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THE EFFECT OF FERTILIZER TO THE BIOGENIC AMINES CONCENTRATION IN WINES

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ABSTRACT
In the last few years food compounds which have a physiological effect (especially wine compounds) came to the front, therefore biogenic animes have gained on importance. In our experiment we determined - for oenological conditions important - histamine, tyramine and serotonin content with HPLC technique. Biogenic amines are produced from amino-acids (precursors) during the decarboxylation procedure. Commercial fertilizers and combinations were independent factors, and biogenic amine concentrations were observed as dependent factors (the Cserszegi fűszeres samples, vintage 2015, were homogenous form in every aspects except the fertilizer usage under alcoholic fermentation=AF). After AF - thus after various fertilizer additions - considerable changes were shown in the amount of the investigated three compounds, although we could not find an obvious pattern. Histamine and tyramine were far away from the toxic limit, serotonin presence was slightly higher than in the earlier results, however serotonin is not a poisonous compound (positive evaluation), hence the ascent was not a problem.

Keywords: fertilizer, biogenic amine, histamine, tyramine, serotonin

INTRODUCTION

Nowadays healthy food - in our case healthy wine- came to the front (in public and scientific context as well). (In our opinion 'healthy' is not a fortunate term, because food strictly cannot be 'unhealthy', like cigarette smoke or mind-altering drugs.) Obviously some foods - also some wines - have higher rate of valuable components, of course these foods can contain compounds from natural origin which have allergenic or other physiological effect. The present experiment is focusing on the biogenic amine content of wine, which is produced during the metabolic activity of microorganisms. In our case the decarboxylation of amino acids is one of the most important way of producing. In relation to other fermented foods the biogenic amine content of wines is low. From oenological aspect important amines are histamine (HIS, toxic limit: 2-10 mg/l), tyramine (TYR, toxic limit: 1-10 mg/l) and serotonin (SER, typical level: 7-10 mg/l) (NAILA ET AL., 2010). Earlier works have been studied the biogenic amine content of various white and red wines, significant differences have been found, together with the influence of the year and the grape variety, these factors did not have main role in biogenic amine level (KÁLLAY and NYITRAINÉ ET AL., 2003; *Table 1*).
Fertilizer usage ensures, together with natural amino acid presence, the required level of yeast assimilable nitrogen (YAN) under AF, these compounds are the precursors of biogenic amines, consequently the HIS, TYR and SER level are influenced.

Table 1. Biogenic amine composition of Hungarian Wines

Biogenic amine	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
Tyramine	0,1-1,1	0,3-0,95
Tryptamine	ND	ND

Source: Kállay- Nyitrainé, 2006

In our present work the amount of the three biogenic amines were measured, and the base analysis were done, so the effect of the fertilizers and combinations were shown up to the biogenic amines and base parameters.
HIS is poisonous over 500 ppm, the poisoning manifests itself as an allergen-type reaction characterized by in allergic reaction, difficulty in breathing, itching, rash, vomiting, fever, and hypertension. The toxic effect of HIS is enhanced, when other biogenic amines are present (synergic relation). The process of HIS forming is called histidine decarboxylation (PRETI ET AL., 2016).
Tyramine stem from tiramin decarboxylation, TYR is reported to be the initiator of dietary-induced migraine and hypertensive crisis, similarly other aromatic biogenic amines. The acute toxicity is 2000 ppm, NOAEL (no observed adverse effect level) is also 2000 ppm (NAILA ET AL., 2010).
SER is an important tryptophan derivative, which has a role in human regulation system of mood, appetite, sleep and temperature. The low level of SER generate migraine, depression and tinnitus. SER (5-HT) in this form cannot get over the blood-brain barrier, however its precursors TPH and 5-HTP can do so, which means they have a physiologic effect (BERGER ET AL., 2009).

MATERIAL AND METHOD

Sample preparation

The samples (vintage: 2015, grape variety: Cserszegi fűszeres) were treated in the course of AF with various type and dose of fertilizers, then we used basic- and fit for purpose analytical measurements. Parameters of must and new wine treatments (our aim were a production of an average white wine): must sulfiting (10 g/q Potassium-bisulfit), enzymatic treatment (1,5 g/q), must clarification (10 °C, 24h), yeast starter (20g/hl), together with the experimental fertilizer dosage under AF. In our work we studied five commercial products, in various combination (*Table 2*). These materials differed from each other (effect/ purpose of dosage): whereas DAP® is an inorganic nitrogen- source, Viniliqiud® and Bioferm® are inactivated and partially/fully autolyzed yeast (organic N- source). Springarom® is not

autolyzed inactive yeast, it has two function not only N-source, but also significant reducer agent, so protect the wine from oxidation, while Springcell® is a yeast hull (cell wall) product, which has detoxification potential, furthermore it helps the yeast survive in the course of AF, but did not change the YAN.
Our samples were homogenous form in every aspects except the fertilizer usage under AF, so independent factor was the effect of fertilizer, while dependent factor was biogenic amine concentration.

Table 2. Fertilizers and combinations

No.	Fertilizer combination	Abbrev.	Quantity (g/hl. or ml/hl)
1	Bioferm®	BD	3x20=60
	DAP®		3x20=60
2	Viniliqiud®	V	50+10=60
3	Bioferm®		3x20=60
	DAP®	BDSc	3x20=60
	Springcell®		1x20=20
4	Viniliqiud®	VSc	50+10=60
	Springcell®		1x20=20
5	Bioferm®		3x20=60
	DAP®	BDSa	3x20=60
	Springarom®		1x30=30
6	Viniliqiud®	VSa	50+10=60
	Springarom®		1x30=30

Method of analysis

- Free- and total sulphurous acid concentration, titrimerty (OIV-MA-AS323-04A)
- Titrable acidity (OIV-MA-AS313-01)
- pH (OIV-MA-AS313-15).
- Ethyl-alcohol concentration (OIV-MA-AS312-01A 4.C.)
- Sugar concentration (OIV-MA-AS2-02)
- Volatile acidity (OIV-MA-AS313-02:R2009)
- Glycerol concentration (OIV-MA-AS312-05: R2009)
- Folin-Ciocalteu index- polyphenol concentration (OIV-MA-AS2-10)
- Chromatic Characteristics (OIV-MA-AS2-07B)
- Biogenic amine concentration, HIS, TYR, SER, HPLC technique (OIV-MA-AS315-18)

Chromatographic conditions:
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 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 technics.

RESULTS

The new wine samples were after AF dry every time, their alcohol content depended on the sugar concentration of the must (about 12-14v/v%), this level was uplifted a bit above the normal level. The titrable acidity and pH were appropriate, so with the currently adequate SO_2 level, thus the samples are defended from oxidation or undesirable activity of presence microorganisms (if this levels will reduce in the future SO_2 completion will be necessary). The volatile acids were on a normal level (about 0,3-0,4 g/l), the total polyphenol concentration was slightly higher than in usual white wines. The investigated base-parameters met the requirements, we obtained our purpose, the normal, average white wine, which was suited for the analysis of the biogenic amine concentration beside various fertilizer combinations.

The description of the biogenic amines

The investigated biogenic amine concentration did not reach the critical level (HIS and TYR toxic limit) and we found significant differences between a pair of samples, but did not find observable obvious trend. The samples HIS concentration was in the lower half of the ordinary interval, and only 1,5 mg/l maximum difference appeared, but statistically it was 50% ascent. The amount of TYR were shown normal level, the values were far away from the toxic limit, in one sample TYR was significant lower than the others, wich can mean VSa is a promising combination (*Figure 1*).

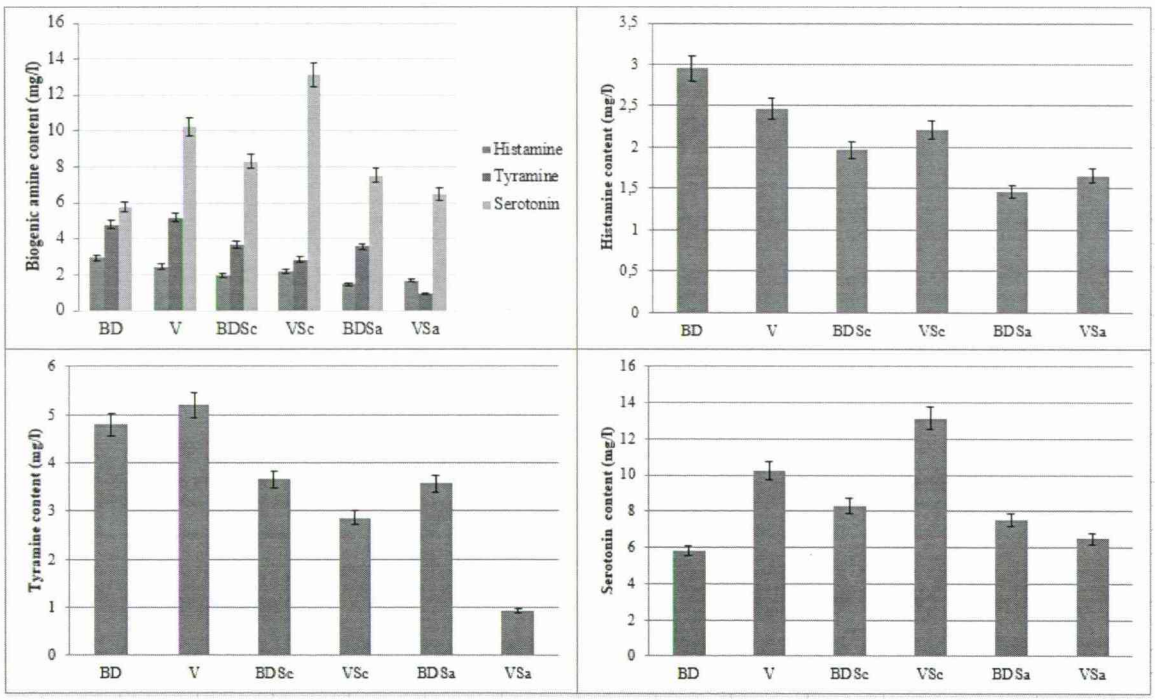

Figure 1. The biogenic amine content by various fertilizer combinations

In case of SER we could speak about typical level, SER concentration was in our samples slightly higher than in the earlier results, but it was not a problem, on the whole presence of SER was desirable. We found significant differences in the biogenic amine concentration of the samples. The BD combination eventuated higher HIS and TYR- and lower SER level in these experimental conditions. Every three measured biogenic amines got into the upper part of the present interval, when we used the V fertilizer. The BSc

combination was shown up as the mean of the investigated fertilizer combinations. The VSc combination seemed to the most effective dose, not only HIS and SER were average, but also SER level was by far the best. The BDSa combination effected lower level in case of all the three biogenic amines. The TYR concentration was on the minimum, the other two amines were average by the usage of VSa combination.

CONCLUSIONS

In these experimental conditions the applied fertilizers and combinations did not make unwholesome/negativ change in the amount of the investigated biogenic amines. About the fertilizers, correlated to each other, we can establish the following statements:

- The use of Viniliquid shifted the concentration of all the three component to positive direction (except one sample).
- The Bioferm, DAP combination was the less successful in itself from the aspect of biogenic amines, however the addition of the third fertilizer (Springcell or Spring arom) in every case yielded advantageous changes (lower HIS and TYR and higher SER concentration).
- The use of Springcell generated diffrent results, because with BD combination the biogenic amine content decreased, but with V fertilizer we measured higher amount of SER and lower amount of HIS and TYR (this was the desired option).
- The concentration of biogenic amines were decreased uniformly by Springarom.

All in all the use of the investigated feritlizers did not make negative change in biogenic amine content, I would go further than that: several combinations had advantageous effect (for example VSc). The variability in the concentrations are explained on the one hand with the incomparable processes of fermentation (two same fermentaition never existed, because the amount of metabolits were controlled- one by one- by the yeast, the purpose of the whole system was the balance), on the other hand the fertilizers were not homogenous, for that reason it was not possible to show wich compound(s) was responsible for the changes in biogenic amine content.

At the same time from a practical point of view our experiment proved that the conscious usage of fertilizers could influence the level of valuable/underirable compounds in wine.

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