BIOGENIC AMINES AND SEROTONIN IN HUNGARIAN WINES PRODUCED WITH ORGANIC YEAST STARTERS

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Recently, certified organic yeast starters have been put on the market, but most organic producers in Central Europe still prefer spontaneous fermentation. Biogenic amines, physiologically active compounds, are formed from precursor amino acids, partially by yeasts. In this study, two organic yeast starters were examined for biogenic amine production, in comparison with spontaneous fermentation. The biogenic amine profile showed considerable differences between the organic yeasts under investigation and spontaneous microbiota, but the biogenic amine production of the two organic yeasts was similar, except for tyramine and ethylamine. Significantly lower histamine and higher serotonin amounts were produced by the organic yeast starters compared with spontaneous fermentation. Organic yeast starters offer an opportunity for organic producers instead of spontaneous fermentation, to ensure the quality and safety of their products in regard to biogenic amines.
Keywords: economic sustainability, Hungarian resistant grape variety, 'Bianca', serotonin, histamine, HPLC

Biogene Amine und Serotonin in ungarischen Weinen, die mittels biologischer Starterhefen vinifiziert wurden. Seit einiger Zeit sind bio-zertifizierte Starterhefekulturen auf dem Markt verfügbar, aber die meisten Bio-Wein-Produzenten in Mitteleuropa bevorzugen noch immer die spontane Vergärung. Biogene Amine sind allergene Verbindungen und werden aus Aminosäuren gebildet und teilweise von Hefen gebildet. In der vorliegenden Untersuchung wurden zwei biologische Starterhefekulturen auf ihre Produktion biogener Amine im Vergleich zur Spontanvergärung untersucht. Das Profil der biogenen Amine zeigte beträchtliche Unterschiede zwischen den untersuchten biologischen Hefen und den spontanen Mikrobioten, aber die Produktion biogener Amine der zwei organischen Hefen war, mit Ausnahme von Tyramin und Ethylamin, ähnlich. Im Vergleich zur Spontanvergärung produzierten die biologischen Starterhefen signifikant niedrigere Histamin- und höhere Serotoningehalte. Biologische Starterhefen stellen für Bio-Wein-Produzenten eine Alternative zur spontanen Vergärung dar, um die Qualität und die Sicherheit ihrer Produkte in Bezug auf biogene Amine zu garantieren.
Schlagwörter: wirtschaftliche Nachhaltigkeit, ungarische resistente Rebsorte, 'Bianca', Serotonin, Histamin, HPLC

Organic producers have two aims: as profit oriented organizations, they want to ensure product quality; and they must comply with the standards of organic farming (Miele et al., 2015; Provost and Pedneault, 2016). European Union legislation provides clear provisions relative to prohibited, limited or permitted additives, processing aids, plant protection products, etc. (EC Regulation No. 834/2007; EC Regulation No. 889/2008). For example, conventional active dry yeast is allowed as a starter culture in organic wines. Despite this, a number of organic producers in Central Europe refuse to use a conventional starter and prefer spontaneous fermentation, which is a risky decision from several standpoints. There are numerous advantages of starter cultures as a quicker start and safer management of fermentation, and an acceptable rate of fermentation products and by-products (Degre 2002).

A few years ago certified organic yeast starters were put on the market alongside conventional starter cultures. The requirements of the selection are listed in Table 1. Earlier studies confirmed that there are significant differences between the diversity of the selected yeast population – Saccharomyces and non-Saccharomyces strains – between conventional and organic grapes (Cabras and Conte, 2001; Komárek et al., 2010; Cordero-Bueso et al., 2011).

Table 1: Steps of organic yeast selection (Regulation (EC) 889/2008)

Agricultural origin	New selection from organic grape
Source of sugar	Certified organic media
Source of nitrogen	Certified organic media
pH-regulation	Not necessary
Nutrient addition	Naturally present in the media
Washing	Not necessary
Waste water	Reduced amount
Additives	No emulsifier
Packaging	Certified organic packaging

In wine, there are three potential origins of biogenic amines: formation in grape/must by its own enzyme system, formation by yeasts during alcoholic fermentation from nitrogenous compounds, and formation by lactic acid bacteria during malolactic fermentation from nitrogenous compounds (Torrea and Ancín, 2002; Smit et al., 2008; Binner et al., 2013). On the question of which living organism is the main producer of biogenic amines, the experimental results show discrepancies, and the publications have divided opinions as well. The most important pathway of biogenic amine formation is decarboxylation with substrate-specific decarboxylase enzymes, along with transamination, reductive amination, and degradation of certain precursor amino compounds (Beneduce et al., 2010; Mendelez et al., 2016). In wines, both the quantity and the composition of biogenic amines are influenced by many factors, such as soil type and composition, soil fertilization, climatic conditions during growth, degree of maturation, residual microbial population, clarification process, conditions of the alcoholic fermentation, as well as the yeast strain used during the fermentation (Eder et al., 2010; Mendelez et al., 2016).

The presence of these bio-active compounds – for example histamine, tyrosine, cadaverine, etc. – is usually undesirable, but in wine these appear in low concentrations of a few mg/l. In higher concentrations in the human body, they can cause difficulty in breathing, itching, rash, vomiting, fever, dietary-induced migraine, hypertensive crisis, and allergic reactions (Naila et al., 2010). Many countries have recommended upper limits for some biogenic amines – most often for histamine between 2 and 10 mg/l. Also, the International Organization of Vine and Wine in its OIV code of good viniculture practices has recommended reducing the biogenic amine content of vine-based products (OIV-CST 369-2011). Despite this, serotonin is an important tryptophan derivative which has a positive role in the human regulation systems of mood, appetite, sleep, and temperature. Low levels of serotonin lead to migraine, depression and tinnitus. Serotonin in this form (5-HT) cannot penetrate the blood-brain barrier, however its precursor

5-hydroxytryptophan can do so, which means they have a physiological effect on the body (Berger et al., 2009). The aim of this paper is to investigate and describe the biogenic amine composition and serotonin in wine fermented with organic yeast, compared with spontaneous fermentation.

MATERIAL AND METHODS

Grapes from the variety 'Bianca' were used (vintage: 2015), which is a Hungarian resistant grape variety, hybridized from Seyve-Villard 12375 E.2 x 'Bouvier'. It is well-suited for organic farming and widely cultivated in Hungary (Hajdu, 2012). Laboratory-scale fermentations were carried out under oxygen-limited conditions in 1000 ml flasks containing 750 ml aliquots of must with the organic yeast inoculum: Be-Red, Oenoferm-bio (Erbslöh, Geisenheim, Germany) and as control without inoculum, closed with fermentation locks. All fermentations were performed in duplicate. The fermentation temperature was kept at 20 °C. So after 15 days of fermentation, all the samples were membrane-filtered (0.6 µm) and stored at 4 °C until analysis (2 to 4 weeks). Standard methods of analysis were used for general wine composition: total acidity (OIV-MA-AS313-01); pH (OIV-MA-AS313-15); ethyl alcohol (OIV-MA-AS312-01A 4.C.) volatile acidity (OIV-MA-AS313-02:R2009); glycerol (OIV-MA-AS312-05: R2009); acetaldehyde (OIV-MA-AS315-01); biogenic amine (ethylamine, methylamine, putrescine, β-phenyl- ethylamine, cadaverine, histamine and tyramine) and serotonin content: all with HPLC technique (OIV-MA-AS315-26; OIV-MA-AS315-18; Kállay and Sárdy, 2003).
Analytical data were evaluated with one-way ANOVA, using Microsoft Office Excel 2013 (v. 15.0.4849.1003; Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

In the present study the biogenic amine profiles of two commercially available organic yeasts were investigated in comparison with spontaneous fermentation. Table 2 shows the values of the general analysis: the ethanol and glycerol production showed normal levels. The level of total acidity was high, considering that the 'Bianca' wines usually have reduced acidity. Measuring the volatile acidity (Table 2), a significant difference could not be found, however the level of volatile acids increased just to the organoleptic threshold (0,8 g/l) in every sample, a later increase definitely should be avoided. The acetaldehyde values are slightly high for new wines, which depend on the properties of the fermenting strains.

Table 2: New wine general analysis; SP: spontaneous, Bio 1. and 2.: commercially available active dry organic yeasts; SD: significant difference: * = $p < 0,05$; n.s.= not significant

Sample	Titratable acidity (g/L)	pH	Ethanol (v/v%)	Volatile acidity (g/L)	Glycerol (g/L)	Acetaldehyde (mg/L)
SP/A	7,0	3,54	12,70	0,80	7,25	54,97
SP/B	7,0	3,62	12,64	0,83	7,08	65,72
Bio 1./A	7,0	3,63	12,26	0,87	7,97	65,19
Bio 1./B	7,0	3,64	12,23	0,84	7,83	70,12
Bio 2./A	7,3	3,62	12,02	0,83	7,56	64,48
Bio 2./B	7,3	3,61	12,11	0,86	7,42	69,77
SD	*	n. s.	*	n. s.	*	n. s.

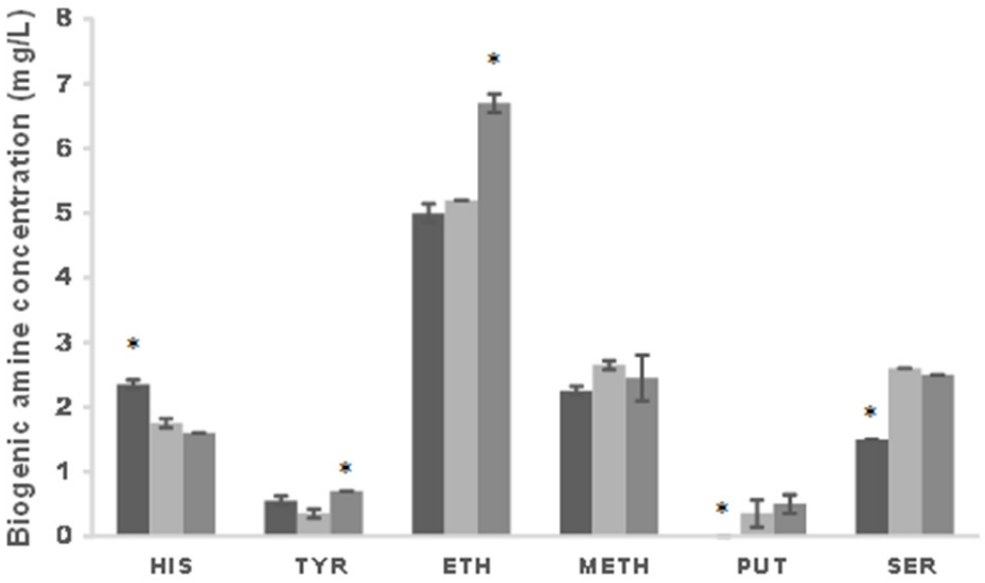

Fig. 1 Biogenic amine and serotonin composition of the samples;
SP=spontaneous; Bio 1. and Bio 2.
= commercially available dried active organic yeasts;
HIS= histamine; TYR= tyramine; ETH ethyl-amine;
METH= methyl-amine; PUT= putrescine; SER= serotonin;
* means significant difference, $p < 0.05$.

Figure 1 shows the biogenic amine and serotonin profile of the new wines. The amount of ethylamine and methylamine is considerably higher in every sample, in disagreement with the literature findings, the other investigated biogenic amine levels confirm earlier reports, which are related to conventional yeasts (Kállay and Nyitrai-Sárdy, 2003; Smit et al., 2008; Mendelez et al., 2016). The highest histamine amounts were produced by the spontaneously fermented samples, whereas the organic yeast starters produced significantly lower levels. The histamine values of samples Bio 1-2 did not reach the strictest recommended upper limit (2 mg/l). The Bio 2 samples contained significantly more tyramine than the Bio 1 samples, and much more ethylamine than the SP and Bio 1 samples.

The lowest serotonin production was measured in the spontaneously fermented samples, while the organic yeasts produced the same higher amounts. Putrescine was not detected in the SP samples, but a normal (typical) amount was produced by the organic yeasts (Fig. 1.), while 2-phenyl-ethylamine and cadaverine were not detected.

CONCLUSIONS

The biogenic amine profile globally presented considerable differences between the investigated organic yeasts and the spontaneous microbiota. In total we could not notice an obvious trend. The histamine and serotonin production proved to be advantageous in the case of organic starter usage, while the ethylamine and putrescine production was unfavorable.

Under these experimental conditions, the biogenic amine profile of the organic yeasts we investigated suits the food-safety requirements. Organic yeast starters are an opportunity for organic producers to ensure product quality and safety in regard to biogenic amines.

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Received January, 13th, 2017