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The effect of yeast autolysis on the composition of wine

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ABSTRACT

The experiment aims to monitor the amino acid content, total polyphenolic components, and antioxidant activity values of wines that have matured on yeast and non-yeast sludge. The grape varieties used in the experiment, which lasted 300 days, were (*Vitis vinifera* L.) Chardonnay, Riesling rhinestone, and Veltliner green. During this time, both the measured parameters and the characteristics of the wine gradually changed. The total amount of amino acids in the wines aged on yeast sludge was more than 200% greater than that found in wines aged without yeast sludge. A 30% decrease in the total polyphenolic component content was noted for wines produced with yeast lees. The antioxidant activity levels correlated with the total polyphenol content, with the levels in wines made with yeast lees on average 13% lower. The experiment showed that wines produced by these different methods have different mutagenic characteristics. Sensory analysis of the wines demonstrated that wines matured on yeast sludge have better organoleptic properties. These wines were sturdier, fuller, and more harmonious than wines aged without a yeast sludge.

Keywords: autolysis, sludge, wine, amino acid, polyphenol

INTRODUCTION

The release of yeast compounds into wine during autolysis is a current major trend in the production of wine. The principle is that these wines remain in contact with the yeast for several months or years, as with Burgundy wines designated "sur lie" **[1]**. Autolysis is a very slow process of yeast degradation induced by increased temperature by adding plasmolysers through mechanical intervention or other factors that facilitate the activation of lytic cell enzymes **[2]**. Still, wines are not a suitable medium for the use of the autolysis process, which takes a long time due to their ethanol content (10-12%), low pH (3.0-3.5), and low maturation temperature (usually below 15 °C). The duration of autolysis depends on the yeast strain **[3]**. Most yeast autolysis studies are performed under conditions that accelerate the process to obtain results in a reasonable period. Tests almost always occur at relatively high temperatures, usually above 40 °C **[4]**. Temperature influences the process of yeast autolysis as the intracellular enzymatic activities are temperature-dependent. However, not all studies on this topic have produced final results that agree, and some have even been contradictory. For example, while **[5]** induced autolysis assays performed between 40 and 70 °C did not detect enzymes in the extracellular medium **[6]**, in autolysis assays with brewer's yeast at 45 °C and a pH of 6.5, say that it is clear that quite extensive proteolysis occurs outside the cell. During yeast autolysis, the individual constituents in the wine change. These substances have a major influence on the sensory profile of the wine. The compounds affected by autolysis include phenols, volatiles, polysaccharides, DNA, RNA, lipids, mannoproteins, amino acids, and other nitrogenous components **[3]**.

During fermentation, the yeasts use the amino acids present in the wine. After fermentation, when all the sugar has been consumed, the yeast releases the amino acids back into the wine. This movement of amino acids from the intracellular environment to the surrounding medium is passive and results in a higher amino acid content in the wine **[7]**. The amino acid content of the wine remains relatively stable, usually for three to four months. After this time, the amino acid content of the wine begins to increase. This increase is attributed to the autolytic process, where cellular proteins are degraded, dissolved, and released into the wine **[8]**. Prolonged maturation on lees with

regular stirring causes a reduction in the concentration of polyphenols in the wine. The yeast cell walls absorb the polyphenols, and the mannoproteins released during autolysis are incorporated into the lees. In this way, the yeast leaves act as a refining agent that reduces the tannin content of the wine **[9]**.

The present work aims to study the effect of autolysis on the content of selected substances found in the studied wines over 300 days and the changes in their sensory characteristics.

Scientific Hypothesis

After ethanol fermentation, the yeast dies and becomes a fermentation sludge. In an alcoholic environment, it decomposes and releases substances into the wine. These substances should bind polyphenolic compounds, increase the level of amino acids, and have a major influence on the sensory profile of the wine.

MATERIAL AND METHODOLOGY

Description of the experiment

Three varieties of grapes were used in the experiment: green Veltliner, Riesling, and Chardonnay, all came from the vineyards of the Institute of Viticulture and Enology (Faculty of Horticulture, Mendel University in Brno, Lednice, vineyard tract "Ve starých," Czech Republic) and were harvested by hand. The grapes were de-stemmed and crushed, and each variety's grape must be left to macerate for 2 days at 12 °C. Each must be then separated into two types of containers:

Variant A: the must was decanted into stainless steel containers, and the leaves were removed from the wine immediately after fermentation (control).

Variant B: the must was put into 600l wooden oak barrels, and the wine was stirred on the lees

Fermentation of all musts (spontaneous fermentation at 15 °C) continued for 20-25 days. In the stainless-steel containers (variant A), the wine was racked off the lees immediately after fermentation, and 40 $mg.l^{-1}$ of sulfur dioxide was added. The free sulfur dioxide was subsequently maintained at 25-30 $mg.l^{-1}$.

Variant B (wooden barrels), battonage began after the fermentation and continued for 300 days. The frequency of stirring was based on the sensory organoleptic characteristics of the wine (approximately weekly). Samples were taken from all wines and analyzed for the whole 300-day period.

Samples

Total number of variants: 2

Number of repetitions of each variant: 3

Number of repetitions: 20

For the first 63 days, samples were taken every 7 days.

After that, from day 64 to day 150, samples were taken every 14 days.

From day 150 to day 300, samples were taken every 30 days.

Biological Materials

Vitis vinifera L., variety Chardonnay, Veltiner green, Riesling.

Instruments

Gas chromatograph: Shimadzu (GC-17 A) equipped with an autosampler (AOC-5000) and connected to a QP detector (QP-5050 A) with GCsolution software (LabSolutions, version 1.20, Kyoto, Japan).

Spectrophotometer: (SPECORD 210, Carl-Zeiss, Jena, Germany).

Automatic spectrometric analyser: Miura one® (I.S.E. S.r.l. Via Luigi Einaudi, Italy).

Chemicals

Folin-Ciocalteu phenolic reagent (Sigma - Aldrich, St. Louis, Missouri, USA), sodium carbonate pa (99%) (Sigma - Aldrich, St. Louis, Missouri, USA), gallic acid (3, 4, 5-trihydroxybenzoic acid monohydrate, 99%; Alfa Aesar Thermo Fisher (Sigma Aldrich, St. luis, Missouri, USA)) were used to determine the total polyphenols. All reagents were dissolved in distilled water. The crucial reagent used in the measurement of total antioxidant activity was the Fe3+-2,4,6-tri(2-pyridyl-1,3,5-triazine) complex (Fe3+-TPTZ) (Sigma Aldrich, St. luis, Missouri, USA).

Laboratory Methods

Determination of the total quantity of polyphenolic compounds: The Folin-Ciocalteu method was used to determine the total quantity of polyphenolic compounds. All samples were analysed in triplicate; the final value was taken as the average of these measurements. A 40 µl sample was pipetted into a cuvette (3 ml) and diluted with 1960 µl of distilled water. Subsequently, 50 µl of Folin-Ciocalteu reagent was added to the cuvette. The mixture was thoroughly shaken. After three minutes, 300 µl of 20% sodium carbonate decahydrate solution (Na_2CO_3) was added. The reaction mixture was again shaken and then incubated at 22 °C for 120 minutes. Absorbance was measured using a dual beam spectrophotometer (SPECORD 210, Carl-Zeiss, Jena, Germany) at A = 750 nm. against a blank. The results were expressed as gallic acid equivalence.

Determination of the antioxidant activity by the FRAP method: Three solutions were used to determine the antioxidant activity by the FRAP (ferric reduction antioxidant power) method – (1) TPTZ solution: 10 mM TPTZ (m = 78.02 mg) dissolved in 25 ml of 40 mM HCl; (2) FeCl3 solution: 20 mM $FeCl_3$ (m = 135.13 mg) dissolved in 25 ml of distilled water; and (3) Acetate buffer solution: 0.02 M acetate buffer pH 3.6 (m = 775 mg sodium acetate dissolved in 250 ml of distilled water, pH 3.6 adjusted with acetic acid). The three solutions were mixed in a 1:1:10 ratio (TPTZ:$FeCl_3$:acetate buffer). 150 µl of the reagent was pipetted into plastic cuvettes, and 3 ul of the sample was added. The absorbance was measured for 12 min at A = 605 nm. The antioxidant activity was calculated from the calibration curve using gallic acid as a standard (10-200 $mg.l^{-1}$). The results were expressed as gallic acid equivalence.

Analysis of total amino acid content: Primary amino groups were derivatized using o-phtaldialdehyde and N-acetyl-L-cysteine (OPA/NAC) form isoindoles on a base medium. These derivatives underwent spectrophotometric detection at 340 nm. The absorbance is proportional to the total amino acid content in the sample. The analysis was performed using a Miura one® instrument (I.S.E. S.r.l. Via Luigi Einaudi, Italy), a spectrophotometer equipped with an autosampler. The determination was performed in triplicate on 2 ml collected samples and either immediately frozen or immediately analysed.

Number of samples analyzed: 120.

Number of repeated analyses: 120.

Number of experiment replication: 0.

Statistical Analysis

The results were processed in Statistica 10 (Czech Republic, Statsoft). The values obtained were plotted on graphs. The values used were the mean of the measurements of the three variants, and the standard deviation was calculated using a multivariate ANOVA analysis. Tukey's test and multivariate ANOVA were implemented.

RESULTS AND DISCUSSION

The tables and graphs below show the total amino acid content, antioxidant activity values, and phenolic compound content for the 300 days of the experiment for all three grape varieties that were divided into two types of containers:

(A) decalcified must was put into stainless steel containers, and immediately after fermentation, the leaves were withdrawn from the wine (control).

(B) the most were put into 600-litre wooden oak barrels, and the wine was stirred with the lees.

Amino acid content values

Amino acids can be released into the extracellular environment prior to autolysis. This is a cellular response to the lack of nutrients in the wine. Peptides with high molecular weight (mainly hydrophobic) are released in the first autolysis processes **[10]**. These peptides are subsequently hydrolysed, resulting in the production of smaller molecules and the release of amino acids. Therefore, the concentration of free amino acids decreases compared to the total amino acids as peptides are released into the wine and are only subsequently cleaved into amino acids **[11]**.

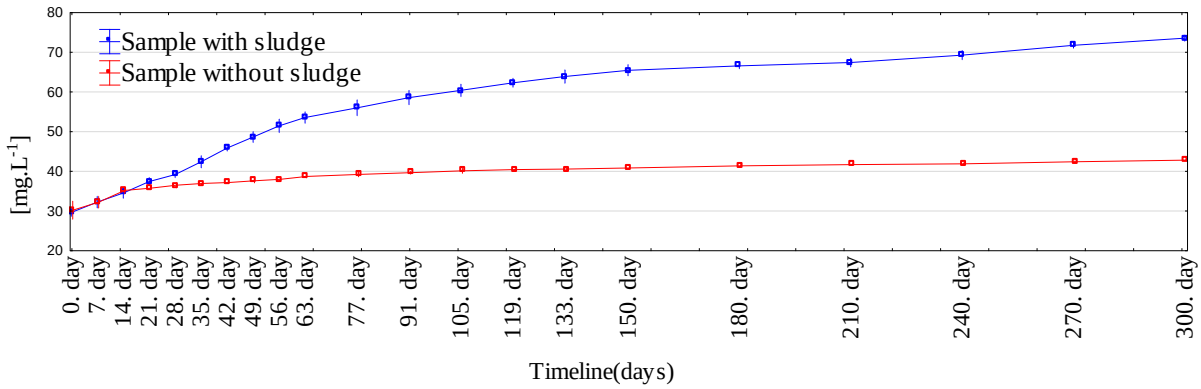

Figure 1 Total amino acid content of Green Veltliner during the maturation of wine on and off lees.

Figure 1 shows a large increase in the amino acid content of the variant with yeast sludge, which increased by 298% (day 0 versus day 300). However, for the variant without sludge, we can see a much smaller increase in value (a 33% increase from day 0 to day 300). The variant with yeast sludge had an amino acid content that was 265% higher on the last day of the experiment in comparison to the variant without sludge. The average total increase in the variant with sludge was 44 mg.l$^{-1,}$ while the variant without sludge only increased by 13 mg.l^{-1}.

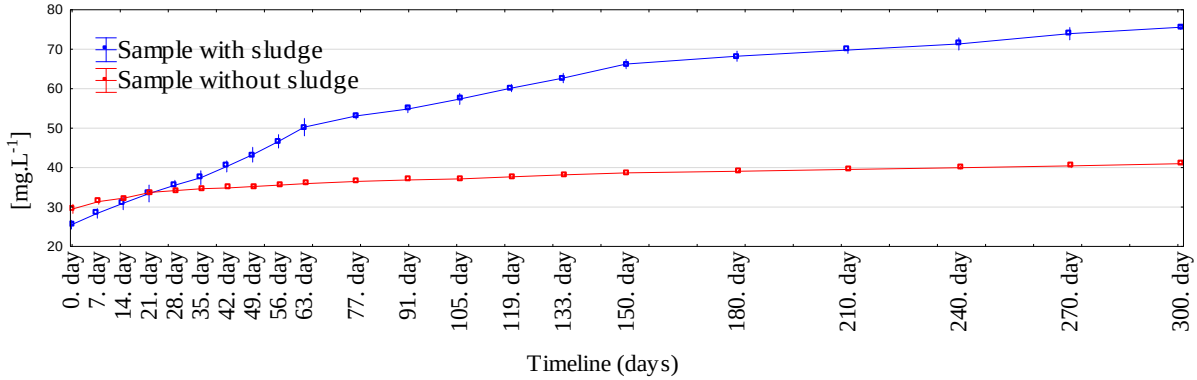

Figure 2 Total amino acid content of Riesling during the maturation of wine on and off lees.

Figure 2 shows a minimal increase of 37% in the amino acid content of the wine without lees (day 0 versus day 30). At the end of the experiment, the difference between the two variants was 164%. The amino acid content of the variant with lees increased by 202% (day 0 versus day 300). At the beginning of the experiment, the amino acid content measured in the variant with sludge was 25 mg.l^{-1}, and the variant without sludge was measured at 29 mg.l^{-1}. The average total increase for the with sludge variant was 50 mg.l^{-1} and for the without sludge variant, 11 mg.l^{-1}.

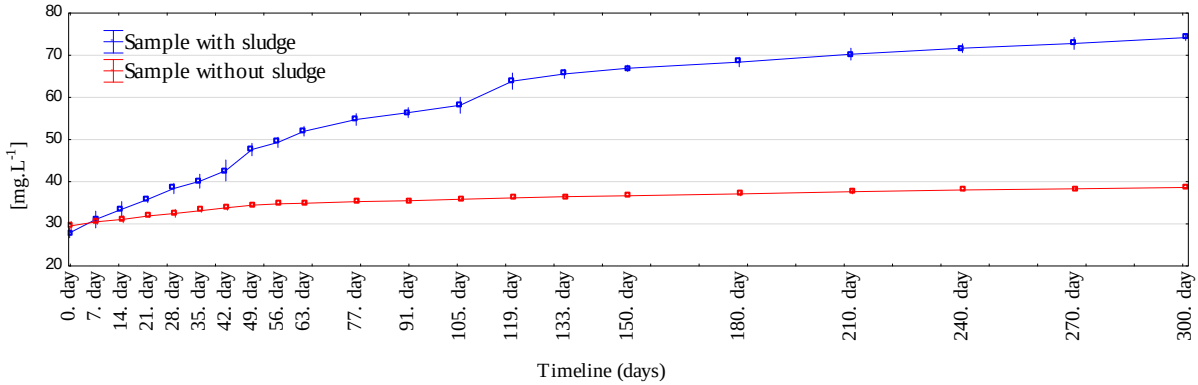

Figure 3 Total amino acid content of Chardonnay during the maturation of wine on and off lees.

It can be seen from Figure 3 that in the vessel without sludge (A), the amino acids increased by 31% (day 0 versus day 300), and in the vessel with sludge (B), the total amino acids increased by 254 % (day 0 versus day 300). The difference was 223% at the end of the experiment. At the beginning of the experiment (day 0), the measured value of amino acids was 28 mg.l^{-1} in the with sludge variant and 29 mg.l^{-1} in the without sludge variant. The average total increase was 46 mg.l^{-1} for the with sludge variant and 9 mg.l^{-1} for the without sludge variant.

The release of nitrogen in the form of amino acids is due to two factors: firstly, passive exorption of the internal contents of the yeast, and secondly, the process of proteolysis itself. Endogenous autolysis of wine yeasts during maturation on lees primarily involves the excretion of nitrogenous compounds, e.g., as the amino acid content gradually increases during lees aging, Sur-lie wines (analyzed at different stages of aging) can consider to be a suitable matrix for testing the impact of yeast autolysis **[12]**. Based on the ongoing autolysis that naturally occurs in wine with lees, it is a good matrix that may be useful for detecting emerging correlations due to changes in amino acid concentrations or other substances that may interfere **[13]**, **[14]**.

Determination of the total quantity of polyphenolic compounds

Polyphenols are the most important and very interesting compounds in the oenological aspects of wine. These compounds come from different parts of the grapevine and are extracted and moved into the wine during grape

processing and aging. Polyphenols and their compounds are directly linked to the wine's final quality. They contribute to the organoleptic characteristics of the wine and influence the color of the wine. Polyphenols are important antioxidants in wine **[15]**.

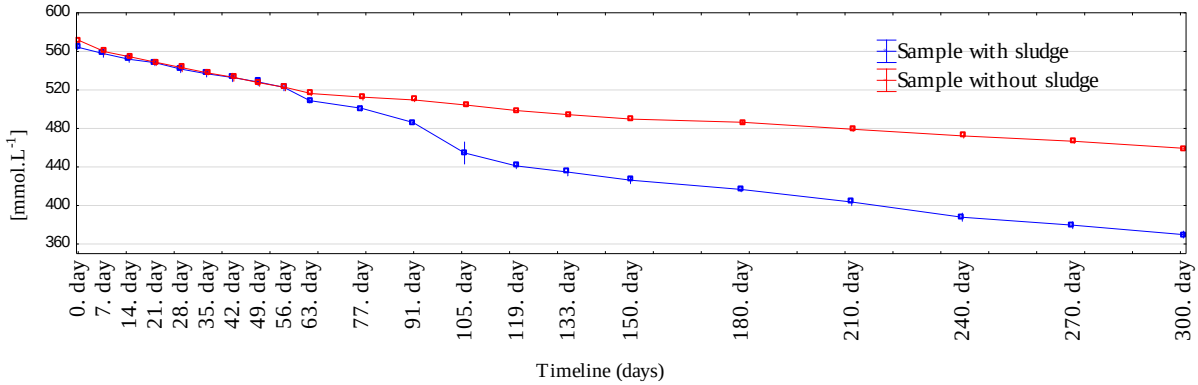

Figure 4 The measured values of total polyphenolic components of the Veltliner green variety, of both variants, with or without lees.

From Figure 4, at the end of the experiment (300 days), we can see an average value of 370 mmol.l^{-1} was measured for the sludge variant, whereas the value at the beginning was measured at 535 mmol.l^{-1}. In the without sludge variant, there was a more moderate decrease from a measured value of 550 mmol.l^{-1} at the beginning to a measured value of 459 mmol.l^{-1} at the end of the experiment.

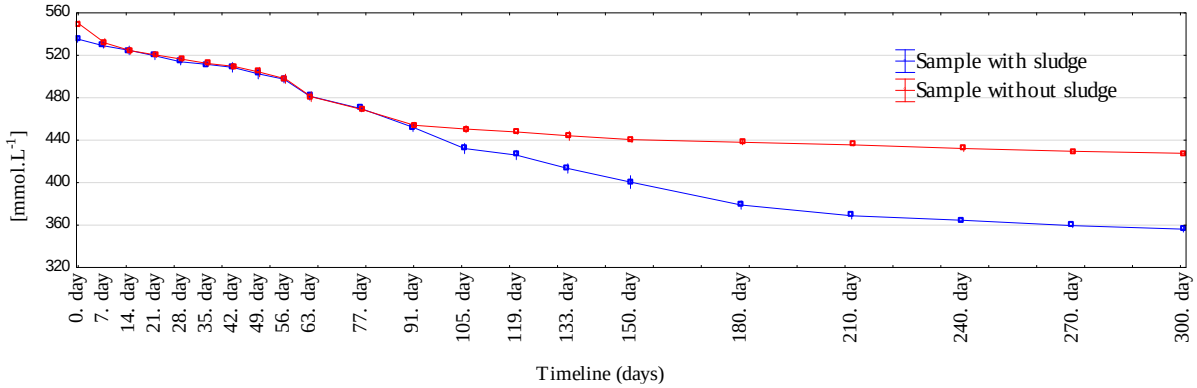

Figure 5 The measured values of total polyphenolic components in the Riesling variety of both variants, with or without lees**.**

Figure 5 shows that at the end of the experiment, the variant without sludge had an average measured value of 427 mmol.l^{-1} (556 mmol.l^{-1} at the beginning) and the variant with sludge had an average measured value of 587 mmol.l^{-1} ^{at} the beginning and 356 mmol.l^{-1} at the end of the experiment. Figure 5 shows a more moderate decrease observed in the variant without sludge (a 22% decrease in value at the end of the experiment compared to the first day). There was a more significant decrease of 33% in the sludge variant.

Figure 6 shows that at the beginning of the experiment (day 0), a high value of 587 mmol.l^{-1} was measured in the with sludge variant and 556 mmol.l^{-1} in the without sludge variant. The decrease in the total phenolic content of the with-sludge variant (B) was 34%; in the without sludge variant, there was a more moderate decrease of 22%. The average value, measured at the end of the experiment, in the with sludge variant, was 357 mmol.l^{-1}, and in the without sludge variant was 434 mmol.l^{-1}.

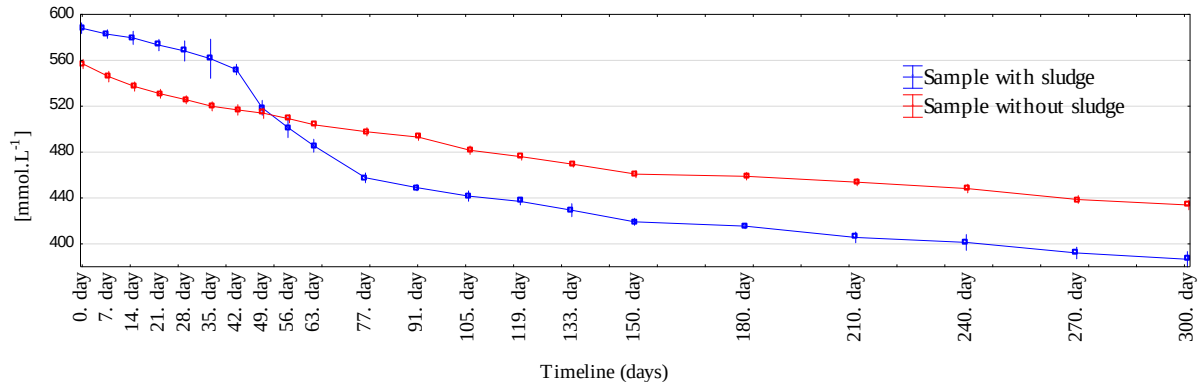

Figure 6 The measured values of total polyphenolic components in the Chardonnay variety of both variants, with or without lees.

The presence of lees protects the wine from oxidation, gives the wine its astringency, and increases the feeling of fullness in the mouth. This can be explained by the reaction of proanthocyanidins with compounds released by yeast autolysis, such as proteins and mannoproteins **[16]**, **[17]**. Figures 4-6 show the evolution of the total polyphenols during the experimental aging for variants with and without sludge over 300 days. The decrease in phenolics as the wine ages with lees could be due to the enzymatic activities of yeast and lactic acid bacteria from the lees **[18]**. Lees play an essential role in the aging of wine, mainly due to their ability to adsorb phenolic compounds **[19]**, **[20]** and release enzymes (after autolysis) that can modify the phenolic fraction **[14]**. Although wine polyphenols interact with yeast lees to a limited extent, such interactions have a significant impact on the reactivity of wine polyphenolic compounds and yeast lees with oxygen **[19]**.

Determination of antioxidant activity

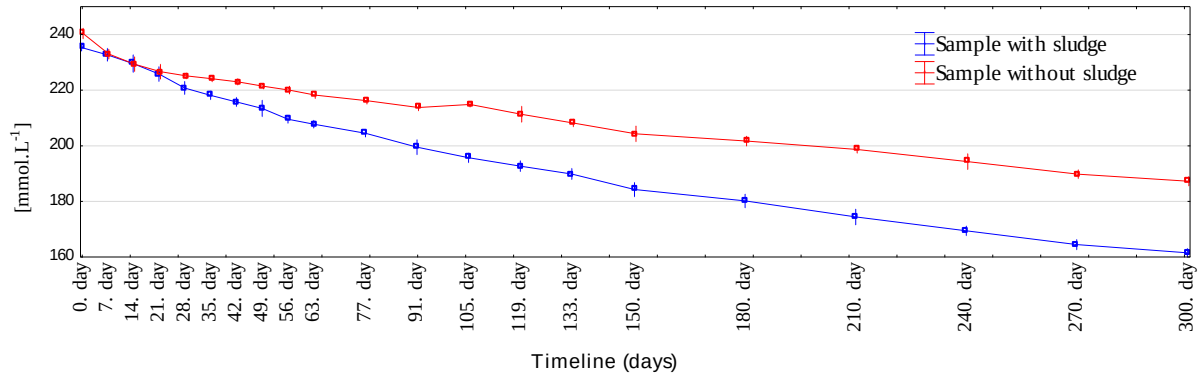

Figure 7 Development of the antioxidant activity in the Veltliner Green variety.

From Figure 7, we can see that the with sludge variant has a more significant decrease in antioxidant activity, of 32%, compared to the without sludge variant, which had a less marked reduction of only 20%. On experimental day 28, it can be seen that there was a very significant difference between the two variants of 5 mmol.l^{-1}, which steadily increased. By the end of the experiment, the value measured in the with sludge variant was 162 mmol.l^{-1} and the without sludge variant 187 mmol.l^{-1}.

Figure 8 shows that at the beginning of the experiment, the average value of antioxidant activity in the with-sludge variant (day 0) was 246 mmol.l^{-1}, and in the without sludge variant, it was 249 mmol.l^{-1}. At the end of the experiment (day 300), the average value in the with lees variant was 169 mmol.l^{-1} and in the without lees variant 198 mmol.l^{-1}.

From Figure 8, we can see that the without sludge variant exhibited a decrease in the antioxidant activity of 16%, but the variant with sludge exhibited a more significant drop of 32%. The average reduction in the value for the variant with sludge was 76 mmol.l^{-1}, and for the variant without sludge, 40 mmol.l^{-1}.

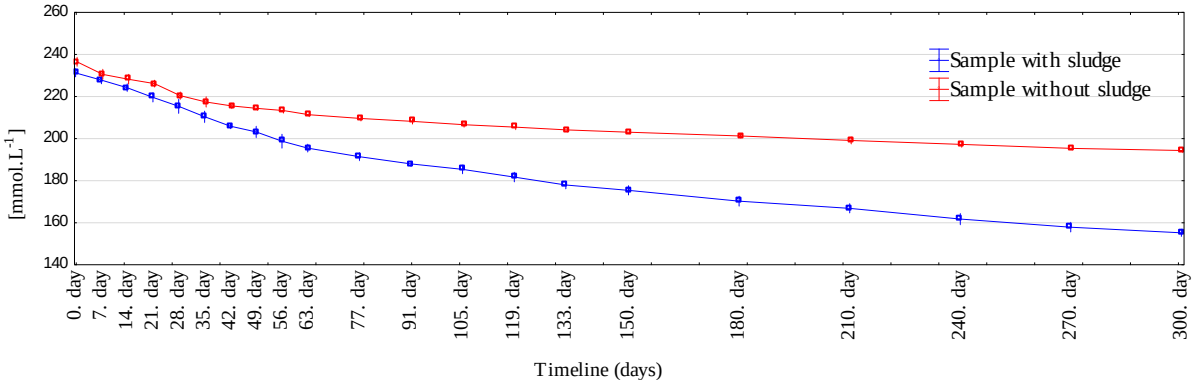

Figure 8 Development of antioxidant activity in the Riesling variety.

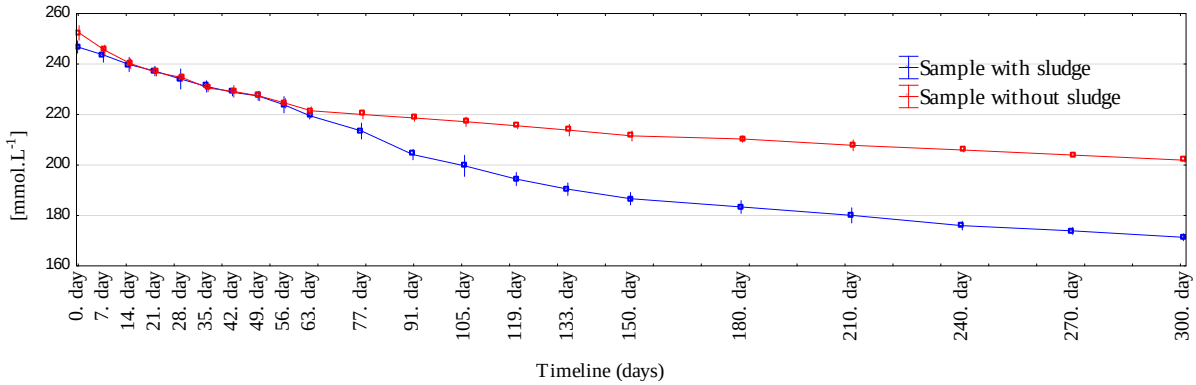

Figure 9 Development of antioxidant activity in the Chardonnay variety.

Figure 9 shows that at the beginning of the experiment, the average value of antioxidant activity in the with lees variant (day 0) was 235 mmol.l^{-1}; in the without lees variant, a value of 240 mmol.l^{-1} was measured. At the end of the experiment (day 300), the average value measured in the with lees variant was 161 mmol.l^{-1} and in the without lees variant 187 mmol.l^{-1}. Figure 9 shows an overall decrease in antioxidant activity in the without sludge variant of 19% and in the with sludge variant of 31%. The average overall reduction in the sludge variant was 70 mmol.l^{-1} and 50 mmol.l^{-1} for the without sludge variant.

Grapes are fruits with a high level of antioxidant activity **[21]**. However, after fermentation, phenolic compounds were lost, which are primarily responsible for antioxidant activity **[22]**, **[23]**. It can be seen from Figures 7-9 that the with sludge variants had a higher decrease in antioxidant activity than the without sludge variants. This can be explained by the fact that the sludge is mainly bound to polyphenols, which gradually oxidize **[24]**. This correlates with Figures 4-6, where a more significant decrease is observed for the sludge variant **[25]**.

Final evaluation

The tables and graphs below show the results of the measurement of total amino acid content, antioxidant activity values, and phenolic compounds at the beginning (immediately after fermentation – day zero) and after 300 days (the end of the experiment) for the three grape varieties divided into two types of containers.

Sensory evaluation of the strength and structure of the wines

The sensory analysis compared the different wine varieties after 300 days. A 10-point scale was selected for this evaluation to evaluate Aroma richness and intensity, flavor richness and intensity, aging potential, complexity, and balance.

Ageing the wine with lees and the associated autolysis of the yeast also impacted the wine's sensory characteristics.

Figure 12 shows, for the Chardonnay variety, that there was a statistically significant difference in all the evaluations between the two variants, but the most significant was in the Potential for maturation evaluation, which averaged 3.2 points. The smallest difference was for the Richness aroma and intensity evaluation, which was 1.2.

For Riesling, there was a statistically significant difference in the scores of all the evaluations of the two variants. The smallest difference was recorded for Richness aroma and intensity, only 0.6 points. However, the most statistically significant difference was recorded for the potential for maturation, which was 4.8 points.

The smallest difference between the two varieties was recorded for the Richness aroma and intensity evaluation, which was 0.4 points. The highest difference was recorded for the complexity descriptor in the variant with a level of 8.4.

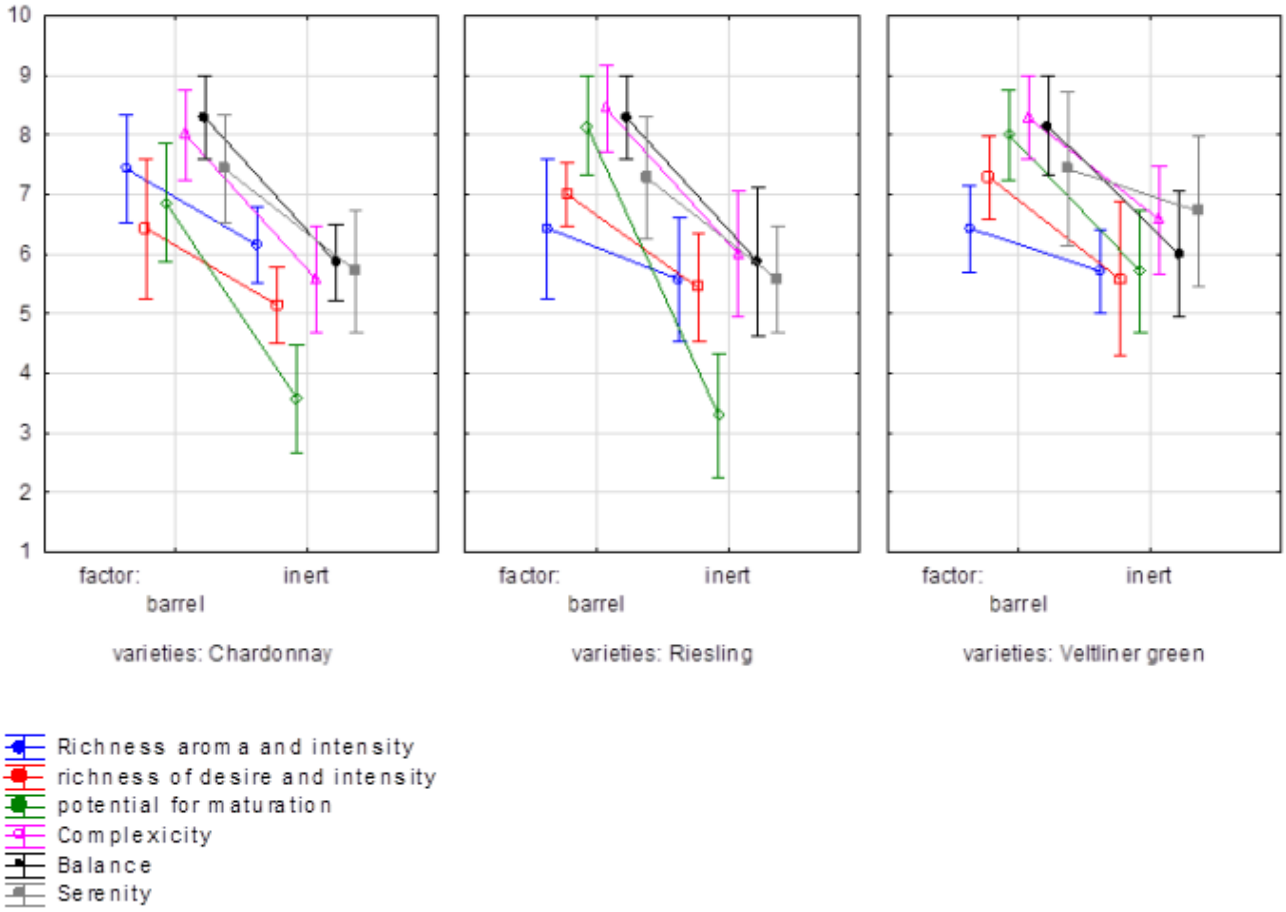

Figure 10 The strength and structure of wines.

All the wines aged on lees were perceived to be more robust, most likely due to interactions between the macromolecules released during cellular autolysis and the wine phenolics **[26]**, **[27]**. Fermentation lees have a high level of reactivity with salivary proteins and induce a feeling of astringency. However, in mannoproteins or polysaccharides, fermentation lees interact with them to form stable macrostructures that cannot react with salivary proteins, thus reducing the astringency **[28]**, **[29]**. Other authors report mannoproteins as active compounds that promote wine stability by reducing the particle size of aggregated tannins **[30]**, **[31]**. Therefore, this technique can be used to smooth these parameters during the aging of red wine. Along with the increased robustness that comes from aging with lees, the overall body and persistence of the wine improved for all varieties. In terms of color, all the wines aged on lees exhibited a greater degree of color intensity than the wines aged in inert containers without lees. This finding agrees with previous results suggesting that mannoproteins and cell wall polysaccharides protect anthocyanin monomers, making aging on lees a novel technique for color preservation **[32]**, **[33]**.

Based on the obtained results, it can be concluded that more biochemical processes took place in wines aged on lees compared to those aged without lees. It is important to note that many factors influence yeast autolysis's effects. The most important ones are temperature, yeast strain and population, ethanol content, vinification time on lees, and the pH of the wine **[34]**. When wines are vinified on lees, there is no need to sulphurise them, as the polyphenols protect them from oxidation through their own antioxidant activity. If wines are aged on lees, it is possible to have a very low sulfur dioxide content in the aged wine **[35]**, **[36]**, **[37]** describes wines, vinified on lees, as sophisticated, full-bodied wines. The sensory analysis of the wine confirmed this. During vinification, oak

lactones, furans, volatile phenols, and others may be released. In my opinion, these can interact with the compounds produced during autolysis. When vinifying on lees, it is also important to be aware of sulfur compounds that can adversely affect the sensory profile of the wine (hydrogen sulfide, mercaptans) **[38]**. In this context, it is important to pay attention to these compounds and take appropriate measures if they are present in the wine **[39]**, **[40]**. One option is to re-homogenize the lees with the wine. These compounds were not detected in the results of the sensory analysis. Otherwise, there are noticeable differences between wine produced with and without yeast lees **[41]**, **[42]**.

CONCLUSION

The experiment aimed to confirm the influence of yeast autolysis on the final composition of the wine. The experiment design was planned for a period when three grape varieties were available for wine production and bottling. Sensory analysis showed that wines that underwent autolysis were more robust, fuller, and harmonious and had a higher potential for archiving. On the other hand, in containers without lees, where autolysis has not taken place, the wines are lighter and fresher, more suitable for early consumption. The increase in amino acids was due to the decomposition of the yeast carcasses. Therefore, their concentration increased in the lees. Amino acids are precursors of aromatic substances, and therefore, wines aged on lees were fuller, more robust, and more harmonious. Polyphenols are antioxidants, and in the lees containers, they oxidized and protected the wine from complete oxidation. In inert containers, sulfur dioxide had to be used to keep the wine from oxidizing. The leaves are very important in the amount of dissolved oxygen in the wine. The experiment also determined the antioxidant activity of the wine. This correlated in all graphs with the polyphenol graphs. This is because polyphenols are the most abundant antioxidants in wine.

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