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# IMPACT OF PECTOLYTIC ENZYMES AND PRE-FERMENT MACERATION TIME ON THE QUALITATIVE PARAMETERS OF MUSTS AND ROSÉ WINES

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**ABSTRACT**

Pre-ferment maceration is a technological process that improves the aromatic and gustatory profile of the wine and increases its archiving potential. The aim of the work was to evaluate the influence of pre-ferment maceration length on sensory and analytical parameters in rosé wines from vintage 2022. In the experiment, 'Cabernet Sauvignon' grapes from the Slovak wine-growing village of Mužla were used. Pre-ferment maceration was not used in the control variant. . In variants A and B, one-hour pre-ferment maceration was used. In variants C and D, two hours of pre-ferment maceration was used. In variants B and D, pectolytic enzymes were added. The use of pectolytic enzymes causes a demonstrable increase in pH in the resulting wine. Variants with longer maceration and the control variant had a demonstrably higher content of total acids than variants with shorter maceration. The highest content of acetic acid (0.25 g/l) was found in the variant with longer maceration and without the use of enzymes. The lowest content of acetic acid (0.20 g/l) was detected in the control variant and variants with a shorter maceration time. Wines with one-hour maceration contained less malic acid compared to variants with longer maceration or variants without maceration. In the content of tartaric acid, the lowest content was observed in variant A (5.20 g/l), while the highest content was in variant C and in the control variant (5.70 g/l). The overall sensory evaluation of the wines was as follows: the best-rated variant was variant B, which reached 86 points from 100-point OIV scale; the worst-rated variant was variant A, with 81.23 points. Based on the results, we recommend an optimal pre-ferment maceration time of 1 hour using pectolytic enzymes.

**Keywords:** rosé wine, pre-ferment maceration, pectolytic enzymes, Cabernet Sauvignon

## INTRODUCTION

Rosé wine production accounted for nearly 10.5% of total world wine production in 2019 (**Masson *et al.*, 2023**). One of the main and most important aspects of modern wine production is especially the use and search for the right technologies to use those properties that the grapes carry with them from the vineyard, which can subsequently improve the quality of the future wine. The quality of grapes is determined by several parameters. These are, for example, pH, organic basic content, and yeast assimilable nitrogen content (YAN) (**Ailer, 2016**). Maceration affects the sensory as well as the analytical protocol of rosé wines. The technique consists of enriching the wine with increased taste, wine complexity, varietal character, and wine stability (**Hernanz *et al.*, 2007**). The length of maceration is defined as the process of contact of the must contained in the grape berries with other parts of the berry after bursting or grinding the grape berries. Maceration as one of the winemaking techniques is commonly used in practice, and its length greatly modifies the aromatic profile of the future wine. Pre-ferment maceration provides positive sensations such as higher antioxidant activity, a longer aftertaste of the wine, phenolic content, a more intense aroma, and a better complexity of the wine. To obtain a pink color, a short prefermentative skin contact period between the crushed red grape skins and the must has become standard procedure in many traditional rose wine-producing areas. A too-short or too-long skin contact time can lead to poor extraction of anthocyanins and aromas or the collateral extraction of undesired molecules linked to astringent and bitter notes (**Baiano *et al.*, 2009**; **Michlovský, 2014**; **Steidl, 2010**). Studies of extraction methods and their conditions play a central role not only in the exhaustive recovery of bioactive compounds from natural matrices but also in the preservation of these compounds in their native form by avoiding alterations (**Naviglio *et al.*, 2018**). Aromaticity is influenced by processing technology. There are mainly the following influences: degree of ripeness, harvesting technique, composition of the microflora of the berries, post-fermentation treatments, and the way the wine is aged. The maceration of the berries and their length are particularly important (**Kozelová *et al.*, 2020**). The processing technology of rosé wine is determined by the health status of the grapes. The formation of volatile acids can already occur on whole bunches imported from the vineyard and can also occur as a yeast or bacterial metabolite during the entire wine production until bottling (**Ferrer *et al.*, 2008**). The effect of pre-fermentation maceration can be increased using pectolytic enzymes. Each group of enzymes acts on different operations. While some hydrolytic enzymes promote sedimentation, others promote pressing or clarification. Such enzymes include, for example, pectinases (polygalacturonase, galactanase), cellulases (endo-(1,4)-beta-D-glucanase), glycosidases (alpha-L-arabinofuranosidase), and the like (**Olejar *et al.*, 2015**; **Balík *et al.*, 2017**). The content of anthocyanins is also determined by their gentle processing. Anthocyanins are contained in the berry skin (**Balík *et al.*, 2017**). The length of the maceration is determined by the health of the raw material, the variety, the area of cultivation, the technology used, and the winemaker's requirements for the character of the resulting wine. The basic quality that defines proper pressing is the minimal content of polyphenols and the low level of must oxidation (**Darias-Martín *et al.*, 2004**). A smaller need for sulfurization occurs in wines produced by carbonic maceration, cryomaceration, surlage, and batonage (**Poláček *et al.*, 2018**). 'Cabernet Sauvignon' is a red wine grape variety (**Pospíšilová *et al.*, 2005**). The Phoenicians brought it to Europe. As late as the eighteenth century, there were eighteen blue and twenty white clones of this variety in Bordeaux. It is not demanding on the soil or location. It needs a lot of sun and is resistant to winter frosts. It ripens late and does not give excessive yields. Musts in climatic conditions of Slovakia reach average sugar content and higher acids (**Malík *et al.*, 2017**; **Hronský, 2014**).

The aim of the work was to assess if the maceration length affected sensory and analytical parameters during the wine-making process, and based on the results, to identify the optimal methods for processing and wine-production.

## MATERIAL AND METHODS

The wine-growing village of Mužla is in the Štúrovo wine-growing district, which is part of the South Slovakian wine-growing region. The cultivation pattern in the vineyard is the Rhine-Hessian line. The grapevines are planted on rootstock SO-4. The year of planting of the vineyard is 2008. The geographical coordinates of the vineyard are: 47.823263 °N, 18.618995 °E. Black earth is the dominant soil type at the site.

The South Slovak wine-growing region is the warmest wine-growing region in Slovakia. The average air temperature during vegetation is 16.9 °C. The annual amount of precipitation is 325 millimetres. Annual sunshine is 1550 hours. The majority of the time, medium-heavy soils without skeletons and light sandy soils

are used to cultivate vines. In the area, viticultural primary production prevails over wine production and grape processing (**SHMÚ, 2023**; **Novotný, 2007**).
Depending on the specified variants, 'Cabernet Sauvignon' grapes for rose-wine production were used. A control sample and four different variants were created, which were then evaluated sensorially and analytically.

**Variants**

Control variant – without pre-ferment maceration, without enzymes.
Variant (A) – without pectolytic enzymes, pre-ferment maceration for one hour.
Variant (B) – with pectolytic enzymes, pre-ferment maceration for one hour.
Variant (C) – without pectolytic enzymes, pre-ferment maceration for two hours.
Variant (D) – with pectolytic enzymes, pre-ferment maceration for two hours.

**Chemicals**

Sulphur dioxide in liquid form (15%; Martin Vialatte, France) was used in the wine production process as a protection against wine oxidation.

**Materials**

250 kg of 'Cabernet Sauvignon' (*Vitis vinifera* L.) grapes.

**Laboratory Methods**

ALPHA Wine Analyzer
It is a Fourier-transform spectroscopy (FT-IR) spectrometer system equipped with a robust ATR (Attenuated Total Reflection) unit for quick analysis of wine, must, or juice. Calibration of finished samples enables parallel determination of various analytical parameters. It guarantees reliable analysis results, extensive calibration, and the possibility of setting up a new calibration database.
On this device with a module for musts and juices, the following contents were analysed: glucose, fructose, total sugars, total acids, malic acid, and pH. The following wine parameters were examined: total acids, total sugars, total extract, pH, glycerol, lactic acid, malic acid, acetic acids, and alcohol concentration.

**Turbidity**

For turbidity measuring a HI-83749-02 portable turbidity meter and bentonite monitoring was used (Hanna Instruments, Bedfordshire, UK).

**Sample preparation**

The grapes were harwested on October 10, 2022, in 20-kg plastic containers in the early morning. The total weight of the grapes was 250 kg. Approximately 50 kg of mash for each variant was taken, except for the control variant, for which 40 kg was used. The total time from picking to processing the grapes was 2 hours.
After pressing, the must was transferred to five stainless steel tanks with a 100-liter capacity, where it was decalcified by static sedimentation, and it was added preparations for desilting (Seporit, Klar Sol Super, and Collagel (Erbslöh, Germany)). After must desilting the volume of the sludge was measured and the juice was transferred to the cooling box, where fermentation of all variants was carried out at a temperature of 18 °C. It was used a noble yeast strain of the genus *Saccharomyces cerevisiae* (Passion Fruit, LaFood, Italy). This yeast is characterized by a high production of esters and can ferment even at low temperatures of around 10°C. The dose was the equivalent of 20 g/hl. For yeast nutrition, it was applied Zimovit (Everintec, Italy) at a dose of 100 g/hl and in later fermentation stage Nutrozim (Everintec, Italy) at a dose of 20 g/hl was applied.
The desilting, short aging, and bottling of the wine took place in the standard way. After filtration, wines of the experimental variants and the control sample were filled into transparent glass bottles of the Bordeaux type with a volume of 0.75 l and closed with a cork. After bottling, wine was left in bottles for a week in cold rooms with a temperature of 10°C. After a week a sensory and analytical evaluation of the variants was performed. The basic physico-chemical parameters of wines using an Alpha Wine Analyser (Bruker Optik, Darmstadt, Germany) spectrophotometer were also evaluated.

**Sensoric evaluation**

It was performed the sensory evaluation using a 100-point OIV evaluation system. Wines were evaluated by 10 tasters, while each taster was evaluated separately. The tasters were not aware of the length of maceration or the technological process of production. The variants were evaluated using OIV glasses, which were washed and freed of unwanted odors before evaluation. The evaluators were familiar with the grape variety and the vintage. The variants were evaluated in the following order: control sample, variant A, variant B, variant C, and variant D. After performing the sensory evaluation, the arithmetic mean of each variant was created.

**Statistical Analysis**

For statistical evaluation of basic physicochemical parameters of musts and wines, the method of analysis of variance in STATGRAPHICS Centurion (Statgraphics Technologies, Virginia, USA) was used. To test the statistical significance of the results, the LSD test (least significant difference test, $P \leq 0.05$) was used. The data distribution using the Shapiro-Wilk test at the 95% level of statistical significance was performed. It was assessed the statistical significance of the difference between samples using the ANOVA-Tukey test.

**RESULTS**

**Volume of sludge in must**

In Figure 1, the volume of sludge in the examined variants is shoved. Differences in sludge volume between the control variant and variants B (14.47 % vol.), C (11.35 % vol.), and D (11.09 % vol.) was found. The evaluation also shows that the musts with a 2-hour pre-ferment maceration produced demonstrably less sludge in comparison with other variants. It is due to shorter contact with the skin of the grape-berry. Similar volumes of sludge in variant A and in the control, variant was found. It means that when the mash was left for one hour without the addition of enzymes, the same amount of sludge was formed as without the pre-ferment maceration.

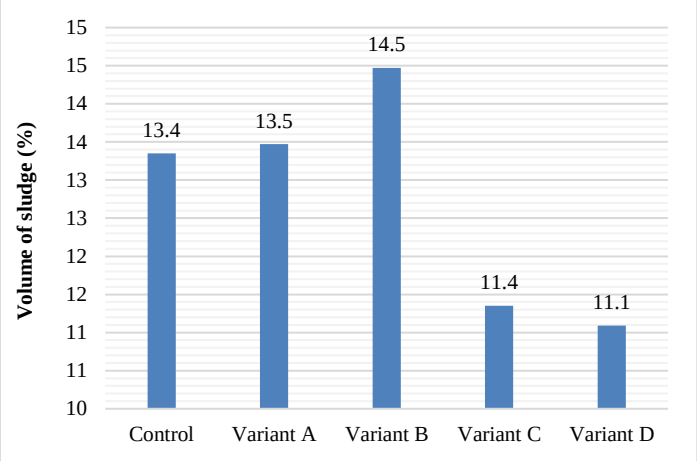


**Figure 1** Volume of sludge in samples of must.
Note: Variant A – maceration 1 h without pectolytic enzymes, Variant B – maceration 1 h with pectolytic enzymes, Variant C – maceration 2 h without pectolytic enzymes, Variant D – 2 h with pectolytic enzymes.

**Must turbidity**

After the musts were desilted, an analysis of the must turbidity was carried out. The turbidity values are given in non-felometric turbidity units (NTU). The lowest turbidity was found in variant A, which has a value of 64.23 NTU. The highest turbidity value was in variant D, with a value of 375.33 NTU. After centrifugation for 12 seconds at 6000rpm, the turbidity was measured again. The lowest turbidity was in variant A, the highest turbidity was in the control variant.
It was found that all variants were statistically significantly different from each other. In the case of the uncentrifuged must, lowest turbidity value was in variant A and the highest in variant D. In the case of centrifuged must, the lowest turbidity value was in variant A, while the highest turbidity was in the control variant. The measured turbidity values are shown in Table 1. After centrifugation, we found statistically significant differences in all variants compared to the musts before centrifugation.

**Table 1** Must turbidity before and after centrifugation.

| | Turbidity in must (NTU) | | | | |
|---|---|---|---|---|---|
| | **Control variant** | **Variant A** | **Variant B** | **Variant C** | **Variant D** |
| **B** | 85.73±2.9 | 64.23±0.0 | 152.33±0.5 | 121.67±6.3 | 375.33±10.1 |
| **C** | 4b | 6a | 8d | 5c | 2e |
| **A** | 27.1±1.91 | 8.05±0.05 | 16.33±0.06 | 10.37±0.29 | 22.83±0.15d |
| **C** | e | a | c | b | |

Note: BC – before centrifugation, AC – after centrifugation; a, b, c, d, e means that lines with a different letter are statistically different (LSD test at 95 % significance level).

**Analytical evaluation of must**

In Table 2 the results of the analytical evaluation of the musts are presented. The highest fructose content was in variant A and the lowest in variant C. The highest glucose content was found in variant A and the lowest in variant C. The total solid content was highest in variant A and lowest in variant C. The highest malic acid content was in the control variant and the lowest in variant C. The highest pH was measured in variant A and the lowest in the control variant. Total acid content was highest in the control variant and lowest in variant B. The highest total sugar content was in variant A and the lowest in variant C.

The fructose content, total soluble solids content and malic acid content was demonstrably lower with longer maceration time of crushed grapes compared to shorter maceration time. The glucose content significantly differs between variants C and A. The pH value was demonstrably lowest when the grapes were not macerated but pressed immediately. The total acid content of the must is demonstrably lower with any maceration time compared with immediately pressing. The total sugar content was demonstrably lowest in the case of longer maceration compared with the variants with shorter or no maceration.

**Table 2** Physico-chemical parameters of musts.

| Parameters | Variants | | | | |
|---|---|---|---|---|---|
| | Control variant | Variant A | Variant B | Variant C | Variant D |
| **FR** | 104.82±0.46b | 108.66±3.15c | 106.86±0.041bc | 101.71±0.17a | 101.87±0.48a |
| **GL** | 94.64±0.06bc | 96.13±5.32c | 92.52±0.09abc | 89.97±0.39a | 90.35±0.36ab |
| **TSS** | 20.28±0.01b | 20.35±1.08b | 19.61±0.07ab | 19.10±0.06a | 19.16±0.07a |
| **MA** | 4.21±0.01d | 3.60±0.09bc | 3.71±0.05c | 3.30±0.08a | 3.54±0.10b |
| **pH** | 3.43±0.01a | 3.48±0.03b | 3.47±0.01b | 3.45±0.01b | 3.46±0.01b |
| **TA** | 6.39±0.021b | 5.09±0.30a | 5.03±0.12a | 5.09±0.09a | 5.13±0.06a |
| **TS** | 197.87±0.18ab | 201.55±8.83b | 195.11±0.70ab | 191.10±0.32a | 191.92±0.76a |

Note: FR – fructose (g/l), GL – glucose (g/l), TSS – total soluble solids (°Bx), MA – malic acid (g/l), TA – total acid (g/l), TS – total sugar (g/l). a, b, c, d means that lines with a different letter are statistically different (LSD test at 95 % significance level).

**Analytical evaluation of wines**

The highest content of acetic acid was shown in the variant with longer maceration time without enzymes; the lowest content of acetic acid was shown in the control variant and in the variants with shorter maceration time (0.20 g/l). The alcohol content was demonstrably lowest in the control variant (11.00 %), followed by variants C (12.10 %) and D (11.90 %). The highest alcohol content was in the variants with shorter maceration times. It can therefore be concluded that pre-ferment maceration for one hour demonstrably increased the alcohol content of the resulting wine compared with wines with no maceration or with maceration for two hours. The highest glycerol content was in the wine, where the mash was macerated for the longest time with the addition of enzymes. The variants with 1 hour maceration demonstrably contained less malic acid compared to the variants with longer maceration time or maceration-free variant.

Regardless of the period of maceration, enzyme-using variations had a pH that was noticeably higher than the other variants. We found that the use of pectolytic enzymes in the process of maceration causes a demonstrable increase in pH in the resulting wine. The lowest tartaric acid content was in variant A (5.20 g/l), while the highest content was in variant C (5.40 g/l) and in the control variant (5.70 g/l). In the case of the total sugar content, all variants differed from each other in a demonstrable way. The highest content of total sugars was in the variants with longer maceration time. The variants with longer maceration time and the control variant had demonstrably higher total acid content than the variants with shorter maceration. Content of total acids is demonstrably lower in the variants with 1 hour maceration.

**Table 3** Physico-chemical parameters of wines

| Parameters | Variants | | | | |
|---|---|---|---|---|---|
| | Control variant | Variant A | Variant B | Variant C | Variant D |
| **AL** | 11.00±0.10a | 12.50±0.20c | 12.50±0.00c | 12.10±0.20b | 11.90±0.20b |
| **AA** | 0.20±0.02a | 0.20±0.02a | 0.20±0.03a | 0.25±0.03b | 0.24±0.03ab |
| **GY** | 6.70±0.10a | 6.60±0.10a | 7.20±0.10b | 7.10±0.10b | 7.50±0.10c |
| **pH** | 3.28±0.02a | 3.31±0.02ab | 3.36±0.03c | 3.35±0.02bc | 3.36±0.03c |
| **TTA** | 2.30±0.10c | 1.94±0.10a | 2.19±0.10bc | 2.24±0.10c | 2.02±0.10ab |
| **TS** | 1.60±0.05b | 1.80±0.05c | 1.30±0.05a | 2.90±0.05e | 2.10±0.05d |
| **TA** | 5.70±0.10c | 5.20±0.10a | 5.20±0.10a | 5.40±0.10b | 5.80±0.10c |
| **TE** | 19.00±0.30a | 19.10±0.40ab | 19.60±0.30b | 20.70±0.40c | 21.20±0.30c |
| **MA** | 2.30±0.10c | 2.00±0.10b | 1.80±0.10a | 2.20±0.10c | 2.20±0.10c |

Note: AL – alcohol (%), AA – acetic acid (g/l), GY – glycerol (g/l), TTA – tartaric acid (g/l), TA – total acids (g/l), TS – total sugars (g/l), TE – total extract (g/l), MA – malic acid (g/l). a, b, c, d, e means that lines with a different letter are statistically different (LSD test at 95 % significance level).

In Figure 2, the evaluation of the sensory analysis across all samples is shown. The highest score was in variant B, followed by variant D and variant C (different homogeneous groups). Variant A scored the lowest poin-score. We can also note that the variants with higher scores in sensoric evaluation were treated with an enzymatic preparation.

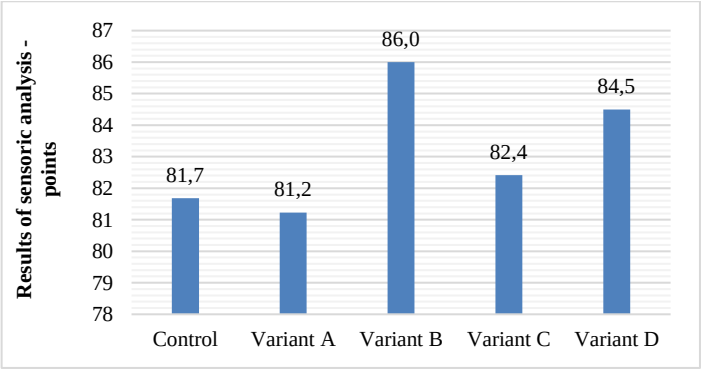


**Figure 2** Sensoric evaluation of the samples using 100-point system.
Note: Variant A – maceration 1 h without pectolytic enzymes, Variant B – maceration 1 h with pectolytic enzymes, Variant C – maceration 2 h without pectolytic enzymes, Variant D – 2 h with pectolytic enzymes.

**DISCUSSION**

**De Santis *et al.* (2010)** said that pre-fermentation maceration positively affects the content of phenolic substances. This technique helps to create an aroma of wine with color stability. Maceration also affects the degree of antioxidant capacity of the wine. However, they add that cold maceration below 6 °C, which is energy-intensive, can be considered the most effective. We can partially confirm this statement because, even though we did not perform cold maceration, the variants that macerated in the presence of pectolytic enzymes were evaluated better. Maceration formed the basis of the aromatic expression, and, in our opinion, enzymes increased the extraction of phenolic substances, which was reflected in the evaluation of variants B and D. According to **Pavloušek *et. al.* (2018)**, most phenolic compounds are found in the skin of the berry. These are mainly stilbenes, hydroxybenzoic acids, flavonols, and others. This statement confirms the justification for the use of pectolytic enzymes, with the aim of disrupting the cell walls and leaching phenolic substances into the must. Confirmation of this statement is the sensory evaluation of the aroma in variants B and D. **Styger *et al.* (2011)** define wine aroma as an interaction of chemical compounds, the concentration of which is defined by the variety and the precursors that are released during fermentation, considering mainly the variety and fermentation as the basis. We can agree with this statement. It is clear from the results that maceration can be understood as a certain superstructure in the production of quality wine, while variety and a mastered fermentation process are the basis for producing flawless wine. The result of this statement is the sensory analysis, which points to higher-point evaluations of the wines produced with the used maceration compared to the

control variant. The glycerol content can be influenced by the length of maceration. We can confirm this statement because by the variants with 2 hours maceration significantly higher glycerol content was measured in comparisom to variants without or with one hour maceration time (table 3). **Parley *et al.* (2001)** claim that enzyme pre-fermentation activity does not increase the extraction of anthocyanins but increases the intensity of the colour, which corresponds to a higher density and the formation of a polymeric extract. They also claim that the content of free $SO_2$ affects the resulting colour of the wine. We cannot confirm this because, from the attached photos, only a minimal colour variation between the wines is visible, except for the control variant, which confirms the effect of maceration on the colour of the wine of variant I, which was produced without the use of pectolytic enzymes and achieved the richest colour.

## CONCLUSION

Based on the results, we conclude that the variants that were not treated with enzymes during maceration showed higher turbidity than the variants treated with enzymes. Maceration contributed to higher clarity of the resulting wine. We can express the opinion that longer maceration contributes to a higher extract in the finished wine. It is also clear from the analytical evaluation that the length of maceration affects the glycerol content in the variants. Lower glycerol content was consistently measured in variants with shorter macerations, length of maceration increases the glycerol content of the wine, respectively. The addition of enzyme preparations during maceration is substantiated and pre-ferment maceration is more effective. The purity of the aroma was mostly evaluated with five or six points for variants with pectolytic enzyme addition, which represents the excellent or very good category. For variants without enzyme addition, the purity of the aroma was mostly evaluated with three or four points, which represent the category of good or sufficient. We can conclude that for the given grapes obtained from the Mužla locality, it is better to use a shorter maceration time of two hour for the resulting quality of the wine. On the other side, maceration with pectolytic enzymes for one hour leads a decrease in sensory parameters of wine compared to other variants.

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