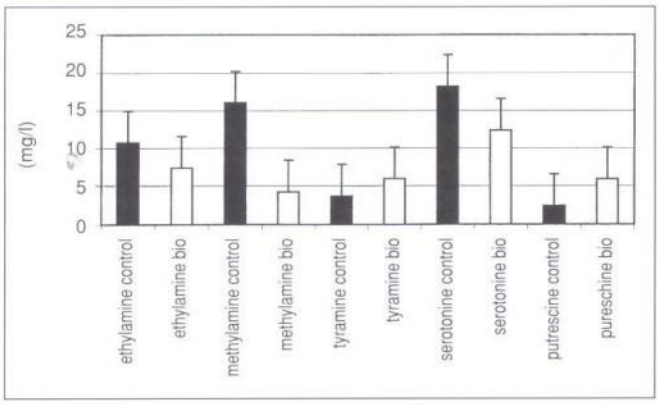

Figure 1. Ethylamine, methylamine, tyramine, serotonine, putrescine in bio and traditional white wines

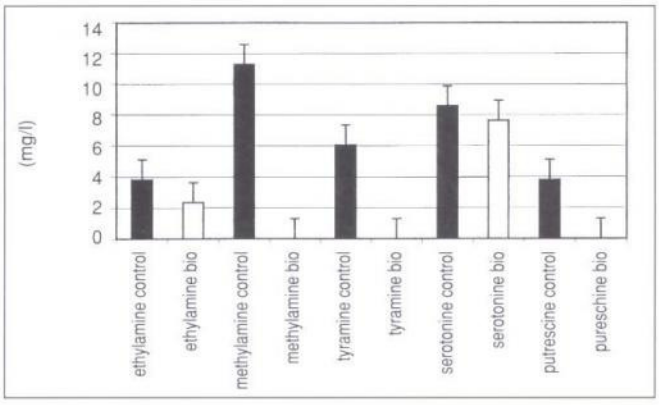

Figure 2. Ethylamine, methylamine, tyramine, serotonine, putrescine in bio and traditional white wines

As for red wines, there is no significant difference in histamine, serotonin, β-phenylethylamine and cadaverine content. The difference is significant regarding ethylamine, methylamine, tiramine and putrescine content. In all cases it is the control samples with the much higher detected amounts of amines *(Figure 2)*.

Conclusion

The above results indicate that we cannot clearly define bio-wines based on biogenic amine content. There is a wide variety of amine concentration in the sample pairs. It is not possible to draw a line marking the border between control and bio-wines based on biogenic amine content.

Neverthelesst we can conclude that the production of bio-wines meets the hygienic requirements, as histamine was only found in one sample. Furthermore, as you can deduce from the data, organic farming does not influence the amine content of wines.

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