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AN ANALYSIS OF THE COMPOSITION OF BIO WINES

Thesis

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1. BACKGROUND AND OBJECTIVES OF THE RESEARCH PROJECT

In recent years in Western Europe and Hungary, people have become aware of the fact that our environment is being more and more affected by the use of various synthetic chemicals for agricultural production. In the interest of reducing this kind of contamination, various philosophies have been proposed, circulated, and put into daily practice. The professional literature mentions three main alternative technologies: integrated, biodynamic, and ecological.

The products created through ecological management are called by several names in the profession: eco-, bio-, and organic (natural) products.

"Ecological agriculture endeavors to establish the kind of sustainable, varied, balanced, environmentally friendly, productive agricultural systems that generate truly valuable food." The following points summarize the basic theories of eco-agriculture.

- stop the use of environmentally harmful technologies
- maintain and improve the natural productive ability of the soil
- reduce use of non-renewable sources of energy, and increase use of renewable sources of energy
- complement the natural needs of various species of plants (SOLTI, 2000)

Ecological practices have also spread to viticulture; more and more natural wines are appearing in the market beside wines made by traditional methods.

The following are the most important characteristics of ecological wine grape production (the list is not all-inclusive):

- use of chemical fertilizer is prohibited; instead, natural fertilizer is to be used (but only in judicious amounts)
- soil supplements may be only natural materials
- synthetic plant protection chemicals are banned
- preparations containing copper or sulfur may be used (in restricted quantities) as fungicides

Winemaking is characterized mainly by reduced chemical additives. As opposed to traditionally made wines, bio wines are lower in sulfur, and this can lead to problems with stability and unpleasant taste or smell (e.g. prohibition of blue fining--using potassium ferro cyanide--to eliminate heavy metal ions. This can create problems, because higher concentrations of heavy metal ions can result in separation).

From this expose we can easily see that bio wine production occurs under much stricter rules, which translates into a smaller margin of error for producers, so theirs is a difficult and risky business.
In the course of my research, I examined the detailed parameters of wines produced under such strict conditions. My goal was to collect a body of statistical data that heretofore has not existed in the literature of viticulture and oenology.

A standard in grape growing are the *resistant* varieties, for the reason that they stand up well to disease. However, resistant varieties often have undesirable fruit.
In the second section of my research, I studied the hyper oxidation wine production process, selecting resistant grapes to see if the amount of undesirable product could be reduced.

I also set the following objectives for studying topics related to organic winemaking from the viewpoint of practical application.

- What is the acid composition of natural wine? Is there a difference between the acid content of natural and traditional wine?
- What is the polyphenol concentration in bio wine? How big an effect does organic grape growing and wine production have on polyphenol content from the physiological standpoint?
- Is the amount of heavy metal-ions (especially iron, copper, potassium, and calcium ions) different in bio and traditional wines?

In wine growing, nitrogen supplements are used differently, so we expect the must and the wine to have different nitrogen-containing substances. I focused on biogenic amines in this respect, and posed the following questions:

- How does organic grape growing affect the biogenic amine content of organic wine? What types of biogenic amines (histamine, tiramine, and serotonin concentrations) are found in organic wine? Do natural wines, which are produced under very strict regulations, conform to hygienic expectations—is the level of histamine sufficiently low?
- Do organic and traditional wines exhibit a difference in their nitrogen, amino acid, and assimilatable nitrogen content?

As mentioned above, the main field of my research was resistant grape varieties. The organic growing of grapes calls for very strict rules, and restrictions on pesticides and other plant protection chemicals demand the more durable wine types – thus, the resistant varieties. On the other hand, these types have unwanted features.

My experiments served to treat the must of less desirable grape varieties with hyper oxidation, and then test for taste, smell, and appearance. The goal was to evaluate to what degree the unwanted features were reduced.

2. EXPERIMENTAL METHODS AND MATERIALS

The first two years of the project involved collecting data and mapping out the plan. The samples used in the study were obteined from commonly available Hungarian bio wines taken from regular production stock wines. Therefore, for the purposes of this project, they were *bio wines* of the years 2000 and 2001.

Based on the first two years' results – according to which the bio wines' content conforms to the technical literature – I brought in control samples from the next vitnage year (2002). From a strict scientific standpoint, the control sample should be grape must (wine) made from grapes grown in a field adjacent to the one the other samples originated from.

However, since domestic winegrowers do only ecological winegrowing and not traditional production, there was no way to get samples from two adjacent fields (traditional and ecological).

In spite of this, my control samples, similar types originating from similar growing areas, provide good background information for evaluation of the results.

<u>Determining amino nitrogen content of musts with titration.</u>

Neutralize 50 cubic cm of must or wine with 1 unit of NaOH so it has a pH of 7,8 stirring constantly. Next, use NaOH to bring the pH value to 8. Then put in two drops of hydrogen peroxide, wait 1-2 mintues, add 20 cubic cm of formaldehyde, stir for a few minutes, and bring the pH level to 8,5 with 0,1 unit of NaOH. Then read the amount of the liquid obtained in cubic centimeters.

Formol calculation: amino nitrogen (mg/l) = 0.1 unit NaOH cm^3 decrease x factor x 28

The optimal quantity of nitrogen is between 200-400 mg/l.

<u>Determining amino acid content of musts and wines</u>

Prior to analysis, the samples were strained through a Sartorius membrane (0,45μm) and then 100 μl of the sample was injected into the apparatus.

Measurements were carried out with an Aminochrom II OE-914 automatic amino acid analyzer (MIM laboratory, Budapest). Durrum DC-4A resin was used for separation. The motion phase contained various Pico (Pierce) buffers, and analysis was done at three specific temperatures.
After determination of origin with a ninhidrin coloumn, we did photometric detection at 570 nanometers (except for prolin, which was measured at 440 nm).

<u>Measurement of biogenic amine content: musts and wines</u>
Preparation of samples: strained the must and wine through a 0.45 μm thick membrane, then caused a reaction with OPA (ortho-phtal-aldehyde) in the presence of a borax buffer.

The following parameters were used for chromatography:

Apparatus: HP type HPLC
Coloumn: Nucleosil 100 C-18 (250x4mm)
Detection: HP1046A fluorescent detector
Rate of flow: 1 ml/minute
Temperature: 30°
λ: 340 nm λ: 440 nm
Effluent: A solution: 0,08M acetic acid
B solution: HPLC-type acetonitrate

<u>Measurement of acid content in musts and wines</u>

- Titrable acidity – according to MSZ 9472-86
- PH measurement with combined glass electrodes – according to MSZ –14849-79

- Malic acid content – Boehringer Mannheim enzyme test, also spectophotometry
- Citric acid content - Boehringer Mannheim enzyme test, also spectophotometry
- Lactic acid content - Boehringer Mannheim enzyme test, also spectophotometry
- Tartaric acid – We created an orange-colored mixture by combining the tartrates of the wine with the vanadation of a reagent that had been added to the wine. Then the color intensity of the mixture was measured, and this measurement was proportional to the concentration of tartaric acid.

Test of polyphenol content of wines

- Total polyphenol content determined by applying Folin-Ciocalteu reagent calibrated for gallic acid, according to MSZ-9474-80
- Leucoanthocyanide content tested with ferro (II)-sulfate-hydrochloric acid-butanol (in a 40:60 proprotion), test done after heating the solution, with spectrophotometry (Aubert, 1970, modified)
- Catechu content tested by thinning the wine with alcohol, then adding sulfuric acid which had been reacted with vanilin, at 500 nanometers, spectrophotometric (Tanner, Brunner, 1979, modified)

Measurement of metal ion content of wines

When preparing the samples, wines were diluted with deionized water 10 parts to 1, in order to avoid the interference of the alcohol content. Measurement apparatus: ICP-AES spectrophotometer ICAP-9000 (Thermo-Jarell-Ash, USA)

Hyperoxidation experiments

100 liters of must were divided into two halves. One half (50 liters) was set aside as a control. The other 50 liters was further divided in half, 25 liters being oxidized so it was just starting to turn light brown; and 25 liters was allowed to turn completely brown. Osygen was introduced by means of a reducer. In addition to oxidation, the samples were fermented as follows:

Sulfites were not added to the cefre of the must. After the must settled, it was fermented with Uvaferm 228 yeast, along with nitrogen supplement(20mg/l). After fermentation, as a trial sulfiting treatment, we introduced free sulphureous acid in the proportion of 25mg/l. Also, the clarification of new wine was done as trial clarification.

Based on the results of the 2001 experiments, work with hyperoxidation was expanded. We could see that complete hyperoxidation was more expedient, so we suspended partial oxidation (for light brown color). The experiments were changed by addition of Olaszriesling and Cirfandli (a curiosity wine from Pécs) as control samples.

The focus of the experiments changed in the following way:

The musts were divided into two parts. One part (the control) was not oxidated; the other part was oxidated (completely brown). Then both samples were treated with 0.5g/hectoliter of Lallzyme HC enzyme, and finally they were fermented in two different ways. One oxidated samples received Uvaferm 228, while the other received 50g/hl Seporito and 20g/hl Kazein—in addition to fermenting yeast. During the wine making process, each sample was treated with 35-40mg/l of free sulphureous acid as a blocking agent.

3. SUMMARY OF RESULTS

Based on my research, I conclude the following:

The statistics and results of the acid tests clearly are influenced by the wine year. The 2000 and 2001 bio wine figures correspond both to the acid content of traditionally produced wines and to the professional literature (Tables 1 and 2). For the year 2002, acid content test results show no significant difference between organic and traditional wines.

Table 1: Values of acid content in bio wines, wine year 2000

wine year 2000	tartaric acid (g/l)	citric acid (g/l)	titrable acid (g/l)	ph	lactic acid (g/l)	malic acid (g/l)
White bio wines	2,84	0,24	6,47	3,27	0,47	1,59
Red bio wines	3,43	0,12	6,26	3,31	0,91	0,57

Table 2: Values of acid content in bio wines, wine year 2001

wine year 2001	tartaric acid (g/l)	citric acid (g/l)	titrable acid (g/l)	ph	lactic acid (g/l)	malic acid (g/l)
White bio wines	2,71	0,35	6,35	3,25	0,83	1,10
Red bio wines	2,42	0,13	6,27	3,41	0,93	1,32

It can be stated from the examination of polyphenol content that the wine year influences the polyphenol content of organic wine. The results reflect the effects of the year 2000, in which red bio wines measured high in polyphenol. Both catechu and leukoanthocyanine were found in appropriate amounts in organic wines. Likewise, both organic and conventional wines were characterized by good levels of phenols (Table 3).

Table 3: Values of polyphenol content in bio wines, wine year 2000

wine year 2000	Catechin (mg/l)	Leukoanthocyanine (mg/l)	Total polyphenol (mg/l)
White bio wines	28,16	12,00	265,41
Red bio wines	692,67	1893,17	1279,33

For the wine year 2001, bio wines show the influence of the year both in the measured levels of catechu and leukoanthocyanine, and in total polyphenol content. For the year 2001, in terms of polyphenol content, red wines were of lesser quality (Table 4). For 2002, there is very little difference between bio musts, bio wines, and traditionally made musts and wines.

Table 4: Values of polyphenol content in bio wines, wine year 2001

wine year 2001	Leukoanthocyanine (mg/l)	Catechin (mg/l)	Total polyphenol (mg/l)
White bio wines	193,76	51,46	325.84
Red bio wines	771,65	376,83	850.57

It was possible to determine from my experiments with metal ion content that in this respect, organic wines for all three years conform to the data in the professional literature. In the case of a few samples, there was a higher concentration of iron ions, but that difference was not caused by organic wine making technology; rather it was due to lack of proper grape processing machinery and other equiment.

Copper ion concentrations likewise measured approximately the same in bio wines and conventional wines, although one might expect higher copper levels in bio wines due to use of materials containing elementary copper and sulphur. Kalium and calcium levels exhibit no difference between traditional and organic wines.

In the course of my experiments with nitrogen-containing compounds in bio wine, I made measurements on the 2000 and 2001 vintages with respect to biogenic amines. According to my data, it can be concluded that the biogenic amine content corresponds to the existing professional data. For the 2000 vintage, white wine biogenic amine content was 6.9-73.9 mg/l, while red wines came in at 42.6-74.6 mg/l.

Histamine, tiramine, and serotonin are three biogenic amine compounds that have significant biological effects. All of these were present in amounts corresponding to the current literature. White organic wine's average histamine content was 1.7 mg/l, and a lightly higher level was found in red organic wines: 1.9 mg/l. Tiramine content: white bio wines, 4.00 mg/l, reds 3.06 mg/l. Serotonin was detected in white bio wines at an average 26.5 mg/l, whereas it appeared in red bio wines at 11.18 mg/l.

The 2001 wine year: white organic wine – total amine content ranged from 11.4-106.4 mg/l; red organic wine – amine averaged 17.0-122.7. Average levels of histamine for white and red bio wines were 2.4 mg/l and 6.4 mg/l, respectively. Tiramine levels in all varieties were similar to levels in traditional wines: white bio wine – 3.8 mg/l, red bio wine – 3.2 mg/l. Serotonin concentrations were higher: 2.3 mg/l in white bio wines, 22.4 mg/l for reds.

It is 95% sure that the 2002 wine year had no meaningful difference between the biogenic amine content of natural wines and those made by traditional methods.

For the 2002 vintage, not only was the biogenic amine content determined, but the biogenic amine precursor compounds and amino acids were measured also. *For amino acid and biogenic amine total content, we can conclude that no obvious connection exists between the amino acids and the biogenic amines.*

In addition, the 2002 bio musts were checked for assimilatable nitrogen content. Using a statistical t-test, I compared the aminonitrogen content of bio and non-bio musts. My results show a 95% probability of no significant difference between bio and conventional musts.

All things considered, we can conclude that organic wines – produced under stricter regulations and narrower parameters – correspond to current professional data and show acid, polyphenol, and iron ion levels closely resembling those in traditional technology wines.

Regarding nitrogen compounds in musts and wines, biogenic content – specifically histamine, tiramine, and serotonin – is practically the same in similar types, years, and places of origin for bio and conventional wines. *The same goes for assimilatable nitrogen content in musts, as well.*

Regarding organic wine growing – due to the strict regulations, particularly those for plant protection materials – it would be wise to grow the so-called resistant types. All three wine years in my study display the result that in the widespread resistant variety, Bianca, hyperoxidation influenced the appearance, taste, and smell (sensory properties) of the wine. The tests done in 2001, using a 20-point ranking system, showed quite a difference between the control and hyperoxidized samples. The hyperoxidized samples clearly fared better; the basic characteristics of Bianca were successfully reduced.

The results of the 2002 and 2003 experiments likewise support the premise that intensive aeration of musts decreases the undesirable features of the wine variety. In measuring polyphenol content, results show success with hyperoxidation and further, the sample that was prepared with combined bentonit and casein for fermentation turned out best – this sample got the highest point value in the sensory ranking system.

4. NEW SCIENTIFIC FINDINGS

1. To date, no one has deterined the general chemical qualities and parameters of organic Hungarian wines. In addition, here in Hungary no one has surveyed general bio wine characteristics from an analytical and sensory perspective. I examined what kind of acid content exists in the natural wines currently being traded. Bio musts and bio wines were tested for tartrate, citric acid, malic acid, titrable acid, and pH value. *I determined that organic wines do not vary in acid content from conventional wines; the deciding point is the wine region of origin and vintage year. We cannot say if a wine is organic or not based on its acid content.*

2. I placed special emphasis on tests of nitrogen compounds in bio wines and bio musts. Bio must aminonitrogen, amino acid, and biogenic amine levels were measured.I especially observed amines having physiological effects: histamine, tiramine, and serotonin. *I established that among organic musts/wines and wines produced by traditional methods, there is no significant difference in aminonitrogen, amino acid, or biogenic amine – in spite of variations in nitrogen replacement during wine growing.* My experiments demonstrate that although organic wines are subject to restrictions concerning chemical additives, they still conform to hygienic requirements.

3. Polyphenol compounds in organic musts and wines from the biological view: three important elements were measured: the whole polyphenol content, leukoanthocyanine, and catechu. *These results also prove that bio must and wines have no particular polyphenol content and are therefore no different from conventional wine in this respect.*

4. Metal ion content was also measured, although some procedures are not allowed in oenology. I paid particular attention to heavy metal ions, which cause separation in wine. *Once again, my experiments showed no significant difference between organic and conventional wines for metal ion concentration.* High metal ion content in several bio wines derives not from their "bio-ness", but from lack of proper wine making technology.
5. I did an experiment to see whether I could reduce the undesirable basic qualities of otherwise advantageous resistant grape varieties. Three vintages of resistant grape must were aerated intensively in order to reduce or eliminate the original aroma. Likewise, control samples of Olaszrizling and Cirfandli received hyperoxidation. Sensory tests for all three years reveal that the unpleasant characteristics decreased. We got better-looking, clearer wine. Conversely, the controls had a negative reaction to hyperoxidation. *Based on my results*, *I believe it is advisable to apply hyperoxidation to resistant varieties when making organic wine*.

5. OUTLOOK FOR DEVELOPMENT AND APPLICATION OF RESULTS

In the past few years, I thoroughly examined the practice of growing and making natural, or bio wine. Based on my measurements and calculation, I arrived at the conclusion that a wine's organic nature is determined not by chemical characteristics, but by the regulations under which it is produced.

The concept of bio wine is not really a scientific category, but rather a commercial – marketing – one. The various experiments described here support this premise. However, we should not lose sight of the fact that bio wine manufacturing – grape growing and subsequent wine making – supports environmentally safe methods.

Moreover, whereas environmentally safe technology produces products equal in quality to those made by traditional methods, it behooves everyone to consider bio viticulture and bio oenology.

The grape, the foundation, first and foremost defines organic wine. Then of course, it is advisable to continue with organic production in the wine making phase. Also, we should push artificial chemicals into the background and emphasize manual treatments (in relation to which there have been previous experiments, even if not specifically with bio wines: non-sulfite containing wines, or cooling, and hyperoxidation).

It is practical to establish resistant types in organic viticulture, since they are tough against certain diseases. Optimally, the unpleasant characteristics are overcome by hyperoxidizing the must.

For the future, research and results will help people to grasp the concept of organic wine, not to mention that reading about the results gives them a complete picture of bio wine. In the future, these results will facilitate the commercial prospects of organize wines.

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