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Examination of nitrogenous compounds in Hungarian bio-musts

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Summary: There has not been any research done on the musts from organic grapes. As a result of the special technologies in organic farming we can expect bio-musts to have a different quantitative and qualitative composition of nitrogen compounds from that of traditional musts. In our present essay we deal with the tests of nitrogenous compounds (assimilable nitrogen content, aminoacid, biogenic amine content) in musts from Hungary's bio-wine cellars. Our results show that the change in procedures has resulted in neither quantitative nor qualitative changes in the nitrogenous compounds of bio-musts.

Key words: amino nitrogen, amino acid, biogenic amine content, Hungarian bio-musts

Introduction

In case of growing organic grapes the method to supply nitrogen is different from the traditional procedures, we consider it important to examine the nitrogenous compounds of bio-musts.

Below you find a short summary of the main tendencies in making bio-wine.

Supplementation of nutrients and manuring 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; *Holb*, 2005).

Based on the above we can expect to find difference between traditional and bio-musts concerning nitrogenous substances. The literature abounds in data on amino nitrogen and amino acid content, but not regarding bio-musts.

The term "assimilable nitrogen content" normally refers to the amount of nitrogen that can be taken up by yeasts. Yeasts can make use of ammonia, ammonium cation and free á-amino acids. Thus for the optimal conditions of alcohol fermentation it is indispensable to have the appropriate amount of nitrogenous compounds present. Previous research has shown that with lower nitrogen concentration yeast fungi produce a bigger amount of hydrogen sulphide (*Vos & Gray*, 1979; *Henschke & Jiranek*, 1993).

It is characteristic of the distribution of amino acids in musts that it is arginine, proline, threonine, serine and alanine that are found in bigger amounts (*Würdig*, 1989).

Goni-Torrea & Ancin-Azpilicueta (2001) did not only measure amino acid and amine content, they also examined their quantitative changes during fermentation in case of different yeast starter cultures. They concluded that there was no significant difference between the samples using different yeast cultures regarding amino acid and biogenic amine content.

After measuring the amino acid content in as many as 43 Portuguese musts they found that arginine and alanine are the ones in biggest amounts. Lysine concentration was only 28mg/l in the samples (*Herbert* et al., 2000).

Assimilable nitrogen content and free amino acid content of musts were examined by *Shievly & Henick-Kling* (2001). They determined the assimilable nitrogen content of 39 musts by formol titration and spectrophotometric methods. They compared the two methods, and concluded that both are suitable for accurate measurements.

Nitrogenous compounds include biogenic amines, which have significant physiological effects. Amines in must and wines are also generally produced through decarboxylation of amino acids. 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).

Bigger amounts of histamine can cause unpleasant symptoms, including 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 of the bronchioles and alveoli (*Falus*, 1994).

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).

Based on the above, we consider it important to examine the nitrogenous compounds in bio-musts. We seek to find an answer to the question whether organic farming influences the production and quantity of nitrogenous compounds in bio-musts. Furthermore, we did some tests on collecting data.

Material and method

Tested materials

We collected bio-musts of year 2002 from Hungary's bio-wine cellars.

Test methods

Determination of amino nitrogen content of musts using formol titration.

Free amino groups in proteins and amino acids can be detected using formol titration. In solutions amino groups form salts, so it is not possible to titrate them directly. Formaldehyde is used to tie amino groups and at the same time the liberated equivalent carboxyl groups are titrated with alkali. The organic acids of must and wine need to be neutralised with the help of NaOH up to a pH of 8. Sulpfur dioxide content is oxydized with hydrogen peroxyde, then NH_2 groups of á-amino acids are tied with formaldehyde and the liberated carboxyl groups are titrated with alkali.

$50cm^3$ of must or wine is neutralised in a process of constant stirring to a pH of 7.8 with 1n of NaOH solution. The pH is then set to the exact value of 8 with 0.1n of NaOH.

Then 2 drops of hydrogen peroxyde are added, a couple of minutes later $20cm^3$ of formaldehyde, and after a couple of minutes of mixing we set the pH at exactly 8.5 with 0.1n of NaOH. Then we determine the amount of alkali used (in cm^3).

Calculation of the formol value:

Amino nitrogen (mg/l)= 0.1n NaOH decrease * factor * 28

Optimal amino nitrogen content is between 200–400mg/l.

Determination of amino acid content of musts and wines

Before analysing I filtrated the samples through Sartorius membrane (0.45µm) and injected 100 µl into the device.

Measurements were done with the automatic amino acid analysator type Aminochrom II OE-914 (Laboratory MIM, Budapest). Durrum DC-4A analyser was used for the separation. The dynamic process included various Pico (Pierce) buffers, which were used at three different temperatures.

After the ninhidrin post-column derivatization photometric detection was performed at 570nm – except for proline, which was measured at 440nm.

Concentration of amino acids was carried out on a Dowek 50W*8 cation substitution column (d=1cm*4cm) as follows:

$10cm^3$ of the sample was put onto the analyser column.

After dripping we washed the column with $40cm^3$ of 0.1M (ph8) phosphate buffer, then with $40cm^3$ of 1M hydrogen chloride solution.

We eluted the amino acids tied to the analyser with $15cm^3$ of 6M hydrogen chloride. The solution we collected was then distilled on a water-bath, and the rest taken up in $1cm^3$ of 0.001 hydrogen chloride.

After use, the column was washed with $20cm^3$ of distilled water. It is advisable to regenerate the analyser after 5 samples by boiling it with 6M HCl and 4M NaOH.

Determination of biogenic amine content of musts and wines.

Membrane filtration of both must and wine through a 0.45ìm membrane was followed by reacting with OPA (ortho-phthalaldehyde) in the presence of borate buffer. 4 minutes after mixing the sample, the buffer and the OPA reagent we injected 20ìl into the device.

Cromatographic 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

The effectiveness of reverse phase chromatography was increased by a technique of gradient elution. The composition of gradients is shown in time in Chart 1.

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.

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

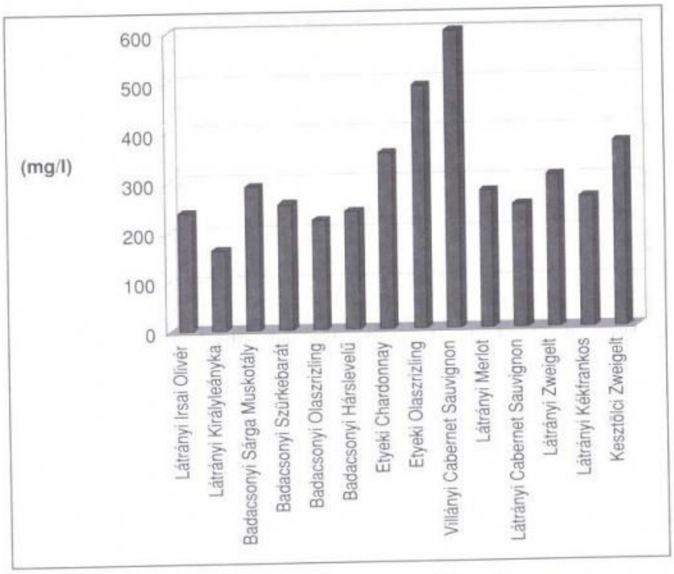

Figure 1. Free amino nitrogen content in natural Hungarian musts

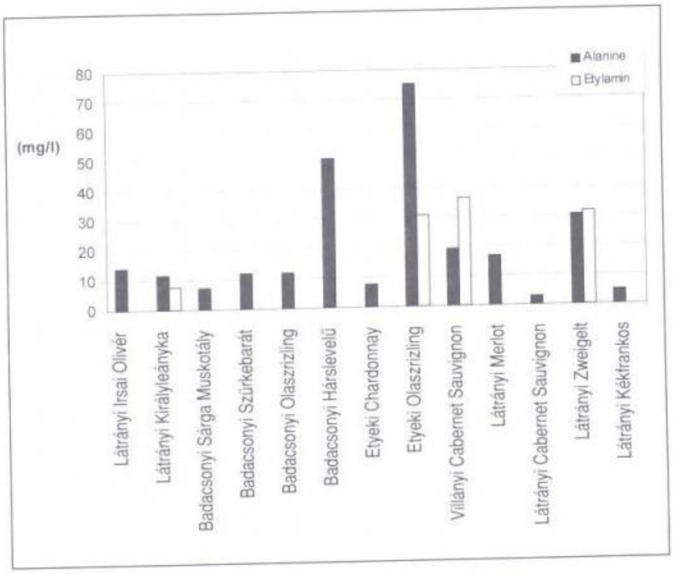

Figure 2. Alanine and metylamine content in natural musts

Results

The amounts of nitrogenous substances indispensable for fermentation are shown in *Figure 1*. The amount of amino nitrogen in white musts is really variable. According to literature data the optimal amount is 200mg/l (*Jiranek* et al., 1995). The amino acid content of bio-musts varied between 163–600mg/l. The highest values were measured in Villány Cabernet Sauvignon, the lowest ones in Látrányi Leányka. The minimum amino nitrogen content of 200mg/l was exceded in all of the samples.

The composition of amino acids and biogenic amines is summarized in *Table 2 and 3*. We considered it important to examine the changes in amino acid composition in bio-musts as well as the quantitative changes in biogenic amines. For a better understanding of the data we have shown the amino acids and the derived biogenic amines on the same diagram.

Alanine and ethylamine contents of white bio-musts are shown on *Figure 2*. All of the musts contain some alanine (8–76mg/l), but ethylamine can only be found in Látrány Királyleányka and Etyek Riesling. In red samples a big amount of alanine was detected even in musts, 15mg/l in Villány Cabernet Sauvignon and 32.2mg/l in Látrány Zweigelt. Among the musts, the same samples also contained a high concentration of ethylamine, 35.5mg/l and 32mg/l, respectively.

The amount of glycine was below 20mg/l in white musts. Methylamine, however, is even detectable in the state of must in several samples; 25mg/l in Látrány Királyleányka, 100mg/l in Badacsony Muscadelle, 101mg/l in Riesling.

Table 2. Distribution of amino acids in natural musts of 2002 (mg/l)

	Látrány Irsai Olivér	Látrányi Király-leányka	Badacsonyi Sárga muskotály	Badacsonyi Olasz rizling	Badacsonyi Szürke-barát	Badacsonyi Hárs-levelű	Etyeki Char-donnay	Etyeki Olasz-rizling	Látrányi Merlot	Látrányi Cabernet Sauvignon	Látrányi Zweigelt	Látrányi Kékfrankos	Villányi Cabernet Sauvignon
ASP	4.51	5.21	1.25	1.23	3.28	1	2.16	4.44	1.25	5.11	4.87	3.98	4.83
THR	1.89	4.21	3.24	2.56	2.65	1.22	3.29	2.86	2.33	2.87	3.66	4.51	2.39
SER	2.87	2.67	2.78	6.21	4.21	2.43	4.11	1.96	1.26	1.67	6.84	3.88	1.73
GLU	3.21	3.12	4.78	3.22	3.85	3.86	2.85	11.8	4.57	3.54	2.21	3.21	4.68
GLY	18.2	9.12	4.81	6.21	8.38	29.7	2.12	15.7	23.6	6.22	1.55	6.89	2.76
ALA	13.9	11.8	7.32	12.1	12	50.4	7.47	75.2	19.4	16.8	2.88	30.4	4.71
VAL	8.51	6.23	2.44	1.97	2.51	14.9	1.29	2.98	10.3	2.89	2.64	1	3.18
MET	7.26	4.54	8.28	1.65	4.15	9.45	12.5	3.74	15.3	2.87	2.69	4.26	3.19
ILE	9.19	8.51	2.06	1.45	6.34	11.1	3.64	5.79	24.3	7.68	2.92	6.13	3.45
LEU	32.5	27.3	18.7	12.1	8.94	48.7	4.99	4.78	54.3	7.83	2.41	21.3	4.43
TYR	21.8	20.1	15.2	18.6	12.1	24.9	4.22	12.8	51.9	11.9	4.17	17.2	13.7
PHE	24.1	21.2	18.6	8.23	7.94	30.1	6.93	2.11	54.9	11.3	10	14.3	6.89
LYS	77.1	62.1	45.7	26.3	33.1	94.8	17	12.6	109	27.2	17.2	22.8	21.5
NH+4	22.3	9.26	2.39	15.4	5.47	10.3	6.81	8.92	7.81	4.91	1.22	7.3	11.3
HIS	20.9	10.2	14.8	13.6	11.2	21.9	4.98	3.16	35.4	11.2	2.8	8.56	3.78
ARG	108	81.3	40.8	257	189	167	10.3	489	74.3	31.9	2.44	278	27.6
PRO	485	496	298	987	852	789	351	643	312	497	195	429	924

Table 3. Distribution of biogenic amines in natural musts of 2002

Cultivar	Etylamin	Metylamin	Histamine	Tyramine	Serotonine	Putrescine	Phenyl-ethylamine	Cada-verine	Total biogenic amines
Látrányi Irsai Olivér	0	0	0	9.69	11.35	13.32	3.01	3.5	40.87
Látrányi Királyleányka	7.77	32.28	0	0	6.61	5.62	3.97	3.97	60.22
Badacsonyi Sárga Muskotály	0	99.16	0	7.67	9.7	13.39	10.21	12.45	152.58
Badacsonyi Szürkebarát	0	0	0	6.69	17	18.26	1.73	2.6	46.28
Badacsonyi Olaszrizling	0	102.28	0	11.27	13.47	14.96	26.59	28	196.57
Badacsonyi Hárslevelű	0	0	0	13.52	16.96	10.09	4.32	5.4	50.29
Etyeki Chardonnay	0	0	0	11.01	18.01	21.41	10.69	12.4	73.52
Etyeki Olaszrizling	30.73	30.73	15.67	7.32	15.74	9.31	5.69	6.3	121.49
Látrányi Merlot	0	61.75	0	9	10.08	15.54	6.83	0	103.2
Látrányi Cabernet Sauv.	0	38.59	23.67	11.02	13.18	16.14	3.42	2.9	108.92
Látrányi Zweigelt	31.47	0	5.71	0	15.21	14.87	3.59	4.8	75.65
Látrányi Kékfrankos	0	0	0	3.42	10.08	4.65	0	3.9	22.05
Villányi Cabernet Sauvignon	36.49	36.49	14.87	9.65	11.1	14.72	0	1.2	124.52

Red musts do not contain a big amount of glycine; their methylamine concentration is high, though *(Figure 3)*.

Among biogenic amines histamine is probably the most significant one. Examining white musts, we can see that all samples contained the amount of histidine described in previous studies. However, histamine was detected in one of the white bio-musts, in Etyek Riesling, in a very big concentration of 15.2mg/l. As regards red bio-musts, we can say that histidine is present in smaller amounts *(Figure 4)*.

White bio-musts have a significant tyrosine content of 12mg/l–25mg/l. The chart shows that tyramine is also present in nearly all of the samples over 5mg/l. Such high concentration of tyramine in musts implies the presence of microorganisms.

The tyrosine content of red musts was in accordance with the average data. Only one sample of Villány Cabernet Sauvignon contains the extremely high concentration of 52mg/l of tyrosine. Also, tyramine was even measured in musts, in an amount of 10mg/l *(Figure 5)*.

White musts have a high concentration of phenylalanine, between 12–30mg/l. We detected phenylalanine in all biomust samples as recorded in literature data. The big amount of phenylalanine may also be responsible for the high concentration of β-phenyl ethylamine.

Large amounts of phenylalanine are measured in red bio-musts, as well. One of the samples, the Villány Cabernet Sauvignon contains a concentration of over 50mg/l. β-phenyl ethylamine can also be found in the musts, but not more than 10mg/l *(Figure 6)*.

Lysine content of white bio-musts varies widely: between 12–92mg/l. Cadaverine is only present in musts in very small amounts, Badacsony Riesling standing out with its 28mg/l. In wines the lysine content is lower, in accordance with recorded data *(Figure 7)*.

Our measurements have shown that there is no clear correlation between amino acid content and the presence of biogenic amines, even though amino acids are percursor compounds of biogenic amines.

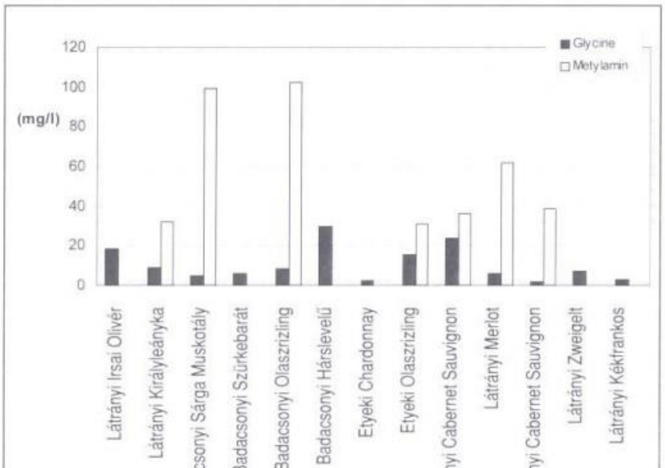

Figure 3. Glycine and methylamine content in natural musts

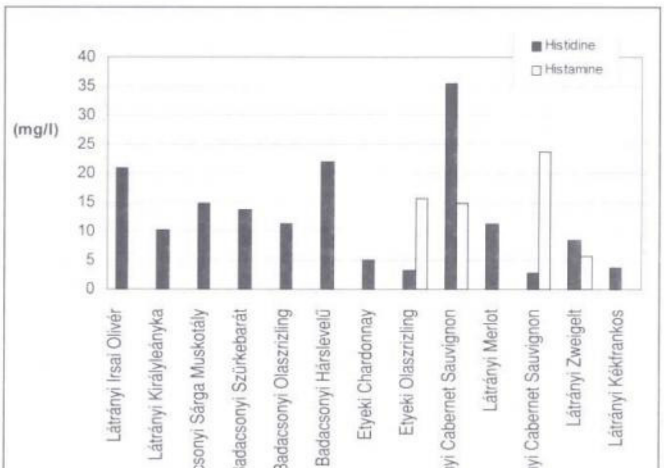

Figure 4. Histidine and histamine content in natural musts

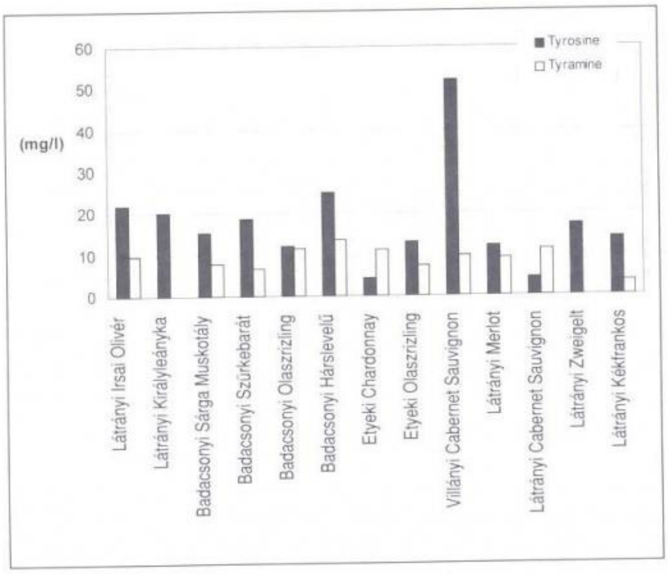

Figure 5. Tyrosine and tyramine content in natural musts

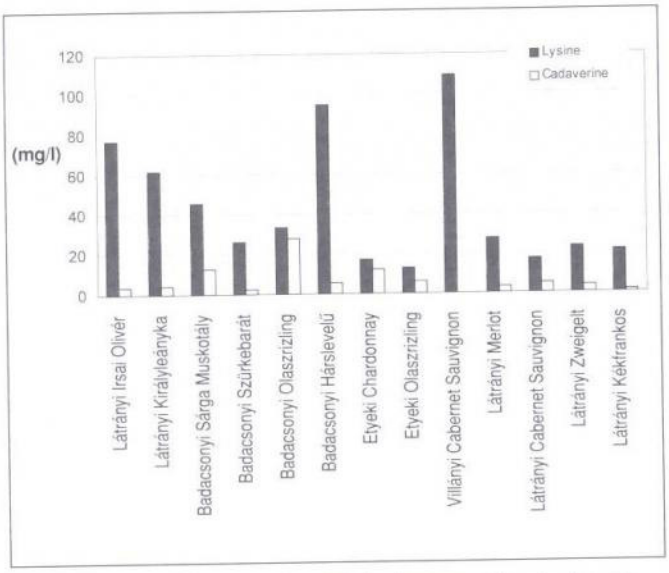

Figure 6. Phenylalanine and phenylethylamine content in natural musts

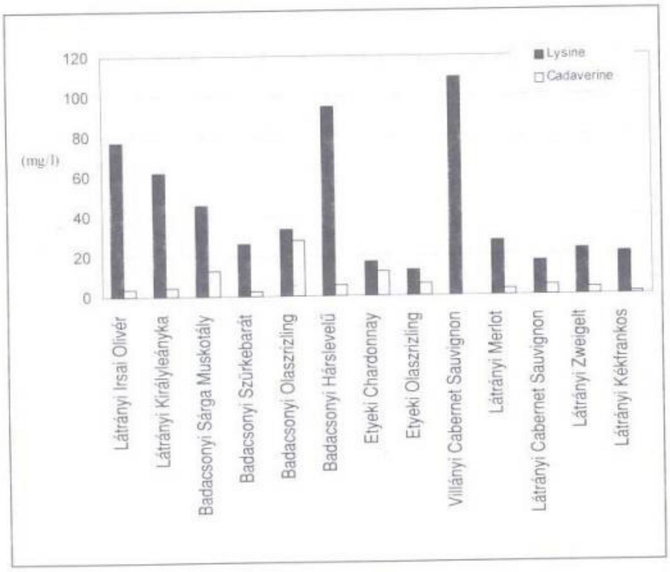

Figure 7. Lysine and cadaverine content in natural musts

Conclusion

It is clear from our data that nitrogenous compounds of bio-musts are in accordance with the recorded data. Organic farming probably does not influence either the qualitative or the quantitative values of nitrogenous compounds of bio-musts. We can conclude that no relationship is implied between amino acids and the composition of biogenic amines, despite the fact that biogenic amines are formed from amino acids.

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