

Article

# Role of Yeast in Wine Fermentation Processes

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**Featured Application:** Examination the decisive role of yeast in optimize wine fermentation process.

## Abstract:

Society considers wine as a special product among food and beverages because of its high gastronomical value and its positively distinctive quality. In recent years, philosophies of the agricultural techniques and development of the oenological technology have been focused on the reduction of wasteful, "polluting" elements, and trends are moving towards an environmental friendly approach.

Due to the stricter regulations and rules (with the limited amount and selection of the permitted chemicals) resistant, also known as interspecific or innovative grape varieties can be the ideal basic materials of alternative cultivation technologies.

In terms of variety selection, innovative varieties can be equivalent to international varieties, although organically their quality could not compete with them. These grapes are more resistant to various fungal diseases and infections than international varieties.

Well-founded analytical and organoleptic results have to provide the scientific background of resistant varieties, as these cultivars with the environmental friendly cultivation techniques, could be the raw material of the future.

**Keywords:** innovative grape varieties, organic wine, biogenic amine, polyphenol

## 1. Introduction

Due to the recent trends of environmentally and health conscious food consumption bio products and organic wines from sustainable farming are gaining larger significance throughout the world. Thus, bio yeast has also appeared among oenological products. Bio yeast is a selected yeast propagated and dried with biological methods. The substrate used for the propagation the bio yeast originates from regulated biological production. Organic wine production requires special attention not only in the vineyard, but in the whole winery. The process of drying is careful, and it is regulated by the relevant EU rules for organic production [1] and the official NOP standards in the US. The packaging of bio yeast is also a certificated organic material. According to the rules and standards bio yeast production utilizes only substrates derived from organic agriculture and GMO-free materials.

Several researches concentrate on the effects of bio yeasts, especially on the amount of biogenic amine, which is a beneficial, physiologically active compound, in organic wines.

Yeast is an oval-shaped unicellular fungus with a eukaryotic structure, which is more developed than that of prokaryotic organisms.

During alcoholic fermentation performed by yeasts the sugar content of grape juice converse into ethanol, carbon dioxide and heat energy [2]. In the beginning stage yeast cells are multiplying which requires oxygen, but the fermentation itself is an anaerob process [3].

For the optimal course of alcoholic fermentation, naturally, several parameters in the juice (carbon resources and nitrogen) and outer conditions (proper temperature) are also necessary. Lack of nutrients can result in fermentation problems, such as slacking or stuck fermentation. Among yeast nutrients the following has an accentuated role: nitrogen to protein conversation, phosphorus to cell respiration, besides minerals and trace elements. During fermentation, yeasts require optimal feeding. There is a significant difference between the nutrient solutions for organic and conventional yeasts, this is the prime factor in the variability of conventional and organic stocks.

Traditionally yeast is propagated on black treacle which is a side-product of sugar refining. The required additional nitrogen is provided artificially with an inorganic form. Synthetic vitamins and minerals, effervescence inhibitors (paraffin oil) are also applied. These are not permitted compounds in ecological food products. PH values are corrected with acids (sulfuric acid) and bases (sodium hydroxide). In order to remove the inconvenient flavours and aromas, conventional yeasts are leached twice, which results in a seriously polluted, hardly treatable wastewater. Traditional yeast production comes with the formation and the residual of the following chemicals: sulfuric acid, ammonia, phosphates, and synthetic oils. With the production of 1 kg yeast 380 kg of slowly degradable waste get into the water. Organic yeast stocks can emphasize the characteristics of the variety and the terroir. It is produced with an environmental friendly method without additional chemicals. Every applied microorganism and material guaranteed GMO-free. The substrate used for the propagation of the yeast derived from certified organic agriculture. This substrate contains all the necessary nutrients, so it doesn't require artificial addition.

Organic producers apply digested grain, which has an advantage compared to black yeast, namely it provides enough nitrogen. Additional nitrogen to the nutrient substrate is necessary only in a few cases, and it is provided in the form of whey or beer yeast. Organic sunflowers oils works as the effervescence inhibitor. PH correction and adjunct materials (artificial vitamins, inorganic salts) are not necessary.

Unlike the conventional method inorganic nitrogen, sulfuric acid and ammonia is forbidden in bio yeast production. Synthetic effervescence inhibitors are also not permitted here. For pH correction sodium carbonate, citric acid and lactic acid are allowed. Potato-starch can function as filter, and sunflower oil as effervescence inhibitor. Since bio yeasts are not leached during the production, and only steam is applied, disinfectants are not necessary. Thus no steps of the process come with wastewater. This fermentation substrate is also suitable for other products, such as organic beverages.

Cells of bio yeast grow slower, and they require more resources. Consequently, their yield is lower, and their price is higher [5]. This is why conventional yeasts are still widely applied [6].

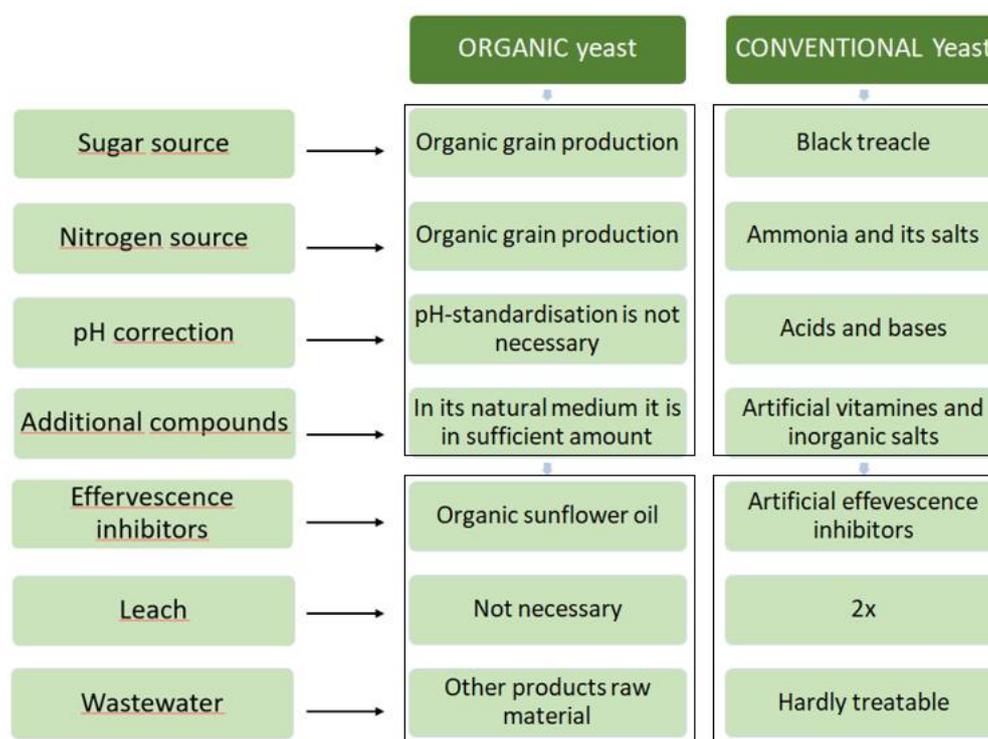


Figure 1: Differences between conventional and bio yeasts

The term directional fermentation in oenology means the application of selected wine yeast stocks. Ideal running of alcoholic fermentation requires several other parameters, such as temperature, carbon source and nitrogen. Grape juice contains the necessary nutrients for the process. Lack of nutrients can result in slacking or stuck fermentation. Nitrogen has an outstanding significance. If there are not enough nitrogen resources for the yeast in the juice, it may come with the risks of fermentation problems (slacking and stuck process). Besides this will increase the chances of hydrogen sulfide production during fermentation, which will lead to certain taste and odour defects (Böckser) [7-8].

The first studies about the biogenic amine content of Hungarian wines were published by KÁLLAY et al. [9]. Biogenic amines consumed by food are degraded in the intestines, frequently by N-acetylation or N-methylation with the involvement of diamine oxidase (DAO) or monoamine oxidase (MAO) enzyme system. It is the responsibility of the latter system (MAO) to prevent these biogenic amines from entering the vascular system. Alcohol or certain widely used antidepressants inhibit MAO, so biogenic amines can accumulate to toxic level in the body [10]. The effects of the biogenic amines studied in our recent publication are detailed here [9]; [11-14]:

- methylamines: methylamines intook by food consist of choline, carnitine, betaine, trimethylamine N-oxide (TMAO) and phosphatidylcholine [15]. Choline is an essential nutrient, which is present in higher amounts in egg-yolk and liver. Together with betaine it has several physiological functions. High TMAO-level of blood protects against hyperammonemia (in case of cirrhosis ammonia entering the vascular system leads to morbidity), and it functions as osmoprotectants in case of osmotic stress [16]. TMAO reduces the neurotoxicity of glutamate, which is related to myocardial infarction and stroke [17]. In spite of these facts it can be detected in higher levels in case of diabetes, Alzheimer's disease, cardiovascular diseases. Its main function in human body is the regulation of glucose-transport in adipose cells, and supposedly the stimulation of the release of neurotransmitters through sodium channels of the nervous system. Neurotransmitters have a significant role in the transport mechanisms through plasma membrane.

- ethylamines: Ethylamines are a component of mammalian, including human urine. According to observations, it is excreted in higher amounts in patients with liver and kidney disease and damage to the central nervous system. It can be detected in plants, food products, animal waste,

even in tobacco smoke. In human physiology, it is connected to anesthetics and recreational drugs. A representative in the first group is ketamine, which is a well-known NDMA-antagonist (N-methyl-d-asparaginate-antagonist). The latter group contains phencyclidine. NDMA is a neurotransmitter, which can substitute glutamate, while ethylamine is a possible precursor for ketamine [18].

- histamine: a derived from histidine. The industry applies it scarcely, it can be a potential ingredient in pesticides and in certain veterinary medicinal products. It synthesises in tissues of living organisms at the meeting of allergens and antibodies, but it can be intook by food also. Histamine has both positive and negative properties regarding its effects on human physiology, depending on the way it enters the body. Its primer function lays in allergic and inflammatory reactions. It participates in brain functions, and as a stimulus transmitter in the nervous system. It also has a significant role in indigestion (gastric acid production, intestinal motility, mucosal functions), in the development of immune responses, in the enhancements of the formation and metastases of tumors. In larger amount histamine intake can cause an allergic reaction. Apporximately 1 % of the population has histamine intolerance (disorder in degradation and accumulation). Histamine poisoning is caused by consuming foods that are too high in histamine, while for histamine intolerance metabolic disorders are responsible, which can occur even after the intake of small amount [9]. Symptoms include (migraine) headaches, flushing, runny or stuffy nose, arrhythmia, low blood pressure, digestive problems (such as diarrhea), hives. Relief of symptoms can be achieved with a low-histamine diet, namely products with a lot of histamine should be avoided. [20-21]. Larger amounts are contained in smoked foods, chocolate and red wines.

- tyramine: tyramine is formed from tyrosine. It is a side product of fermentation and decomposition processes, it functions as a neurotransmitter in human body, besides it is a metabolite of *Escherichia coli*. It can be found in large quantities in the basal ganglia and in the limbic system, so supposedly tyramine is connected to behavior and emotions [12]. It is a precursor in dopamine synthesis, [23-24], which is a neurotransmitter, neuroendocrine-transmitter and neurohormone. It is also called reward-hormone, it has a significance in "reward-mechanism" [25]. The following food products contain extremely lot of tyramine: smoked, fermented or marinated fish and meat, fermented dairy products(tyramine derives from the greek word cheese), fermented vegetables (for example sauerkraut), chocolate, several fruit and vegetables (banana, coconut, raisin, beans etc.), even yeast. Consuming large amounts of food with high tyramine content can significantly raise blood pressure (even by 30 Hgmm or more). Tyramine concentration in wine (~25 µg/g) is considerably lower than in cheese (even 2000 µg/g). Tyramine can cause serious health problems with persons taking certain antidepressants (monoamine oxidase inhibitors).

- serotonin: serotonin is a monoamine neurotransmitter derived from L-tryptophane. It is a basic compound for pharmaceutical industry (antidepressants). It has effects on blood pressure, sleep and sexual behavior, state of arousal, it also affects the manifestation of aggression. If problem occurs in the serotonergic system, several diseases in the nervous system can be detected: depression, generalized anxiety disorder, obsessive-compulsive disorder, post-traumatic stress disorder, phobias and epilepsy. Abnormally low serotonin levels lead depression, which can result in suicide. High levels of serotonin have positive effects on health, such as improved perceptual ability and general well-being [26]. MDMA (3,4-Methylenedioxymethamphetamine) consumption comes with significant release of dopamine and serotonin [17]. During banana ripening large amount of serotonin is produced either.

- putrescine: putrescin is a cadaverine-related polyamine which smells reminiscent of pepper. Industrial utilization: catalyst in resin technology; compound in medicines for hypertrophic scars after breast augmentation surgeries; insect attractants. It promotes cell division in smaller doses, but in larger amount it extents toxic decomposition products. It affects reproduction (spermatogenesis), it functions as nerve protector, but it also has significance in tumorigenesis. During the preparation of fatty foods at higher temperatures putrescine can converse into N-nitrosopyrrolidine, which is a carcinogenic compound [29-29]. It is produced in both living and dead organisms. It is responsible not only for the unpleasant smell of carcasses but bad breath. Cheese (1560 mg/kg) and fermented vegetables (549 mg/kg) can contain it in high amount [30].

- phenethylamine: supposedly phenethylamine (PEA) regulates the effectiveness of nerve cell connections (neuromodulator), it is a neurotransmitter also [31]. It can be often detected in food, especially in fermented products. Almonds and chocolate, even tobacco contain high amount of phenethylamine. Typically people with schizophrenia have high levels of it in the urine, while children with attention deficit hyperactivity disorder (ADHD) have low levels of PEA. There are no verified cure for ADHD [32], a study states PEA intake relieves symptoms almost instantly [33]. At the same time it gets into the brain only in small doses typically, because monoamine oxidase-b converse it to phenylacetic acid. In certain cases phenethylamine produced by human body has similar effects as amphetamine [33-34].

- cadaverine: cadaverine is a foul-smelling („death smell“) diamine compound which is produced by the putrefaction of animal tissue, by the hydrolysis of proteins, but living organisms can emit it also. Primarily it was detected in bacteria, by now it has been found in plant organs [35], for example in soy bean, chick peas etc. It shows an elevated level in urine of persons with lysine degradation disorders. Pharmacological effects of cadaverine include low blood pressure, bradycardia (low heart rate), stiffness or partial paralysis of the limbs [36]. DEL RIO [37] et al. (2019) emphasized that sufficient information about toxicity is not available, NOAEL-value (No Observed Adverse Effects Level) of laboratory experiments with rats proved a daily 180 mg/tskg. Other authors measured the LOAEL (Lowest Observed Adverse Effect Level) values of cadaverine regarding cell cultures, which is proved to be 510,89 mg/kg. In case of putrescine LOAEL was 881,5 mg/kg. This value is significantly lower than in previous presentations (255,45 mg/kg for total biogene amine and 2000 mg/kg for cadaverine) [38]. Cheese can contain cadaverine in large amount (3170 mg/kg), but fish is also significant producer (1690 mg/kg).

## 2. Materials and Methods

The role of the yeast in wine production is crucial. In this present we wanted to find out what kind of 'Bianca' wine was produced when using organic yeast, what kind of dynamics the alcoholic fermentation followed and whether this method could be used in industrial production as well. We fermented wines from the 'Bianca' juice samples three times where model chemical solutions were applied. The initial level of chaptalisation was 20,6 MM<sup>o</sup>, and the initial level of titratable acidity was 6,2 g/l. For the fermentation process 2 l capacity glass carboys were used, inoculation happened with 20 g/hl organic yeast. Wine nutrient salt was added in 15.../hl doses in the middle and at the end of the fermentation process. The control sample was inoculated with yeast Uvaferm CS2, 20g/hl and nutrient salt Uvavital was added at inoculation, mid-fermentation and at the end.

## 3. Results

Results of the primer analysis are summarized in Table 1. The values show that there is a significant difference among the samples regarding alcohol- and residual sugar concentration. In case of the control samples a regular, normal fermentation is detectable, residual sugar content is only 2 g/l. Organic yeast showed partial effectiveness only, 63,4 g/l of sugar remained in the wine. This was the reason for the significant differences in alcohol and residual sugar content.

In wine making technology volatile acid content is a cardinal question. Our present study showed that all the problematic values were under the critical limit (1 g/l). Although the organic samples resulted in a significantly higher level than control wines.

Glycerin content followed the literary values, since it is well known glycerin-pyruvic acid transformation results in a 6-10 g/l concentration.

*Table 1. Results of analyses: wines produced with organic yeast.*

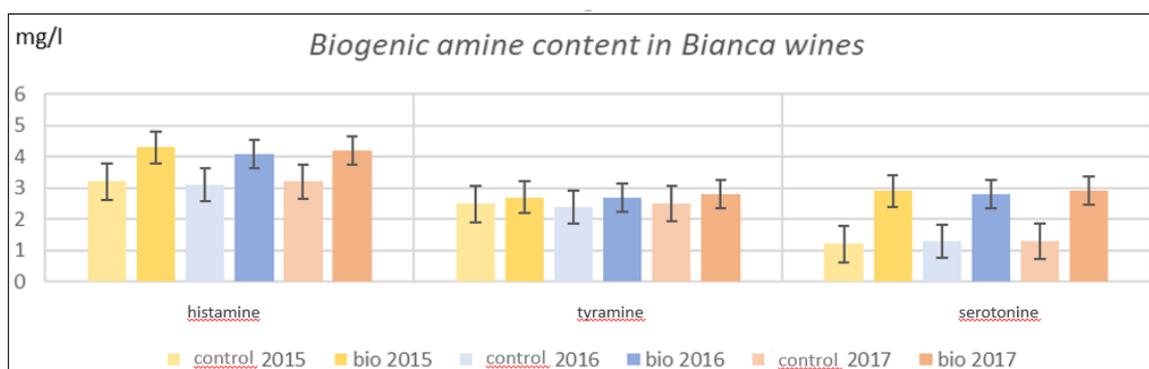
	control		organic yeast	
	average	deviation	average	deviation
free/overall SO <sub>2</sub> (mg/l)	46/105		55/104	
titratable acidity (g/l)	7,1	1,04	7	0,55
pH value	3,36	0,13	3,31	0,08
alcohol (V/V%)	13,89	0,76	9,77	1,2
sugar (g/l)	2,2	0,55	6,34	0,84
volatile acid (g/l)	0,37	0,13	0,59	0,15
glycerine (g/l)	6,16	0,16	6,53	0,08

Histamine, tyramine and serotonin concentration due to their physiological effect are presented on the 2nd diagram.

In our research we wished to know how organic yeast influenced biogenic amine formation in regarding three important compounds: histamine, tyramine and serotonin. We recorded significant differences in the samples produced with organic yeast; the values were higher. In terms of physiological effect, the organic yeast produced serotonin in a higher concentration. We can conclude that the level was below the critical 5 mg/l as suggested in the literature review [39] and it is in accordance with the health prescriptions in the case of the three most important biogenic amines.

We evaluated our results using a T-test. In the case of the histamine concentration it can be stated that there is no relevant difference between the control and the organic samples at the 95% significance level. The histamine concentration is not allowed to exceed 5 mg/l in wines, and both images (Fig. 2.) show that the histamine levels in the two samples were well below that.

The tyramine concentration changed according to the relevant literature [40]. In both samples, we were not able to detect statistical differences. The serotonin content followed as expected, and based on the statistical test we can state that there is significant difference between the traditionally produced wine samples and those ones that were fermented with organic yeast ( $\alpha=0,05$ ;  $s_2=0,49$ ;  $DF=5$ ).



*Figure 2. Biogenic amine content in „Bianca” wines 2015-2017.*

In the case of the other biogenic amines there are relevant differences between the samples produced with organic and traditional yeast fermentation. The amount of amines such as cadaverine, putrescine and ethylamine could be measured in significant quantities ( $\alpha=0,05$ ;  $s_2=0,22$ ;  $DF=5$ ). At the same time, the methylamine level was higher in the control sample (6,5 mg/l). Based on our measurements we could only prove significant differences between the samples in the case of the methylamine concentration. 2 phenylethylamine could not be detected in any of the samples.

Table 2. Results of analyses: wines produced with organic and traditional yeast

	2015	2016	2017	2015	2016	2017
(mg/l)	Control 2015	bio 2015	Control 2016	bio 2016	Control 2017	bio 2017
Methylamine	6,5	3,7	6,2	3,5	6,4	3,8
Cadaverine	1,8	3,2	1,7	3,2	1,8	3,1
Putrescine	0,98	1,1	0,95	1,1	0,94	1,3
Ethylamine	0,87	2,4	0,85	2,4	0,84	2,5

We could not find significant differences in biogenic amine composition between the samples, except for the methylamine concentration. Our results show that these yeast varieties can ensure the same quality as traditional yeast strains. We consider a priority to analyse the so called organic yeasts, as they can be at the base of organic grape production and organic wine production. Our results are that there is no significant difference to be described, even at a very low confidence level ( $\alpha=0,2$ ) in any of the three vintages.

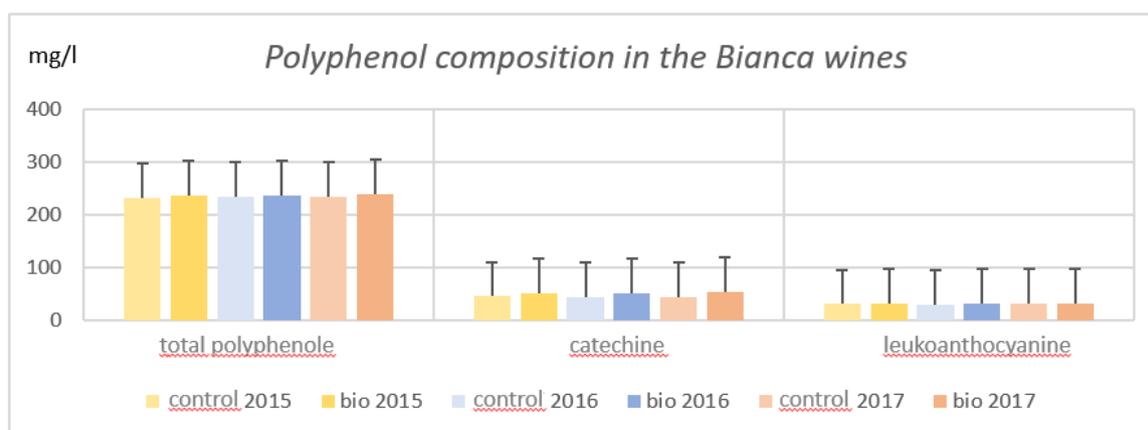


Figure 2. Polyphenol composition in the Bianca wines 2015-2017.

Tyrosol is formed only through alcoholic fermentation, and the amount and formation of the tyrosine is influenced by the yeast. Tyrosol was high in the wines produced with organic yeast in all three vintages.

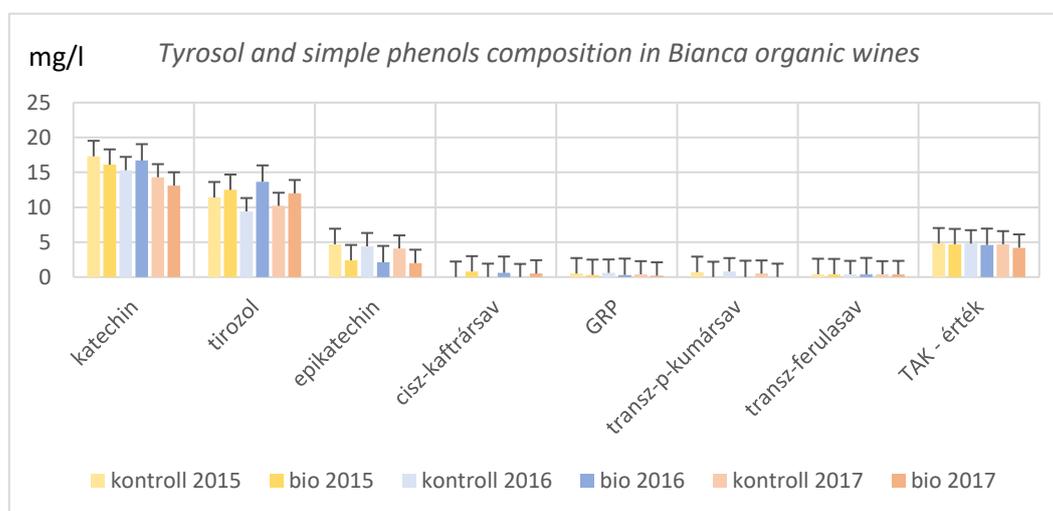


Figure 3. Tyrosol and simple phenols composition in Bianca organic wines

The overall antioxidant capacity value developed evenly as it is formed from polyphenol, so there is no difference. As for epicatechin, in all three vintage years we measured lower values in the organic wines.

2015	2015		2016		2017	
(mg/l)	control	bio	control	bio	control	bio
catechine	17,3	16,1	15,3	16,7	14,3	13,1
tyrosol	11,4	12,49	9,4	13,66	10,2	11,99
epicatechine	4,7	2,4	4,4	2,1	4,1	2
cisz-caftric acid	0	0,8	0	0,6	0	0,5
trans-caftaric acid	0	0	0	0	0	0
cisz-cutaric acid	0	0	0	0	0	0
GRP	0,5	0,3	0,6	0,3	0,4	0,2
trans coumaric acid	0	0	0	0	0	0
fertic acid	0	0	0	0	0	0
caffeic acid	0	0	0	0	0	0
trans-p-coumaric acid	0,7	0	0,8	0	0,5	0
trans-ferulic acid	0,4	0,4	0,4	0,4	0,4	0,4
TAK - value	4,8	4,7	4,8	4,6	4,7	4,2

Table 3. TYROSOL AND SIMPLE PHENOL COMPOSITION IN THE "BIANCA" ORGANIC WINES 2016-2017.

In the samples produced with organic yeast, the catechin, leucoanthocyanin and the overall polyphenol concentration values supported the data in the scientific literature [41]. There was no significant difference between the polyphenol content in the wines from the control or the treated items. The tyrosol concentration was higher in the organic samples. Regarding the simple phenol composition there was no relevant difference among samples. We can state that a considerable difference between the organic and the control samples was in the polyphenol and simple phenol composition and, which is the most important finding, in the amount of tyrosol.

The results of the analysis turned out as expected. There was no considerable difference among the samples. We witnessed a great difference in polyphenol composition, which is a result of the interaction of the yeast with the polyphenol.

The biogenic amine content in the examined wines does not differ from that of the „normal“ wines.

The biogenic amine composition in the resistant vine varieties corresponds to the composition of the biogenic amines in the international varieties. The organic yeast also has an influence on the production of amines, but there is no clear distinction compared to the "traditional" types of yeast, we need further examinations.

From the results of our research we can conclude clearly that organic yeasts produce significant differences in the glycerine concentration. The residual sugar content also showed a difference among the samples. The volatile acid content was measured below the critical level of 1,0 g/l [42] in all cases.

#### 4. Discussion

Out of the physiologically effective compounds it was the biogenic amine composition that gained a closer inspection. The most important allergen, histamine, was measured within the approved range (10 mg/l), which proves that the biogenic amine, which is an allergen, makes Bianca perfectly suitable to produce organic wine. With the other amines I concluded that their values correspond to the previously calculated values appearing in the scientific literature, and there is no significant difference as a result of using organic yeast or the vine variety Bianca as source material.

Regarding the polyphenol composition, we can conclude that it is suitable to produce high-quality Bianca organic wine.

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