SZENT ISTVÁN
EGYETEM

OPTIMISATION OF THE OENOLOGICAL TECHNOLOGY FOR THE GRAPEVINE VARIETY BIANCA

DOCTORAL THESIS

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2 BACKGROUND AND THE OBJECTIVES OF THE RESEARCH

Wine is a special food product that upholds several types of human activity and several types of industries; thus, it plays an important role in local economies, especially in countries such as Hungary. Our wine making technology, our local production traditions and wine culture are deeply rooted. Wine is exceptional among the food products destined for human consumption due to its high nutritional value and appetizing taste, and due to its character, making it qualitatively distinct from the other food products. In the past few years it has become widely known in Western Europe and Hungary that agricultural production puts continually growing pressures on the environment with the use of synthetic substances and other chemicals. In order to reduce the unnecessary pollution, various cultivation philosophies have been outlined, become widespread and have become part of daily practice. In agricultural production and naturally in viticulture, eco-friendly technologies have gained priority.

Considering the ever-stricter rules and regulations, the resistant, or interspecific vine varieties could be the source material for alternative cultivation technologies. Regarding the choice of variety, the interspecific varieties are the the same quality as the international varieties though organoleptically they might not be up to them. These strong vine varieties are more resistant to fungal diseases and infestations than the international varieties. They are easier to involve in organic, integrated or biodynamic systems of cultivation as the need for using chemicals is lower. However, processing these vine varieties into wine has not prevailed owing to the particular and undesirable flavour properties as well as to the aromatic composition typical to primary vine species.

The significance of the theme

In the domestic and the international scientific literature there is no research that could give a comprehensive overview of the relations examined in this paper, or how the possible outcomes could be used in the oenological technology. Notably regarding the specific features emerging while processing the resistant vine varieties and producing the wine types. As the source materials for the wine sector of the future will be these alternative, environmentally friendly vine species, several well founded, analytical and organoleptic results need to be put forward. The aims and obectives of my thesis are the following:

2.1 Aims and objectives of the research

An analysis of the modern viticulture and the evaluation of complex oenological settings based on the following main points:

- The development of the adequate technology in the wine-sector for the resistant vine variety Bianca, with the widest area plantations in Hungary
- Maturation processes and microanalytical examinations of the must
- Wine technology experiments in laboratories and under various largescale circumstances
- The effect of the DMR technology on the analytical composition and organoleptic properties of the must and wine originating from resistant vine varieties, in our case, Bianca.
- Basic analysys of must and wine types (routine)
    - Microanalytics
    - Determining the biogenic amine component of must and wine types (HPLC technique)
    - Determining the poliphenol components (spectrophotometry and HPLC)

3 MATERIALS AND METHODS

The laboratory at the Department of Oenology at Szent István University, while serving scientific theoretical research and students' practical training, is home to numerous wine- and must sample examinations based on a well-established analytical model. Naturally, the incoming samples are from different varieties, but as Bianca is widely produced, a considerable number of samples are available for examinations. There is a vast amount of data accessible related to the vine variety Bianca, originating from various places of production, from various cultivation practices and different vintage years, processed in this paper. As a result, there is a high level of reliability when defining the parameters of the basic analysis regarding the wine and must types from the Bianca variety. We can answer the question whether this variety will meet the expectations of modern wine technologies, or what further technological specifications should be defined so as the variety can gain a greater role on the market.

3.1 Examining dynamic maturation processes

In the present paper I have examined how the grape, as source material, when harvested at different time intervals in relation to the various stages of ripening, affects the parameters of wine analytics. I have chosen the variety Bianca for my thesis, as it is sufficiently resistant to cold and rotting, a quality that makes it suitable for organic production. The fruits of the variety samples were harvested in six vintage years with an approximate difference of seven days. When evaluating the results, we noticed how important it is to determine the right harvest time in preparing the source material for wine production. I compared the results of the six years based on the following criteria: berry size, sugar content, acidity and pH level. After evaluation we can conclude that the composition values corresponded to those presented in the scientific studies. Relying on these measurements and basic analytical data we can state that the variety Bianca is suitable for producing a modern type of wine. In the evaluation process I followed the sampling period daily.

3.2 Organic yeast

The role of the yeast in wine production is crucial. During the research we wanted to find out what kind of Bianca wine is produced when using organic yeast, what the rate of the alcoholic fermentation is and wheter it can be used in the course of mass production as well. We produced wine from the Bianca must samples three times, and we used model chemical solutions. The harvest date was 05.09.2012. The initial level of chaptalisation was 20,6 MM°, the initial level of titratable acid was 6,2 g/l. For the racking 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 of the fermentation process and at the end of it. The control item was inoculated with yeast Uvaferm CS2, 20g/hl and nutrient salt Uvavital was added at inoculation, mid-fermentation and at the end. The vine variety Bianca yeast experiments were carried out with the support of the limited liability company Nyakashegy Kft.

3.3 DMR technology

We had been searching for a wine making technology for the variety Bianca and we expected that a higher quality product could be manufactured as a result. (A desirable sugar concentration level and lasting acidity). Also, we hoped for an output which can be produced in a way to meet modern day oenological requirements, using the variety Bianca. The experiment was implemented on the Bianca plantation situated in the town of Kecskemét, on the grounds of the Research Institute for Viticulture and Oenology (National Agricultural Research and Innovation Centre) in 2015. The grapevines were grown under full operational conditions. The one-year-old cane was cut three weeks before the harvest. We repeated the process twice, in randomly arranged blocks. Next to the control vinestocks we obtained three treated samples within a three-week time period, at one week each. After harvesting, quick destemming and crushing, we produced the wines using identical technologies. Sulfur dioxide (50 mg/l SO2) was added, followed by cultured yeast inoculation. After the primary fermentation we used Bentonite (50 g/hl).

3.4 Research methods

With the assistance of a group of expert evaluators, all of them members of the Department of Oenology, we carried out a sensory evaluation of the fermented wines parallel to the basic and microanalytical measurements. A profile analysis was used for the comparison of the results that emerged in the sensory evaluation.

3.4.1 Examining the biogenic amines in grape musts and red wines

There are multiple methods to measure the level of biogenic amines. Due to the development of the analytical techniques we have effective column chromatography methods (HPLC and IEC) and overpressure layer chromatography (OPLC) procedures at our disposal. Capillary electrohporesis methods are all suitable for defining the amines. The HPLC tehnique is suitable for processing the precise and high quantity sampling data (Kállay-Nyitrainé, 2003).

3.4.2 Examining acids in musts and wines

We implemented practices used in everyday life to examine acids in wine:

- titratable acid content – regulated by the Hungarian Standards Institution bearing the serial number MSZ 9472-86
- pH measurement using a combined glass electrode – MSZ-14849-79
- malic acid content – using the Boehringer Mannheim enzyme test and spectrophotometry
- citric acid content – using the Boehringer Mannheim enzyme test and spectrophotometry
- lactic acid content – using the Boehringer Mannheim enzyme test and spectrophotometry
- tartaric acid content – the vanadate ions of the reagent added to the wine combined with the tartrate ions in the wine turn the liquid into an orange colored complex. We measure the intensity of the color, which is proportional to the concentration level of the tartaric acid. MSZ-9489-78.

3.4.3 Examining the polyphenol content in wines

- Overall polyphenol content defined using the Folin-Ciocalteu reagent, calibrated for gallic acid, in accordance with the Hungarian standard nr. MSZ-9474-80,
- Leucoanthocyanin content examined after warming butanol-hydrochloric acid containing iron (II) sulphate, with spectrophotometry. Ratio 40:60. (Flanzy, 1970 Modified),

- To measure the catechin concentration I used the Rebelein (1965) method, where the liquid, reacting with vanillin, gives a colored product. Other, routine analytical examinations: sulphurous acid content (free/all) - MSZ 9465-85, volatile acid content – MSZ 9473-87.
- To define the overall polyphenol content: Folin-Ciocalteu reagent, calibrated for gallic acid, (Kállay, Török, 1999).
- The amount of leucoanthocyanins was defined by heating hydrochloric acid butanol containing iron (II) sulphate, proportion 40:60, using spectrophotometry, based on the Flanzy (1970) modified method.
- The catechin content with wine diluted in alcohol, after inducing a chemical reaction with vanillin sulphuric acid, on 500 nm, using spetrophotometry (Rebelein 1965)
- The simple phenol component was defined using the HPLC technique. It is possible to establish the level of the cinnamon acid derivatives, like coffee acid or their esters obtained with tartaric acid, such as the caftaric acid, employing a diode string detector.
- We defined the cis-trans-resveratrol content of the wines with direct injection, through the HPLC method (Kállay, Török, 1997)
- Measuring the quercetin and quercetin glucoside content using the HPLC technique, (Purospher RP18, 250×4 (5 µm) (Merck, Germany) column, 0,45 ml/min stream, 30 °C, on 370 nm.
- Biogenic amines defined with the HPLC equipment, 3.6.4. Statistical evaluation

The results were imported into and evaluated in the programs Microsoft Word for Mac 2017 (version 16.9, license Office 365 Subscription) and Excel for Mac.

4 RESULTS

4.1 Examining the dynamics of maturity

Our observations were carried out during four consecutive years (2014-2017) for the vine variety Bianca. We compared the average weight of one hundred berries through the vintage years. The test harvest for sample collection lasted from the third week of August until the end of September. There is a significant difference between the results of the four vintage years described in this paper, based on the weight of the berries of the Bianca variety (a=0,05; s^2=958,7; DF=15).

4.1.1 Changes in the sugar content through the ripening process

On Figure 1. we can observe the sugar content in the Bianca musts (between 2009-2010 and 2014-2017). From burgeoning to full ripening sugar flows into the berries. The period following it is the after-ripening, when sugar growth only happens as a result of water loss, as described in LŐRINCZ ET AL., 2015. Apart from the year 2015, in all six vintage years we witnessed consistent sugar level growth. According to the earlier observations made by HAJDU (2003), the ideal ripening sugar level for the Bianca variety is between 18,2 - 24,3 MM°. The refraction levels were around 20,8 ref % (s^2=117,3) at harvest time in the six vintage years. Statistically it is proven that from the fourth week from burgeoning the refraction values reached the maximum level specific for the variety. Based on the measurement results we can state that in all six vintage years the values significantly increased along the weekly samplings, compared to each other (a=0,05; s^2=37,8; DF=3). The difference in vintages can be statistically proven, (a=0,05; s^2=45,7; DF=5), and there is a measurable difference between the vintages. According to LŐRINCZ ET AL (2015) sugar content higher than 18 MM° is considered a high ripening value. The lowest refraction value was measured in 2009, (16,7%) while the highest was reported from 2017 (21,4%). As the harvest period continued, the sugar content levels increased amid decreasing variability, corresponding to the existing scientific observations and literature.

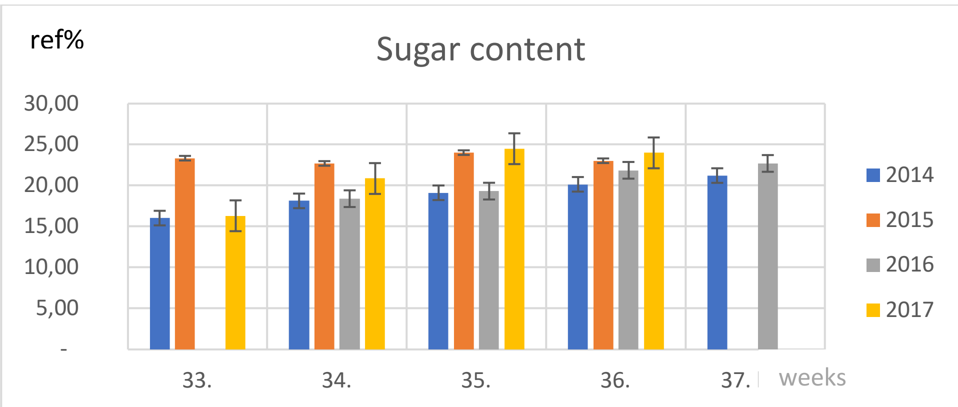


Figure 1. Sugar content in wine musts from the variety Bianca 2014 – 2017.

4.1.2 Titratable acid content change through ripening

In the ripening process the titratable acid content of a grape berry decreases from 40-50g/l to 4-15g/l from burgeoning to full ripening. The decrease is a result of the dilution of the berry liquid with water as well as the acid reduction during the respiration of berries (LŐRINCZ ET AL., 2015).

According to HAJDU (2003), the average titratable acid content in Bianca is around 7,3 (6,5-10,2) g/l. Based on the literature, with the advancement of the harvest there is a decrease in the titratable acid content beginning with the burgeoning. The amount of the decrease, based on our measurements, is significant, from week to week (a=0,05; s^2=32,2; DF=3) and the vintage years also differ provenly (a=0,05; s^2=116,0; DF=5).

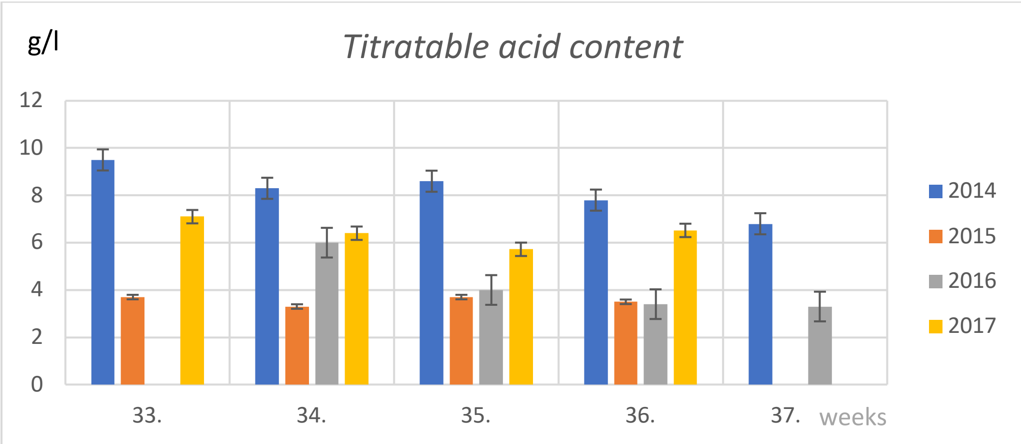


Figure 2. Titratable acid content in the Bianca musts 2014 - 2017.

4.1.3 Change in pH values along the ripening process

Besides the sugar content in the must, the pH value is the most important parameter that defines the quality of the wine, according to KOVÁCS (2016). The pH value indicates the strenght of the acids and it is widely known that it is the negative logarithm of the hydrogen ion concentration. In each vintage, the pH value and the acid content must be kept under close observation. During the observation period a significant difference was stated between the vintage years (a=0,05; s^2=3,2; DF=5). Considering the ripening process, we cannot statistically prove a change, based on data originating from the weekly samplings (a=0,05; s^2=0,04; DF=3).

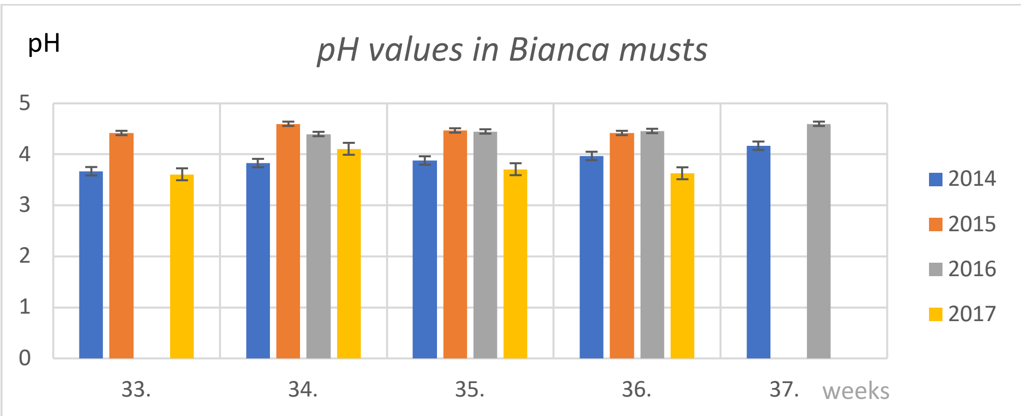


Figure 3. pH values in musts from the variety Bianca 2014 - 2017.

We can confirm that the vine varitey Bianca is suitable for today's modern winemaking technology, considering basic analytical data. Both the acid and the sugar content, as well as the pH value developed in accordance with the existing studies and literature. Winery specialists prefer an earlier harvest because of the higher acit content. In terms of product safety, early ripening and high resistance qualities are positive supportive arguments in the hands of the producers, as they face the requirements of the plants being adaptive to extreme weather conditions. The first step in the winemaking technology is defining the optimal harvest time in order to gain a fresh, fruity type of wine. Measuring the sugar content, the pH value and the titratable acid content, regarding the observed vintage years, we can state that the fruit of Bianca is best harvested when the acid content is higher, and the sugar content is lower. Based on the observations along the vintage years, the optimal date is between 18th and 20th August.

4.1.4 Overall polyphenol content in the musts in the ripening process

Examining the parameters of the fine components in the musts, the first significant chemical group is that of the poliphenols. We can conclude that there is no significant difference in the amount of poliphenol in the ripening process, in the case of the Bianca variety (a=0,05). It is worth higlighting about the poliphenol content, that the group of these compounds is extremely sensitive to oxidation, which may affect the process of adding sulphur to the wine.
There are statistically proven differences when comparing the vintage years. The overall poliphenol content was high in every year, which supported a higher sulphur addition, in the technological aspect. We saw the highest overall poliphenol concentration in 2014, both at the beginning and at the end of the ripening process. Mold appeared as an effect of laccase and tyrosinase in the vintage year, due to the wet weather. In 2015 and 2016 there was not much deviation in the samples, so we can accept the process as balanced.

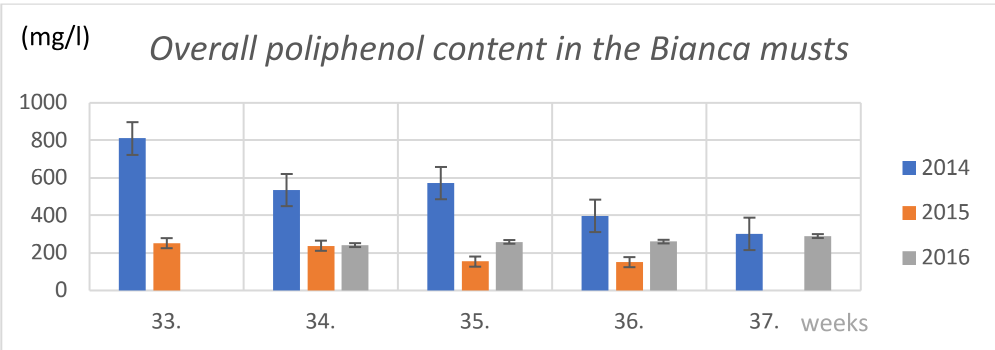


Figure 4. Overall poliphenol content in the Bianca musts 2014 – 2016.

4.1.5 Catechin content in the ripening process

We can state, evaluating the catechin content, that there is a significant difference between the vintage years. In the ripening period (from week to week) the difference is not relevant (a=0,05). In 2014 it showed a decline, and in the following two years a tendency to increase.

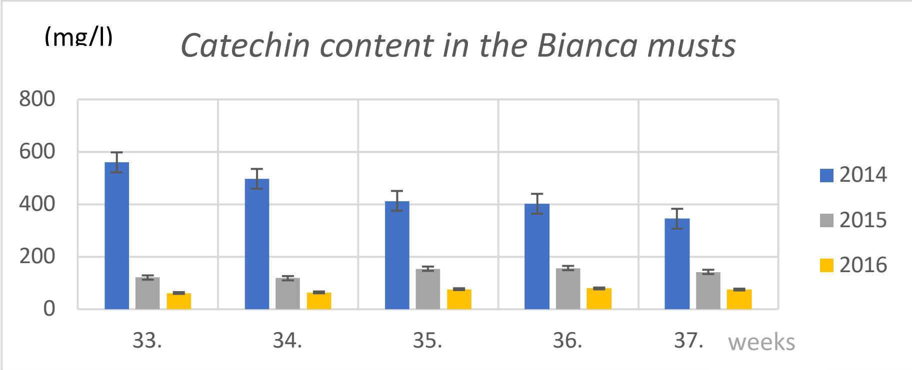


Figure 5. Catechin content in the Bianca musts 2014 – 2016.

4.1.6 Leucoanthocyanin content in the ripening process

Like with the catechin content, when evaluating data on the leucoanthocyanin levels, we see a significant difference between the vintage years. We cannot identify clear and distinct changes from week to week in the ripening stage (a=0,05). In 2014 the levels decreased, and in the following two years the values displayed an increasing tendency.

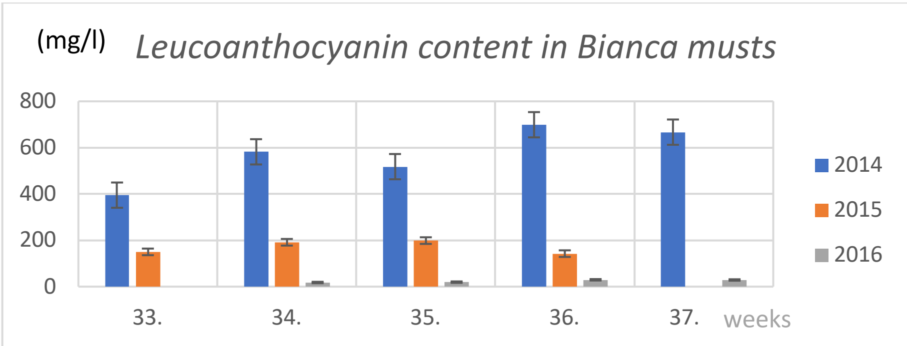


Figure 6. Leucoanthocyanin content in Bianca musts 2014 – 2016.

4.1.7 Titratable acid content in Bianca wines

The significant difference between the vintage years can be proven, as the acid content in the wines originating from the different harvest periods is statistically distinct. The wines produced from the fruit harvested in the third week of August (around the 18th) displayed a fresh, fruity, vigorous acidic taste, also confirmed by the sensory evaluation.

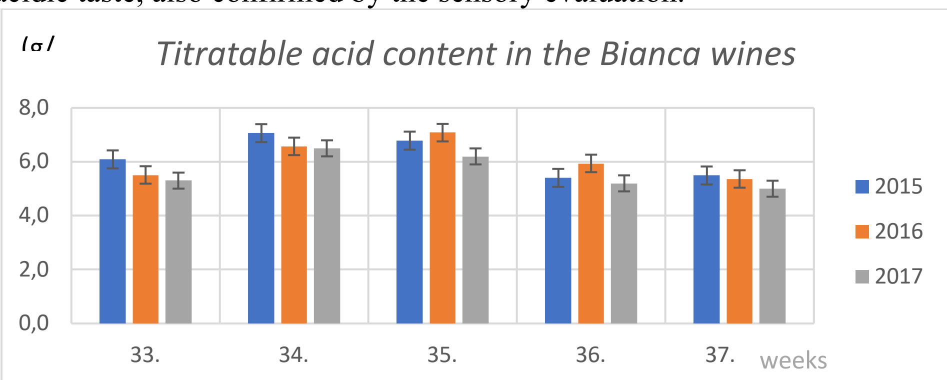


Figure 7. Titratable acid content in the Bianca wines 2015 – 2017.

4.1.8 pH levels in wines

There is no statistically proven difference between the examined wine samples, but the range of the pH values was between 3,32 and 4,11 in the wines produced in the different vintage years.

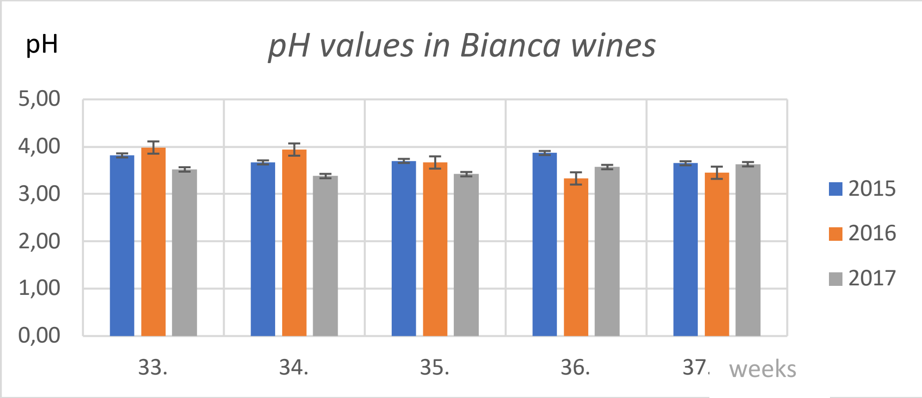


Figure 8. pH values in Bianca wines 2015 – 2017.

4.1.9 Overall polyphenol content in the wines

The effect of the vintage years cannot be detected in terms of the overall polyphenol content in the wines. There is a minimal increase as the harvest proceeds, it can be proven statistically with a 95% confidence level. The overall polyphenol content increased by 70mg/l during the four-week period on average (average in week 33: 242,0 mg/l; in week 36: 312,2 mg/l).

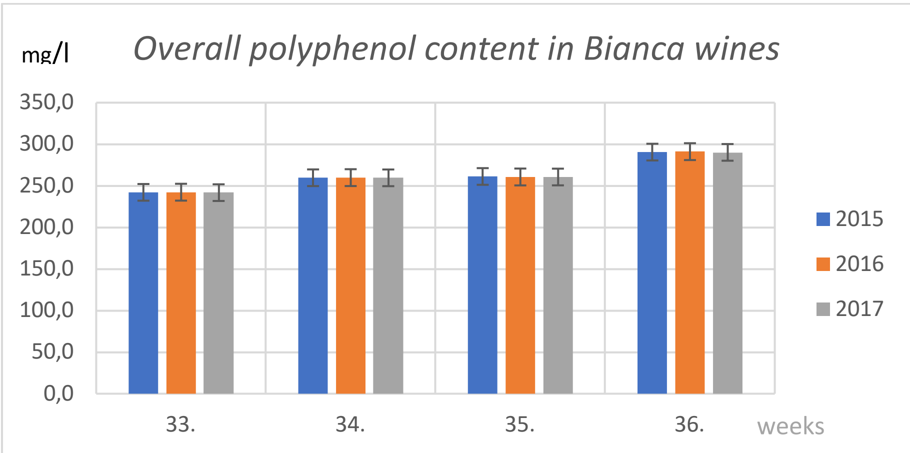


Figure 9. Overall polyphenol content in Bianca wines 2015-2017.

4.1.10 Catechin content in the wines

During the examined period we measured approximately identical values in the wines produced from the harvest of the first three weeks, 204 mg/l on average. In the samples from the last two harvests we measured 251,6 mg/l on average in all three vintage years.

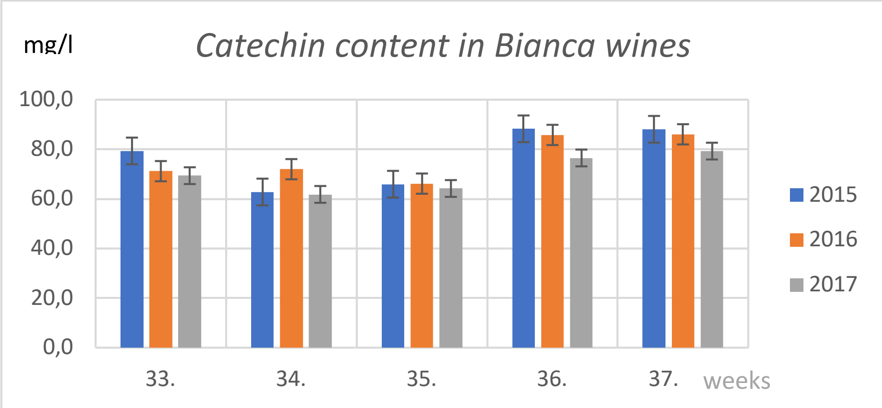


Figure 10. Catechin content in Bianca wines 2015-2017.

4.1.11 Leucoanthocyanin content in the wines

Based on descriptions by Australian authors (Heather, 2017), the leucoanthocyanin content in (dry) wines may range widely between 0-1000 mg/l. The significant difference depending on the weekly harvesting times and the effect of the distinct vintage years can be confirmed. There is a sightly increasing value as ripening proceeds, the outstanding values obtained may arise from the sample selection. The difference between the lowest value (from week 34) and the last value (week 37) was 8,39 mg/l.

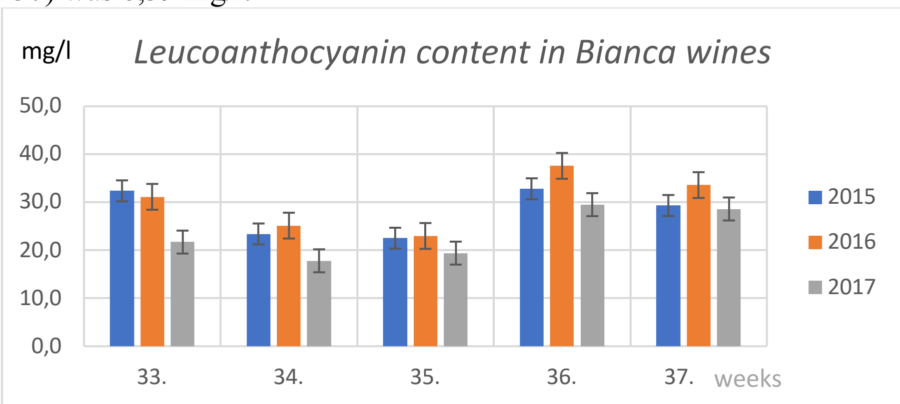
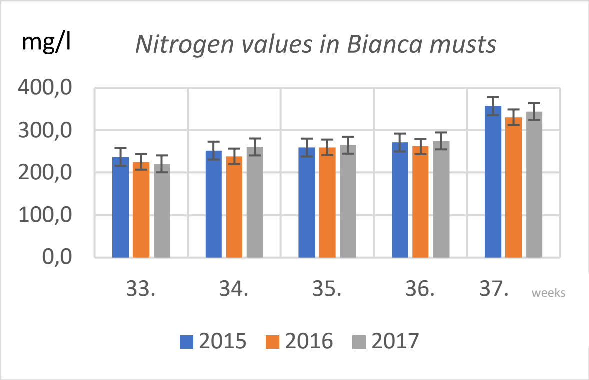


Figure 1. Leucoanthocyanin content in Bianca wines 2015-2017.

4.1.12 Alpha amino nitrogen content in the wines and musts

There is no statistical difference based on either the vintage years or the harvest times at a confidence level of 95%. Increasing residue level can be observed in the year 2017. The Alpha amino nitrogen content was high due to the stress induced by the weather conditions. The precipitation was low, and compounds with nitrogen content accumulated in the plants. The temperatures in 2015 and 2016 were high.



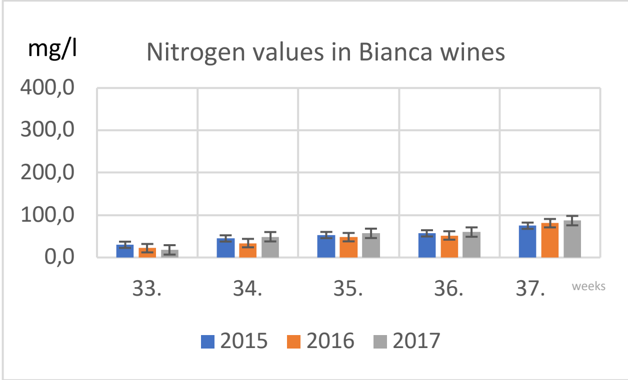


Figure 12. Alpha amino nitrogen values in Bianca musts 2015-2017.

Figure 13. Alpha amino nitrogen values in Bianca wines 2015-2017.

4.1.13 Proline content in wines

There is no difference in the proline levels based on the harvest time and vintage. As the harvest proceeded, we could observe a minimal rise in the value, but it cannot be statistically proven at a confidence level of 95%. During the ripening period, proline is increasingly present in musts. The yeast does not use up the whole proline content, that is the explanation for the increase in the values. In other words, the differences in values are the result of the ripening process. If we examine the years 2015 and 2017 separately, we can conclude that Nitrogen was accumulated in the harvest season of 2017.

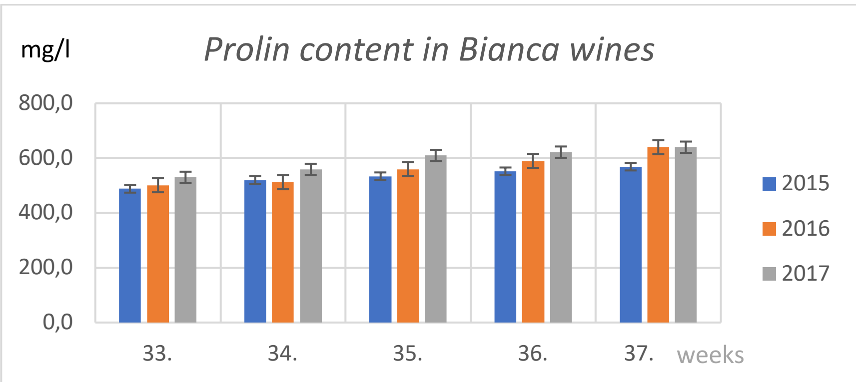


Figure 14. Prolin content in Bianca wines 2015-2017.

4.2 Wines produced with organic wine yeast

The yeast is a selected type propagated and dried in organic conditions. The substratum used for propagation is from a certified organic culture. The wines produced from organic grape require a distinct attention and care, not only on the bearing surface but in the whole winery.
In our present experiment, our main aim was to check how strongly the organic yeast influences the production of compounds with nitrogen content, whether there are differences compared to the formerly used brewer's yeasts. Directed fermentation is a basic element of the modern winemaking technology.

We wanted to know in our research in what ways organic yeast influenced the production of the three main biogenic amins: histamine, tirozine and serotonine. We found relevant differences in the samples produced with organic yeast, and the values were higher along the vintage years. In terms of the physiological effect, based on the three main biogenic amine concentration levels, the organic yeast produced serotonine in a higher concentration. We can conclude that in the wines manufactured with organic yeast the levels of all three biogenic amines was below the critical 5mg/l, in accordance with the scientific literature (ESPOSITO ET. AL., 2019) and the health prescriptions in effect.

4.2.1 Results of the basic analyses of wines produced with organic yeast

The results of the analyses have been summarized in Table 1. Our results prove that there are significant differences between the samples regarding alcohol content and residual sugar concentration. The control sample shows that fermentation took place properly, and the residual sugar content is only 2 g/l. However, the organic yeast only partially used up the sugar in the fermentation process, as 63,4 g/l sugar was left in the wine, that is why there is a considerable difference in alcolhol content and residual sugar content.

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

	control		organic yeast	
	average	deviation	average	deviation
free/overall SO_2 (mg/l)	46/105		55/104	
titratable acid (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

4.2.2 Biogenic amine content in the wines produced with organic yeast

In this experiment our main aim was to examine how organic yeast influences the production of nitrogen based compounds, and whether there is a difference between organic yeasts and the yeast stocks used previously. Directed fermentation is a basic element in modern wine making technology.

Earlier experiments have proved that with low nitrogen concentration the yeasts produce hydrogen sulfide in larger amounts (VOS and GRAG, 1979; HENSCHKE and JIRANEK, 1993). The explanation for that lies in the synthesis of amino acids. Amino acid precursors are insufficiently produced in the yeast cells, to which sulphide from sulphur reduction could attach, so hydrogen sulphide leaves the cells and it accumulates in the wine.

In the musts characterized by nitrogen deficiency, besides hydrogen sulfide production, there is also an increase in higher order alcohol types during the fermentation period (OUGH ET AL., 1980). I am presenting the histamine, tyramine and serotonine concentration, due to their physiological effect, all of them at the centre of my research, on a separate diagram (Figure 15).

In our research we wished to know how organic yeast influenced biogenic amine formation in the three main cases: histamine, tyrosine and serotonine. We recorded significant differences in the samples produced with organic yeast; the values were higher in each vintage year. In terms of physiological effect, the organic yeast produced serotonine in a higher concentration. We can conclude that the level was below the critical 5 mg/l as suggested in specialzed literature (Esposito et. al., 2019) 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 we can state that there is no relevant difference between the control and the organic sample at a 95% significance level. The histamine concentration cannot exceed 5 mg/l in wines, and both images show that the histamine levels in the two samples were well below that.

The tyramin concentration changed according to data present in scientific literature (Martuscelli et. al., 2013). In both samples, we were not able to detect relevant differences. The serotonine content was 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 (a=0,05; s^2=0,49; DF=5).

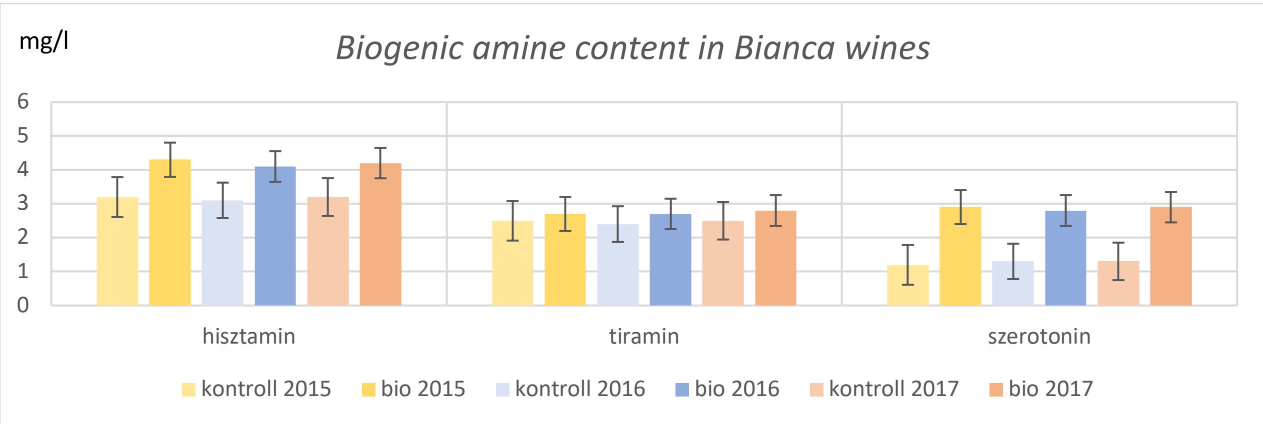


Figure 15. Biogenic amine content in Bianca wines 2015-2017.

In the case of the other biogenic amines there are also 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 (a=0,05; s^2=0,22; DF=5). At the same time, the metilamin 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 metilamin concentration. 2 phenylethylamine could not be detected in any of the samples. Both types of yeast used up all the nitrogen that can bond in the fermentation process; so a difference between the samples could not be observed. The prolin concentration presented itself as expected, it is widely known that in anaerobic conditions yeasts cannot utilise prolin.

4.2.3 Polyphenol content of the wines produced using organic yeast

We could not find significant differences in biogenic amine composition between the samples, except for the methyl amine 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 (a=0,2) in any of the three vintages.

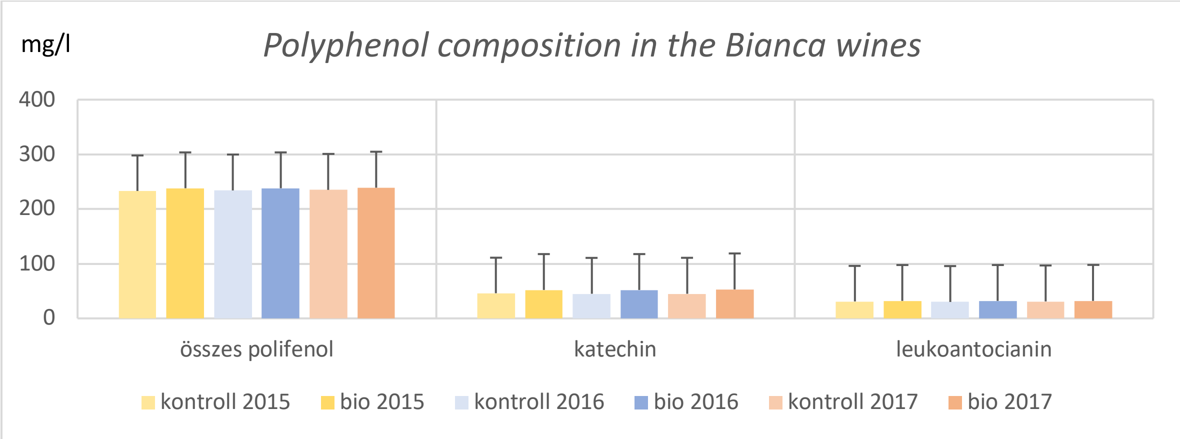


Figure 16. Polyphenol composition in the Bianca wines 2015-2017.

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.

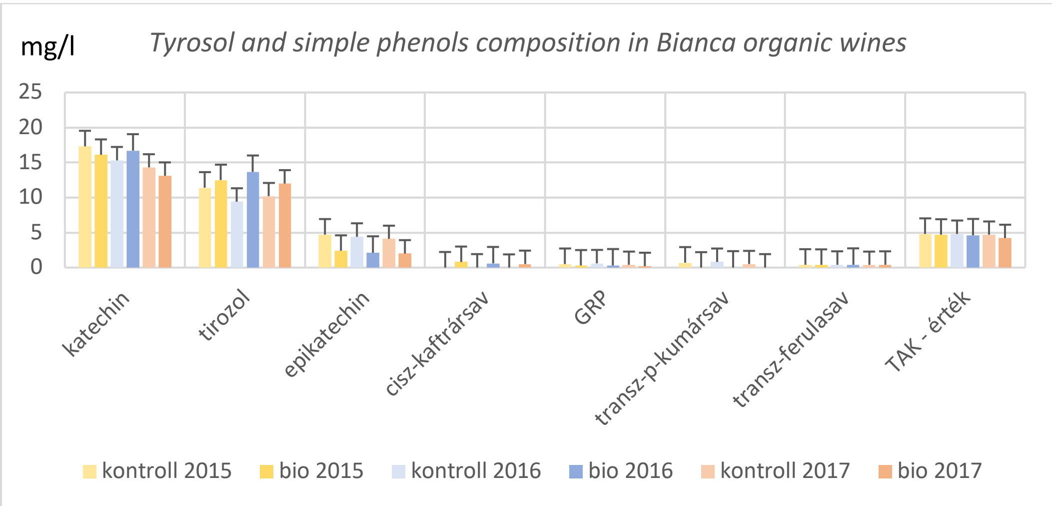


Figure 17. Tyrosol and simple phenols composition in Bianca organic wines

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 vines.

In the samples produced with organic yeast, the catechin, leucoanthocyanin and the overall polyphenol concentration values supported the data in the scientific literature (Freitas, 2019). 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 interacton 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 (Kállay, 2010) in all cases.
The biogenic amine composition also produced relevant differences among the samples. Overall antioxidant capacity can play an important role in eliminating the harmful effects of the free radicals in the human body. Independently of the polyphenol composition, in former experiments (Kállay et. al., 1999) there were no high values measured in the overall antioxidant capacity in the premium quality wines from the Tokaj region. In the wines named Tokaji Aszú (many of which are produced by leaving the grapes on the vine long enough to develop the "noble rot") the overall antioxidant capacity was independent from the sugar content and sugar-free extract (puttonyszám in Hungarian) in the mature wine. However, the length of the ripening period did influence the overall antioxidant capacity.

4.3 The results of the DMR experiments

It is widely known that high quality wines can be produced from good quality crops and source materials, so if there exists an oenological treatment that has been widely adapted in the technology, it is reasonable to inspect the effects of that technology in the final product, in our case in the must and the wine as well. The DMR treatment affects the wine products largely and positively and it has a beneficial effect both on the organoleptic qualities and the chemical compostion of the wines (RUSJAN ET. AL., 2017; RESCIC, 2016). Several scholars have been inspecting the effects of the DMR treatment, but not many have researched it in relation to the Bianca variety. It is already a resistant variety. The extra efforts invested are to bear fruit in the high nutritional value and the taste of the final product. I evaluated the results of the DMR treatment along these principles.

4.3.1 The titratable acid content in the musts as a result of the DMR treatment

The acid content in the musts produced from harvesting after cutting the one-year-old cane of vines showed decrease. We need to remark that the acid content in musts varied on a wide scale, between 3,5 and 7,5 g/l, and in the last two harvest periods 4,0 g/l or values below that were observed in the most vintage years. Those values can be regarded as very low for the domestic wines according to Kállay (2010). Opposite to the findings in the specialized international literature (CORSO ET AL, 2013; RESCIC ET L 2016) as a result of the DMR treatment the values of the acid content decreased in the Bianca grapes after cutting the cane and after the harvest. Choosing the harvest time and scheduling the DMR treatment with consideration for the acid content is important. It should be dated for the first and second weeks of August (11-25), after cutting the cane, based on our data. We were not able to find significant differences between the vintage years.

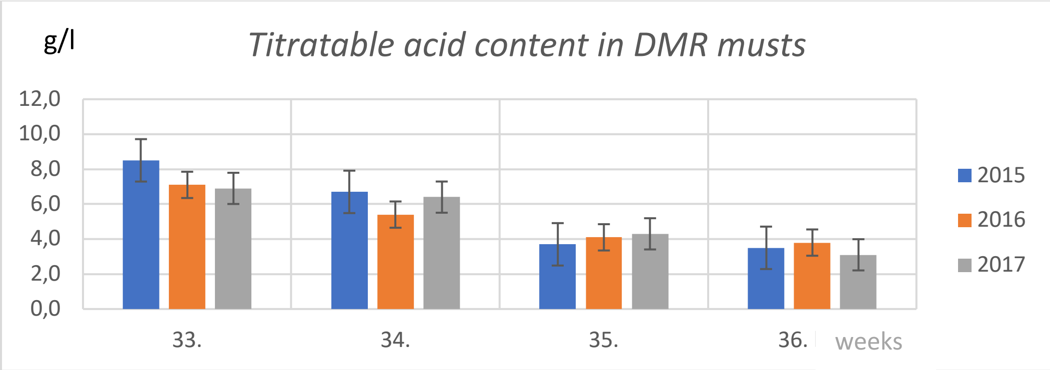


Figure 17. ábra: Titratable acid content in musts following DMR treatment 2015-2017.

4.3.2 pH values in DMR musts

The characteristic features of the must, the wine and the processes within them depend largely on the acid content. The titratable acid content in a solution accounts for the sum of the free and the half-combined acids without taking into consideration their strength. The real acidity or hydrogen ion concentration (expressed by the pH value) depends on the volume and strength of the acids present (Kállay, 2010). The pH value in the must defines the pH value of the wine fermented from it, though through the process of fermentation some changes in values may occur. On Figure 39. we can see the pH values of musts produced from DMR treated grapes. We can see that along the consecutive harvest sessions the pH values increased slightly. The values ranged between 3,5 and 3,7 corresponding to the specialized literature (2,80 - 3,70 as discussed in Kállay, 2010).

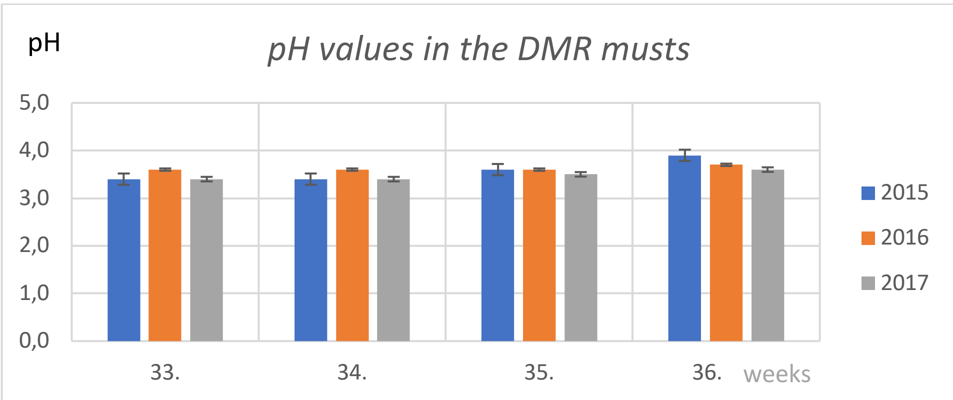


Figure 18. pH values in the musts following DMR treatment 2015-2017.

4.3.3 Sugar content in the musts formerly treated with DMR

After cutting the cane, in the bunches and berries, the sugar concentration increases due to water loss, as a result of the DMR treatment. Based on our examinations we find it proven that with the progress of the harvest, sugar content considerably increased: from 19,7 ref% to 26,0 % on average in the three years. Other scholars arrived at the same conclusion, Rusjan et. al. (2017) measured 20% increase in the dilutable dry matter within the same circumstances. The differences between the vintages can be regarded as balanced because no differences could be observed in the average values along the years.

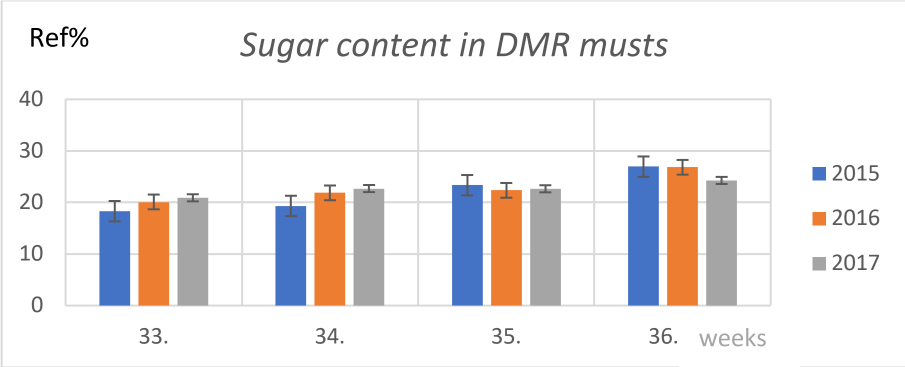


Figure 19. Sugar content in musts following DMR treatment 2015-2017.

4.3.4 Overall polyphenol content in musts following the DMR treatment

The overall polyphenol content in the musts showed a variety along vintages and harvest times. We measured different average values yearly (2015: 250,1mg/l; 2016: 314,4mg/l; 2017: 382,8 mg/l). As grapes ripened, the overall polyphenol content increased.

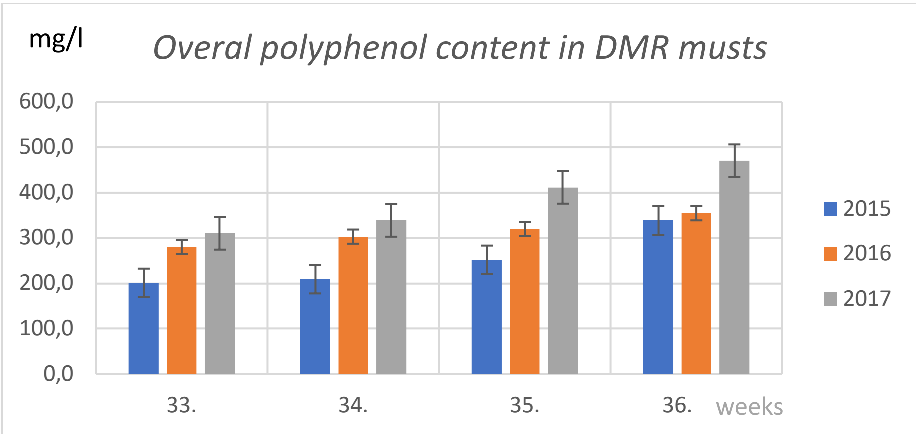


Figure 20. Overal polyphenol content in musts following DMR treatment 2015-2017.

4.3.5 The catehin content in the musts following the DMR treatment

Flavonoid compounds in white wines usually consist of flavan-3-ol catechins and flavan 3,4 diols (leucoanthocyanins). They enhance the full-bodiedness of the wine and contribute to quality. However, flavonoids have a role in discolouration, too, turning the product brown and in developing a bitter flavor (around 40 mg/l). Their presence is desirable only to a limited extent (Kállay, 2010). The catechin conent in the examined must samples was diverse.

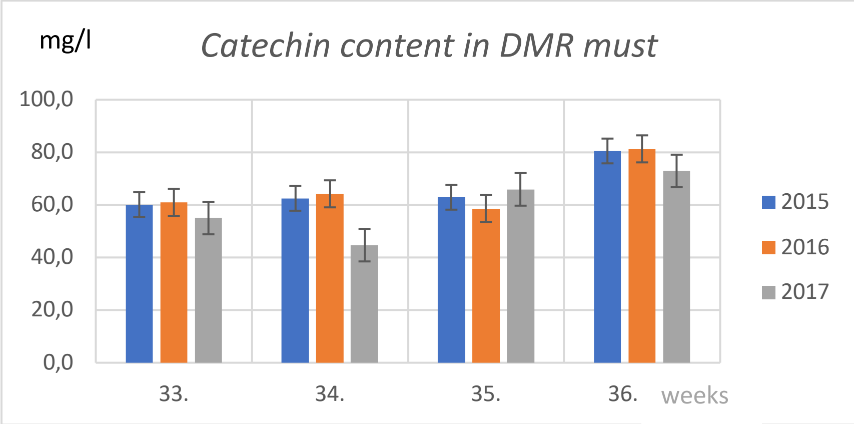


Figure 21. Catechin content in musts following DMR treatment 2015-2017.

4.3.6 Leukoanthocyanin content in the musts following the DMR treatment

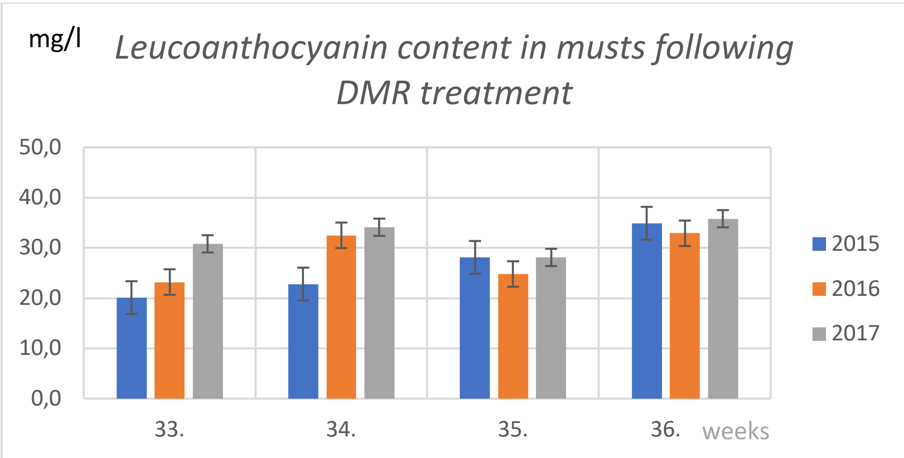


Figure 22. Leucoanthocyanin content in musts following DMR treatment 2015-2017.

Leucoanthocyanin compounds take part in the defensive mechanism of the grapevine. The higher the amount in the plant the higher is the resistance to drought and pathogens. Experiments were carried out in Dagestan where they examined the leucoanthocyanin content in the must produced from the vine variety named Refosk (Refosco) under DMR treatment. Bakhmulaeva and Colleagues (2019) measured values between 183,8 and 282,2 mg/l. In the case of the variety Bianca the values were diverse in one year (2016) and displayed increase in the other, during harvest. We can observe the variation in results in figure 43.

4.3.7 The titratable acid content in the wines following the DMR treatment

The acid content in the wines manufactured from the samples was between 5,4 and 6 g/l. Comparing the vintages, the wines from 2015 and 2016 had 6,4 g/l acid content uniformly, while in the year 2017 the acid content was 1 g/l lower (5,2 g/l) on average. Alcoholic fermentation influences the acid content in wines; other types of acid are formed as well, such as succinic acid, lactic acid, etc. The results of the oenological experiments can be attributed to certain processes only if we disambiguate the various effects of the fermentation in the samples. Ensuring the same conditions, (same yeast, nutritive salts, sulphurizing dose, temperature) we can exclude the distorting effect of fermentation. We can compare the wines and the samples produced under similar conditions and arrive at undistorted results. As an effect of the DMR treatment, in the present experiment, we could not describe any changes, though in the international literature (Rusjan et. al., 2017) a rise in the alcohol level and a harmonic and more full-bodied appearance is mentioned.

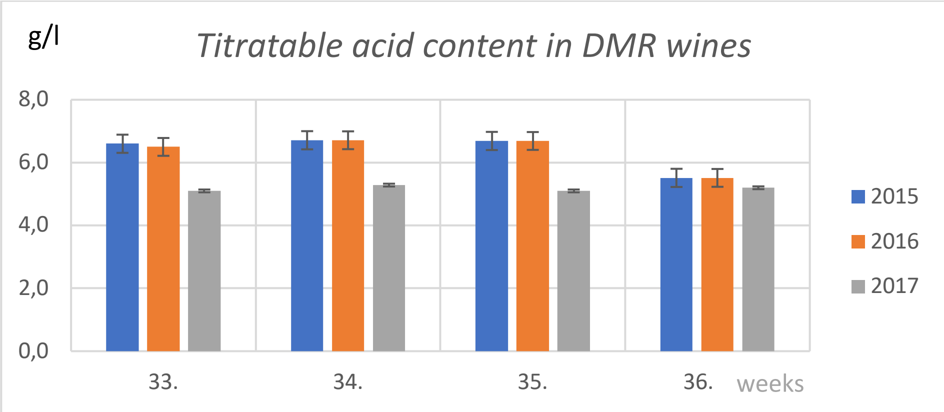


Figure 23. Titratable acid content in wines (Bianca, DMR) 2015-2017.

4.3.8 Ph values in the wines produced using the DMR technology

The pH values in the wines produced with the DMR technology were 3,5 on average; in the year 2017 there was a negative deviation (3,4) and in 2016 a positive one (3,6) for most of the samples. The wines did not display a significant difference as a result of the DMR technology.

4.3.9 Alcohol content

As an effect of the DMR treatment, due to water loss, sugar concentrates in the berries. From higher sugar content higher alcohol levels may be expected. We experienced this from the third week after cutting the cane, both in the case of the musts and wines. Figure 46. shows that the fruit harvested in the 3rd and 4th weeks produced high alcolhol levels in all vintages in similar fermentation circumstantces (yeast included). We could measure high values exclusively in the wines produced from 3rd and 4th week's harvests of year 2016 and the 4th week's harvest of 2015 (above 14 V/V %).

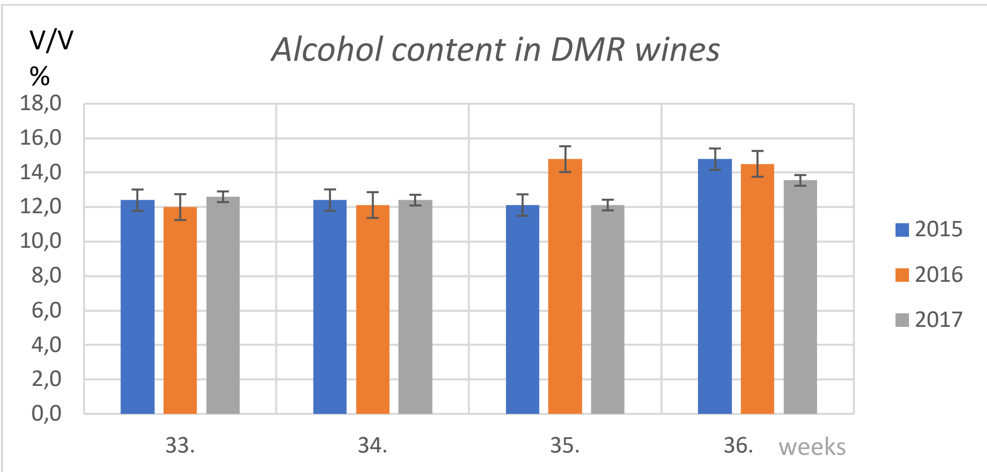


Figure 24. Alcohol content in wines (Bianca, DMR) 2015-2017.

4.3.10 Color intensity

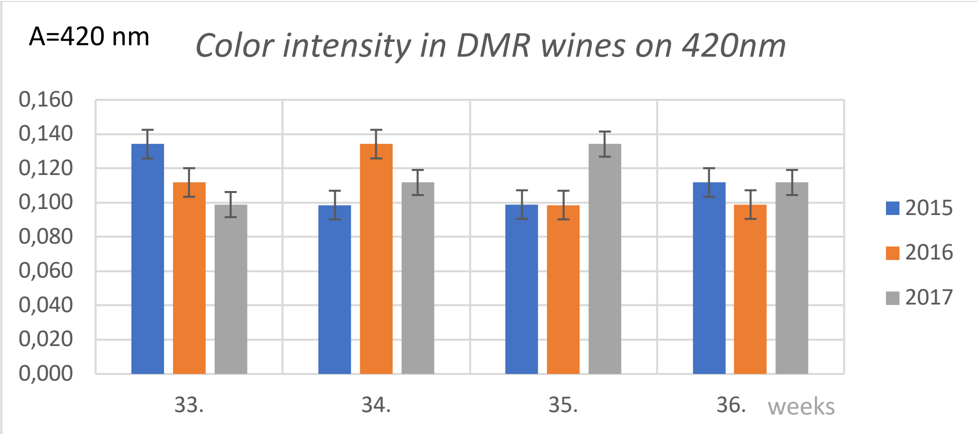


Figure 25. Color intensity in wines on 420nm (Bianca, DMR) 2015-2017.

Inspecting the color intensity, we focused on the compounds responsible for the wine turning brown, in terms of quantity. The higher the absorbance, the more the brown colored compounds appear, as summarized above. Examining the color intensity (Figure 25.) we can state that given the same sulphur level (50 mg/l), the results depend on the oenological technology. The color intensity values measured in the wines varied as the harvest proceeded; a slight increase can be observed taken into account the three years' average values.

4.3.11 Overall polyphenol content in wines

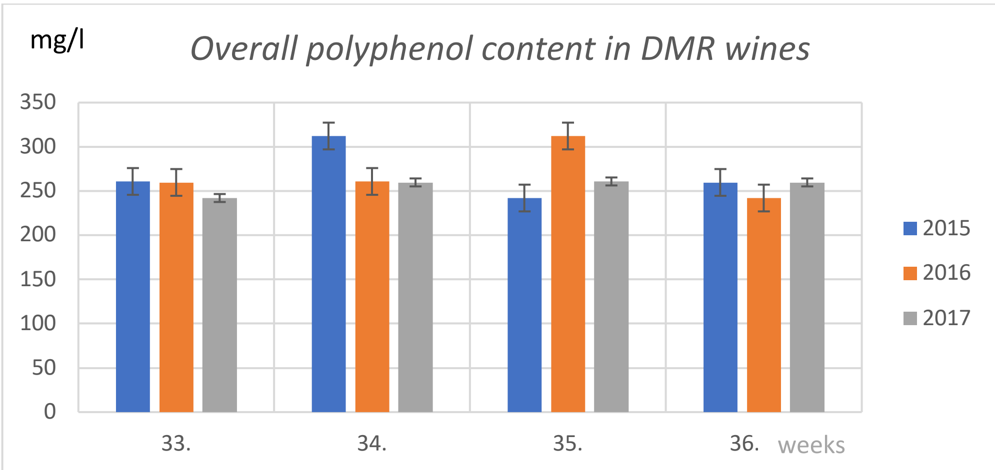


Figure 26. Overall polyphenol content in wines (Bianca, DMR) 2015-2017.

The polyphenol type of compounds is extremely sensitive to oxidation and it can influence the amount of sulphuring in musts and wines. In the given wine sample, the higher the amount of the polyphenol concentration is, the darker the color of the liquid. A more substantial amount of sulphurous acid (H2SO3) might result in wines losing freshness and their fruity character. Rusjan et. al. (2017) mention that as a result of the DMR treatment more polyphenol can transfer into the must from the husk of the grape berry. In our research we could not clearly connect the effect of the phytotechnical treatment with the change in the various measured values. As the harvest proceeds, the oxidation of polyphenols can be an explanation for the decreasing values. The overall polyphenol value range was between 242 and 312 mg/l (Figure 31.).

4.3.12 Catechin content in DMR wines

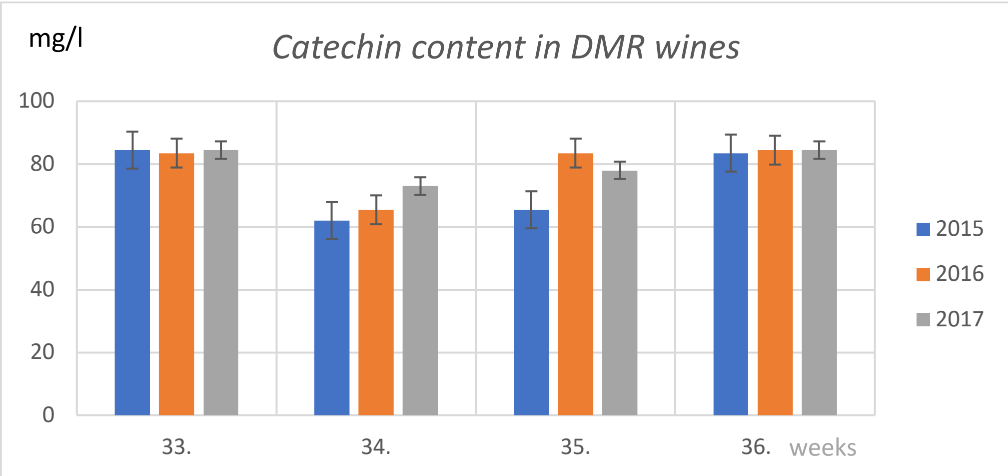


Figure 27. Catechin content in wines (Bianca, DMR)2015-2017.

In the white wines the flavonoid concentration consists mainly of catechins (flavan-3-ol) and leucoanthocyanins (3,4 – diol) according to Caro et. al., 2010. However, these compounds bring along a bitter flavor, and their presence is desirable only in limited amounts. With the presence of these compounds the roughness in taste may increase. If we compare the catechin levels in the musts to the wines, we can conclude that we experienced a growth linear with the harvest dates in the case of musts. We could observe the same phenomenon in wines, too, within the vintage, linear with the passing of time, as a result of the DMR treatment.

4.3.13 Leuocoanthocyanin content in DMR wines

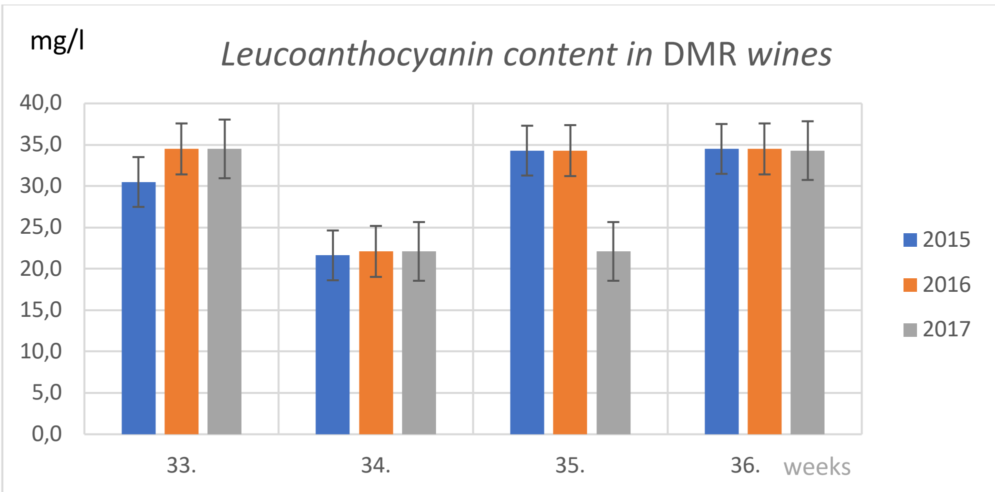


Figure 28. Leucoanthocyanin content in wines (Bianca, DMR) 2015-2017.

Leucoanthocyanin is a compound in wines that considerably influences their organoleptic properties. The austerity characteristic of the taste depends on the polymerization grade. Compounds with low condensation levels and molecular size have acidic, austere taste characteristics. Other scholars concluded that after the DMR treatment the outcomes were 1,7 higher in values (Petrovsek, 2017). Based on the present research the leucoanthocyanin values, except for the sample harvest in week 2, developed uniformly.

4.3.14 Assimilable nitrogen content in DMR wines

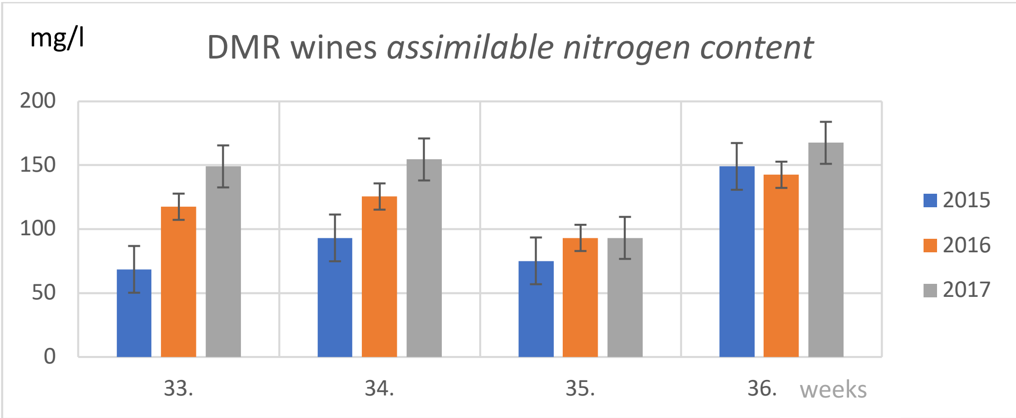


Figure 29. Assimilable nitrogen content in wines (Bianca, DMR) 2015-2017.

We can observe the assimilable nitrogen values on Figure 51. The The assimilable nitrogen content in the wines can change compared to the content in the musts due to the yeast and to clarification. The values were between 93 and 209 mg/l with high variability. The weekly average harvest showed around 131 mg/l, only the 3d week was a lot different, 113 mg/l. When producing the samples, we provided similar conditions using the same type of yeast. As we used similar yeasts, presumably the nitrogen used by each of the samples was a similar amount. As a result, the assimilable nitrogen values can differ only due to the unequal initial amounts. So, differences in the value might account for the differences in treatments and samples. In all three years the assimilable nitrogen value slightly increased. We need to add, that in every vintage, the data for the 35th week stands out from the rest, it might happen that an error of measurement occurred.

4.3.15 Proline content in DMR wines

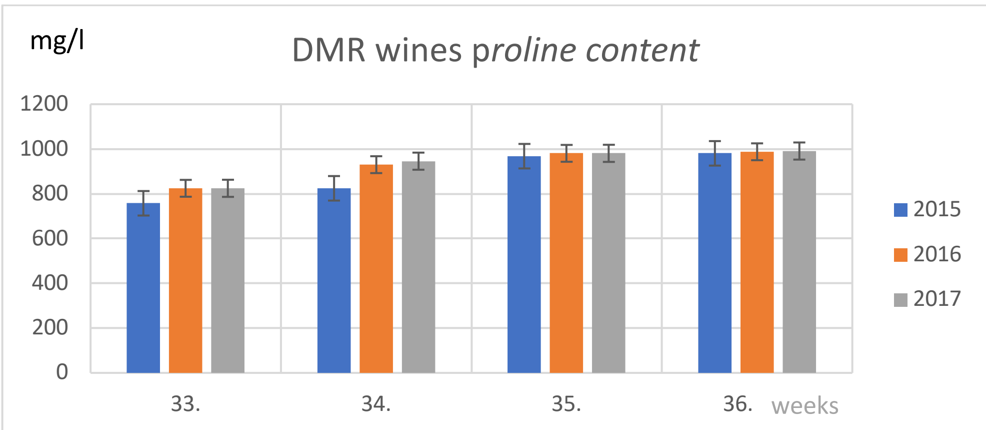


Figure 30. Proline content in wines (Bianca, DMR) 2015-2017.

In the context of proline content, we can conclude that the effects of the DMR treatment cannot be detected in the wine samples. All samples were uniform.

4.4 RESULTS OUTLINING NEW SCIENTIFIC ACHIEVEMENTS

1. In the samples discussed in the Bianca maturing dynamics research, based on primary data and data from fine analytics, I wished to find the optimal harvesting time for the vine variety. The aim is to produce a fruity type of wine while preserving the harmony and inner values of the berries and, at the same time to prevent the dominance of the undesired flavor of the variety, though the grapes are susceptible to becoming flabby.

After examining the Bianca wines with fine analytics, for four years, we can conclude that they are not significantly distinct from the wines produced from traditional vine varieties. The polyphenol component in the Bianca wines in the vintages observed by me is as follows: the overall polyphenol content is 273 mg/l on average, the leucoanthocyanin content is 27 mg/l on average and the catechin concentration is 112 mg/l on average. After analyzing the fine components in the Bianca wines from the four-year-harvest, we arrived at the conclusion that there is no significant difference compared to the wines oringinating from traditional vine varieties.

2. I examined the fruit of the Bianca vine variety through the various stages of ripening, in four years, being the first in our country to do so. I searched answers to my quest of whether we can contribute to the production of high-quality wine source material if we have a resistant type of vine and a well-chosen harvest time. *Doing my research, I concluded that the polyphenol composition of the Bianca vine is suitable by meeting all standards for the manufacture of high-quality wines. The polyphenol content, compared to white international species, did not present significant differences or discrepancies. In the Bianca musts, from the fruit harvested in its fully ripened stage, the average overall polyphenol content was 304 mg/l, the catechin content was 218 mg/l and the leucoanthocyanin concentration was 270 mg/l.*

3. Tyrosol, the compound responsible for the bitter taste, was examined in the process of making organic wine. Its appearance and quantity first and foremost depend on the yeast activity. *The level was higher in the case of the organic yeast in the vintages in question, but it never exceeded 25 mg/l.*

4. The undesirable "resistant" flavour is detrimental to Bianca's role on the market. From the fruit collected during the different harvest times, wine was produced, and it was judged by experienced, recognized professionals, scientists and university educators. *As a result of the sensory evaluation, the wines produced from the crops harvested between August 1^{4th} and 28^{th} were outstandingly successful in terms of overall impression and fruitiness, supporting the conclusions listed above.*

5. I examined the fine analytical parameters of the winemusts and wine, inspecting the effects of the alcoholic fermentation in the case of a resistant vine variety. Concerning the nitrogen-based compounds in the Bianca wines, we could see that the assimilable nitrogen was 270 mg/l and from the amino acids out of which *prolin is the most relevant, as it is not used during the alcoholic fermentation, came with an average value was 561 mg/l.*

6. I defined the biogenic amine and the polyphenol content as the main fine analytical parameters in the production of the absolute organic wine. *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 amins 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.

7. In defining the optimal production technology for the Bianca vine variety, I examined the effects of the DMR treatment – presuming that it would enhance its quality – along several vintages. We can conclude that the most harmonic Bianca wines are produced one or two weeks after cutting the cane. In the wines produced from harvests after that period we could not detect improvement in the inner qualities. It is no use leaving the bunches for a longer time on the cane, because due to water loss we gain less fruit and the concentration in sugar results in residual sugar content in the wines.

5 CONCLUSIONS AND RECOMMENDATIONS

As the growing season shortens, the bunches and and berries reach the parameters for the optimal inner values earlier. It is not only the Bianca variety for which it is important to define the optimal harvest time to obtain quality wines; with the climate change the harvest season starts earlier for grapes and for most of our other outdoor crops. In the case of the Bianca, the appearance of the aromatic constituents has a diminished role as we wish to avoid the undesirable aroma character. It is not enough to set the harvest time based only on the sugar and acid content. Relying on our measurements in the four vintages as well as on the scientific literature we can say that the ideal harvest time for the Bianca varitety is in the third week of August (between 18th and 22nd). The acid and sugar content and the inner characteristic features of the fruit turned out to be ideal when harvested in that period. As a result of the sensory evaluation we saw that the wines produced from the third and fourth harvests (3rd and 4th weeks of August) were given the highest marks for the overall impression and fruitiness. We can conclude undoubtedly that for the vine variety Bianca the optimal harvest time is mid August, one week before or after it, depending on the vintage.

The analysis of compounds containing nitrogen moved to the forefront as they inform about the food hygiene conditions. They are not only examined in meat or cheese products or other fermented food types but also in wines. We need to test the resistant vine varieties suitable for organic wine production, among them the Bianca variety, in order to be aware of the nitrogen amounting capacity in the berries. The presence of nitrogen-based compounds in the berry and the must will influence the nitrogen-based compounds in the wines, among them the biogenic amins. Based on my research on the four vintage years on the biogenic amin composition we can state that neither histamine nor other amins are formed in significant amount of concentration.

The optimal polyphenol content and the Sulphur ratio must be defined so as the fruity and fresh aroma character can be preserved in the wines. The optial polyphenol content in the Bianca wines is 240-280 mg/l, based on our measurements and the samples from the examined vintage years.

Considering the regulations for organic wine production, with a lower sulphuric acid level we can ensure the hygienic conditions for organic wines (see in the case of biogenic amins above), and we can ensure the exclusion of polyphenol oxidation. Based on my experiments and analyses, this optimally lowest sulphuric acid dose is 100 mg/l. Naturally, the rules of Sulphur addition need to be observed, out of which a Sulphur addition test is inevitable.

As an effect of the DMR treatment, in accordance with the scientific literature, sugar content increased after cutting the one-year-cane. The most harmonious wines were produced one or two weeks after the cut. The inner values of the wines manufactured after that period did not increase any further, it is not recommended to leave the grape bunches on the stem for longer than that. Water loss leads to a decline in crop yields and high sugar content leads to residual sugar in wines. I wish to mention that the proposal discussed above is a guideline only if the technological aim is not to produce wine high in sugar content.

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