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IMPACT OF FERMENTATION TEMPERATURE AND DURATION ON ANTHOCYANIN CONCENTRATION AND ON QUALITY OF CABERNET SAUVIGNON WINE

Géza BALLA, Tekla Amália LUNKA*, József JOÓ, Csaba MOLDOVÁN, Zsolt SZEKELY-VARGA, Artúr Botond CSORBA, Endre KENTELKY*

Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Department of Horticulture, Calea Sighișoarei 2, 540485 Târgu Mureș, Romania

Corresponding authors email: tekla.lunka@gmail.com and kentelky@ms.sapientia.ro

Abstract

Romania has a good geographical location and favourable climate and soil properties for grape growing. The main reason for this is the high anthocyanin content in red wine, which acts as an antioxidant. The aim of the experiment was to examine the effect of changes in fermentation temperature and duration of fermentation on the anthocyanin content and quality of the resulting varietal wines. Also, to improve the quality during the fermentation process of one of the main red wine varieties of the Miniș wine region, Cabernet Sauvignon. The laboratory tests showed that none of the parameters were affected by the fermentation temperature or the duration of the fermentation. In terms of anthocyanin content better results were obtained with longer fermentation period and higher fermentation temperature. Cabernet Sauvignon had an anthocyanin content of 485 mg/L after 15 days of fermentation at 30°C. During the sensory evaluation it can be stated that wines fermented for a longer period of time and at higher temperature proved to get higher score.

***Key words**: Cabernet Sauvignon, fermentation, Miniș, wine.*

INTRODUCTION

Winegrowing and winemaking have a very long history in certain areas of the world where the climatic conditions are favourable. The wine was the jewel for Egyptian, Greek and even Roman cuisine. For a very long time, wine was even more popular for noblemen than water because it was cleaner than the water that was accessible (Kádár, 1973).

Red wine is not just an article of pleasure, it also has a medicinal effect. At the end of the 20th century, the millennia-old fact that wine has an antibacterial effect was scientifically proven. Pasteur's opinion was that wine is the most hygienic and healthiest drink (Stájer, 2004; Lugasi, 2007; Yoo et al., 2010).

According to today's understanding, the moderate and regulated consumption of wine has a positive effect on the cardiovascular, digestive, central, and peripheral nervous systems (Antoce & Stockley, 2019; Teissedre et al., 2018). It has been reported that wine consumption has an inverse association with colorectal cancer and light wine intake could protect even against non-alcoholic liver diseases (Kerr & Greenfield, 2007; Dunn et al., 1947). The polyphenol content of wine may be the primary reason for the effects in these studies (Newcomb, 1993). Most of these potentially bioactive compounds are found in grapes, majorly in red grape varieties (Anderson et al., 2005). For example, anthocyanins found in Cabernet Sauvignon, Syrah or in Merlot are effectively extracted into wine (Romero-Cascales, 2005).

This can especially be said about red wines, mainly grown in the Miniș-Măderat wine region. One of the red wine varieties very popularly grown here is the Cabernet Sauvignon (Balla, 2003).

Cabernet Sauvignon comes from southwestern France and is one of the world's most widely recognized and cultivated red wine grape varieties. It is a natural hybrid of Cabernet Franc and Sauvignon Blanc. Cabernet Sauvignon is considered the "king of wines". Even before the phylloxera blight Cabernet Sauvignon was a very popular variety in the Miniș-Măderat wine region. In recent decades, it has become an even more important variety in this wine region.It is due partly to the ease of its cultivation: the grape's thick skin makes it resistant to rotting and the vines are hardy and naturally budding

late so they are more tolerant to frost, and partly to the recognizable character and outstanding quality of the wine made from this grape (Bowers & Meredith, 1997).
In 1979 the area of Cabernet Sauvignon vineyard in the Miniș region was 286 ha, by 1989 it decreased to mere 155 ha. Currently, due to a new wave of popularity continuous replanting takes place in this region (Balla, 2003).
Cabernet Sauvignon has a moderate water requirement and likes a warm climate. Requires long-stem pruning and a lot of canopy management. This variety adapts well in terms of soil, yet produces high-quality fruit on a well-drained gravel soil.
The grapes have a significant pigment content, and the sugar content fluctuates between 208 g/l and 240 g/L, so the wine has a potential alcohol concentration of 12.2–13.9% vol.
The wine is deeply coloured, has delicate fruity aromas, and taste notes reminiscent of blackcurrant, black cherry, or plum. Its darker colour often indicates a higher tannin content (Mihailca & Adam, 1980).
One of the most popularly noted traits of the wine made from this variety is its affinity for oak due to its strong character and high acid and alcohol content. Barrel fermentation softens the grape's naturally high tannins and it also gives the wine unique wood flavours of vanilla and baking spices that complement the grape's typical flavours of black currant, prunes, and tobacco (Dallas et al., 1996).
The other main trait of this variety is that it has a high content of a specific polyphenol called anthocyanin. The phenolic content of wine refers to phenolic compounds, natural phenols and polyphenols, which form a large group of hundreds of chemical compounds that affect the taste, colour and mouthfeel of the wine (DiStefano & Gonzalez-Sanjose, 1991). These compounds include phenolic acids, stilbenoids, flavonols, dihydroflavonols, anthocyanins, flavanol monomers (catechins), and polymers of flavanols (proanthocyanidins). This large group of natural phenols can be roughly divided into two categories: flavonoids and non-flavonoids. Flavonoids include anthocyanins and tannins, which contribute to wine colour and mouthfeel. (Bowser & Meredith, 1997; DiStefano & Gonzalez-Sanjose, 1991).
The grape's phenolic compounds are distributed in various tissues of the berry (skin, pulp and seeds). These phenolic compounds are extracted into the wine during successive fermentation and maceration steps. Depending on the location of the polyphenolic compounds their diffusion into the must-wine and after that their final concentration at the end of the fermentation process can be different. (Pérez-Navarro et al., 2018).
Several studies have investigated the effect of the duration and temperature of maceration on red wine and most importantly on anthocyanin composition (Gil-Muñoz et al., 1999; Gómez-Míguez et al., 2007; Budic-Leto et al., 2008). Amerine (1955) found both colour and flavour were best at temperatures of 21°C and 27°C.
In another study, was found that fermentation at a temperature of 30°C could reach higher results regarding the colour density and flavour than at 20°C (Girard et al., 1997; Gao et al., 1997). In cases when the colour extraction is not appropriately elevated, fermentation temperatures could be applied (Reynolds et al., 2001). Izquierdo-Cañas et al., (2020), mention that the fermentation temperature can have a significant effect on the volatile compounds. Furthermore, the temperature can have a greater impact on the aroma composition of the wines (Sereni et al., 2020). However, Terpou et al., (2020) concluded that at low temperature pine sawdust could be helpful to produce high-quality sweet wine.
In a different study (Pérez-Navarro et al., 2018), it was found that increasing the fermentation temperature at the end of the process leads to more intense colour and higher concentration of phenolic compounds in Pinot noir wines, but at the same time, there is a chance of an increased volatile phenol concentration as well.
In a review by Şener (2018) it was stated that the duration and the temperature of maceration significantly influence the wine's quality and higher temperatures increase the extraction of phenolic compounds. However, fermentation at a lower temperature leads to delayed fermentation, which is a reason for a higher concentration of total phenolic compounds.
The aim of the present study was to determine the influence of changes in fermentation

temperature and duration on the anthocyanin content and quality of the resulting wine when using the technology of red wine production with skin fermentation of Cabernet Sauvignon grape variety.

MATERIALS AND METHODS

The experiment was carried out in 2020 at Wine Princess S.R.L., 26 km far from Arad, in Păuliș. Păuliș has been one of the most significant settlements of the Păuliș (Miniș) wine region since the 17th-18th centuries. The vineyard's area is of approximately 70 ha, on which various vine varieties can be found: Cabernet Sauvignon, Cabernet Franc, Kadarka, Pinot Noir, Merlot, Tramini, Muscat, Fetească regală, Italian Riesling. The storage capacity is 5000 hl. The wines are stored in wine cellars dug into the hillside, some of them in barrels, others in bottles depending on the technology involved.

The experiment started with the harvest on the 20th of September 2020 and continued until the 17th of March 2021.

Considering the climatic characteristics, the temperature did not show extreme values in this duration. Negative temperatures could be measured until March, so the sprouting of the grape buds was undisturbed. The average annual temperature was 12.1°C, and the average temperature of the vegetation period was 17.6°C. The absolute maximum temperature was 35.6°C, measured in August, while the absolute minimum temperature was −15°C measured in January.

In terms of precipitation, the average amount of precipitation was 883.8 mm. During the vegetation period, the average precipitation was 559 mm. The average annual air humidity was 73%, and 71% during the growing season.

Grape processing and fermentation

After measuring the quantitative and qualitative properties, the samples were randomly taken from 17,000 kg of grapes. Sugar content was measured with a refractometer and titratable acid content by titrating the samples.

Two variants were separated: Variant 1 (V1) was fermented at 25°C for 10 days, and Variant 2 (V2) at 30°C for 15 days. After the fermentation ended, the sugar content was measured and then the free-run wine was separated and the marc was pressed at a maximum of 1.5 bar pressure. The free-run wine and the press wine were blended in a proportion of 80/20. The quantity of wine produced was 11,050 litres.

After the malolactic fermentation on December 20–21, the first organoleptic examination took place and when the wine's alcohol, titratable acid, volatile acid, free sulphur dioxide content, pH level, and anthocyanin content were determined.

These measurements were made with the multiparameter analyser OenoFoss (FOSS, Hilleroed Denmark), which can instantly measure all the important parameters (ethanol, total acidity, malic acid, lactic acid, volatile acid, glucose, pH, density) from only a few drops of wine, approx. 1–2 mL.

The new wine was poured into medium toast barrique barrels. After three months of barrique fermentation the organoleptic and laboratory examinations were repeated with both variants.

For the evaluation, a simplified 100-point evaluation system was used, issued by the International Organisation of Vine and Wine (O.I.V., 2007). The colour, clarity, intensity (which is determined by the amount of anthocyanin), aroma and bouquet, as well as the taste, overall impression and harmony were examined.

An average was calculated from the results obtained during the evaluation. The objectivity of wine judging is ensured by statistical results. We processed the collected data, treated the organoleptic evaluations of the wine judges as repetitions and recorded them in a computer database.

The significance of the differences between the treatments was tested by applying one-way ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

RESULTS AND DISCUSSIONS

Alcohol content

There is no difference between the variants fermented for 10 days at 26C and the variants fermented for 15 days at 30°C (Figure 1). Cabernet Sauvignon V1 and the Cabernet

Sauvignon V2 were measured on the 20th of December with an alcohol content of 14.1% vol. volume. Since the alcohol content is primarily determined by the amount of sugar accumulated in the grapes, the temperature and duration of fermentation do not affect the alcohol content.
It can be determined that the results from the 15th of March, measured after three months of ageing, do not show any significant differences either. In the case of V2, a change in alcohol content of 0.1% vol. () was observed during the second analysis. This slight increase in alcohol is attributed to residual sugar after fermentation, which was converted to alcohol during malolactic fermentation.

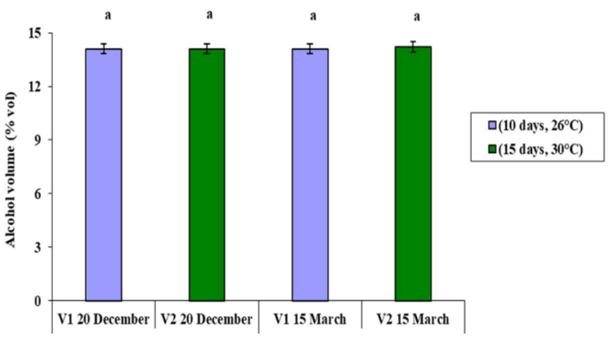

Figure 1. Changes in alcohol content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on titratable acidity

Examining the titratable acid content (Figure 2), the results showed differences between the variants, however no statistically significant differences were observed. The titratable acidity of the wine fermented for 10 days at 26°C was 7.2 g/L tartaric acid, while the titratable acidity of the wine fermented for 15 days at 30°C was 7.6 g/L tartaric acid. The measurement was made on the 20th of December. The analysis on the 15th of March gave the following titratable acidity results: V1 6.8 g/L tartaric acid and V2 7.2 g/L tartaric acid. It can be determined that there is a difference between the two variants even after three months of ageing. The variant fermented for a longer time and at a higher temperature has a higher titratable acid content. At the same time, a decrease in acidity can be observed in the case of both variants during maturation, which was caused by the precipitation of tartaric acid during malolactic fermentation.

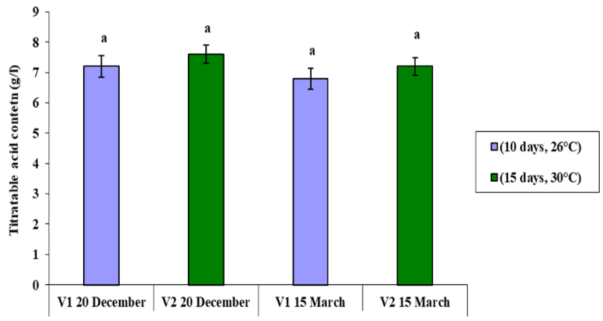

Figure 2. Changes in titratable acid content. Different letters indicate significant differences between treatments ($p < 0.05$).

The effect of changes in fermentation temperature and duration on the volatile acid content

The results of volatile acid content differ between the V1 and V2 variants (Figure 3). The first laboratory test gave the following results: 0.6 g/L acetic acid for wine fermented for 10 days at 26°C, and 0.57 g/L acetic acid for wine fermented for 15 days at 30°C. March results: V1 0.61 g/L acetic acid, V2 0.59 g/L acetic acid. The results also reported that the volatile acid content of both variants increased by 0.01 and 0.02 g/L acetic acid during the three-month maturation period.

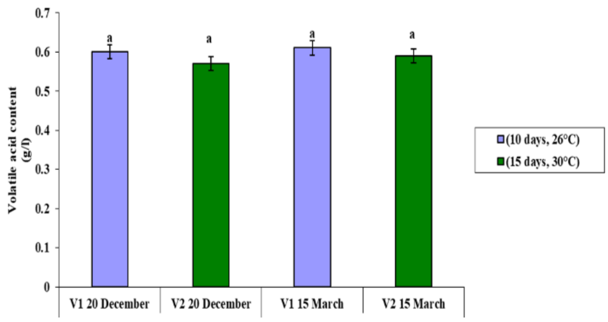

Figure 3. Changes in volatile acid content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on free sulphur dioxide content
During the examination of the free sulphur dioxide content (Figure 4), it can be determined that the V1 shows a higher value than the V2. Furthermore the 10 days of fermentation at 26°C recorded statistically significant differences, when the two periods were compared. Their values after the December analysis: 27 mg/L for V1 and 21 mg/L for V2. After three months of maturation, the free sulphur dioxide content also remained different considering the two variants.

23 mg/L for wine fermented for 10 days at 26°C and 21 mg/L for wine fermented for 15 days at 30°C. Examining the V1, a decrease of 4 mg/L can be observed during storage, while the free sulphur dioxide content did not change in the case of the V2.

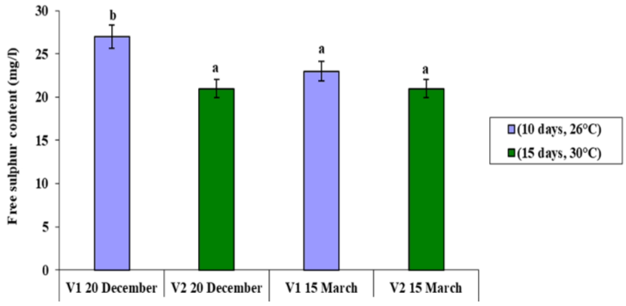

Figure 4. Changes in free sulphur dioxide content. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on pH content

The pH contents (Figure 5) were between 3.1 and 3.3. During the December test, a pH of 3.3 for V1 and 3.1 pH for V2 was measured.. March results were 3.2 and 3.1 pH. in the case of the Cabernet Sauvignon V1, a pH drop of 0.1 was observed during the maturation period, and in the case of V2, the pH content did not change during storage either. No statistically significant differences were recorded.

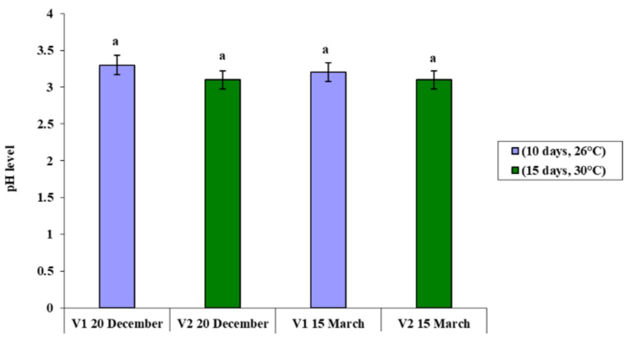

Figure 5. Changes in pH level. Different letters indicate significant differences between treatments ($p < 0.05$).

Effect of changes in fermentation temperature and duration on anthocyanin content

There is a difference between the V1 and V2 in terms of the anthocyanin content, one of the significant factors determining the colour of red wines (Figure 6). The anthocyanin content of the wine fermented for 10 days at 26°C was 420 mg/L, while the anthocyanin content of the wine fermented for 15 days at 30C was 485 mg/L after malolactic fermentation. The results from March were the following: Cabernet Sauvignon V1 with 400 mg/L and V2 with 453 mg/L. The anthocyanin content of the variant fermented at a higher temperature and for a longer time was higher and showed a decrease of 32 mg/L during maturation. The decrease can also be attributed to the anthocyanin content of Cabernet Sauvignon V1. The anthocyanin content of the V2 showed a higher value than the V1, despite the greater decrease. During ripening, highly reactive anthocyanins form anthocyanin-tannin complexes with tannins, as a result of which the anthocyanin content decreases. In the case of our experiment, the decrease in anthocyanin content during storage can also be attributed to this.

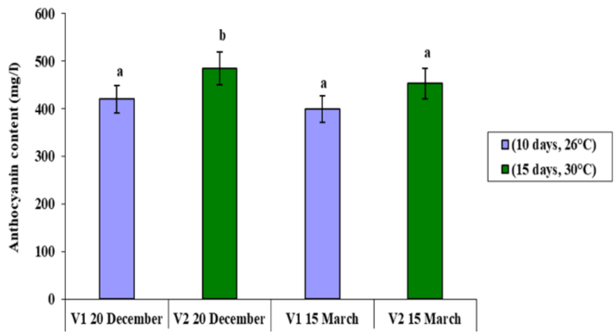

Figure 6. Changes in anthocyanin content. Different letters indicate significant differences between treatments ($p < 0.05$).

Results of the organoleptic examination

From the results of the first sensory test (Figure 7), V2, fermented for a longer time and at a higher temperature, received a higher evaluation. The colour parameter significantly influenced the total score. Since the anthocyanin content of Cabernet Sauvignon V2 was higher, the colour and clarity parameters also had a higher average than in the case of the wine fermented for 10 days and at 26°C. The averages of the organoleptic evaluation of the other parameters were similar, and a very slight difference (1 point) can be seen in the scoring of the aroma. The total score: V1 was evaluated by the judges at 80 points and V2 at 86 points.

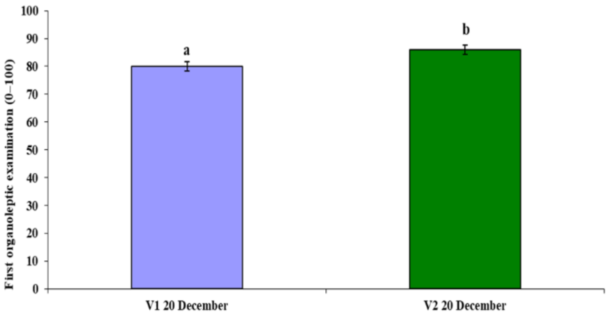

Figure 7. Ratings from the first organoleptic examination.

On the occasion of the second examination on the 15th of March a greater difference in the total score between the variants can be seen compared to the first organoleptic examination (Figure 8).

During ageing in barrels, the intensity of the colours decreased, which resulted in a decrease in the anthocyanin content, but the quality of the colours improved, which is also shown by the assessment of the March test. For both variants, the colour and clarity parameters received more points during the December evaluation. At the same time, it can be seen that there is a 4-point difference between the Cabernet Sauvignon V1 and V2 variants, in terms of the colour parameter. The V2 proved to be better, receiving a 25-point rating. During storage, in addition to the aroma, the bouquet of the wines developed, this factor was also evaluated by the inspection committee. These values also turned out to be better than the first organoleptic test, and here again, the values of V2 were higher.

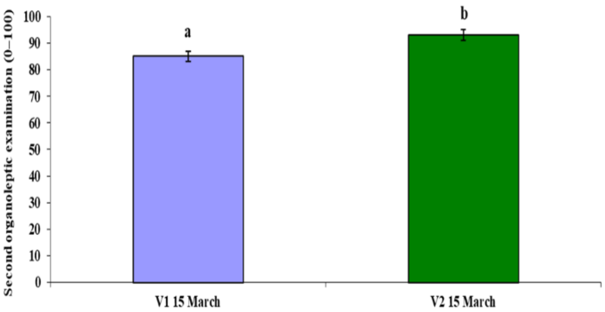

Figure 8. Ratings from the second organoleptic examination.

Anthocyanins are water-soluble vacuolar pigments, and they are responsible for the initial colour of red wines (Kong et al., 2003), furthermore they are strong antioxidants and their quantity is activated under different stress conditions, like extreme temperature, bacterial and fungal infection (Silva et al., 2017). According to Medina-Plaza et al., (2020), the anthocyanin concentration is significantly influenced by a higher temperature and the ethanol concentration. In another study they concluded that the kinetics of anthocyanin adsorption and desorption are clearly dependent on the temperature, ethanol concentration and also cell wall materials composition (Medina-Plaza et al., 2019).

CONCLUSIONS

After examining the obtained results, we can conclude the following: none of the measured parameters (alcohol, titratable acid, volatile acid, free sulphur content and pH level) was affected by the temperature of the fermentation. The anthocyanin level was higher with a higher temperature. In the case of V2, the anthocyanin content was 485 mg/L after 15 days with a fermentation temperature of 30°C. Moreover, lowering of the anthocyanin level while fermenting did not change the quality of colour, only its intensity. The wine had a deeper colour after 3 months of fermentation in the barrique barrels.

Concerning the results of the organoleptic examination of Cabernet Sauvignon, it can be stated that the wine fermented for a longer time and at a higher temperature (Cabernet Sauvignon V2) proved to be better (93 points). A higher fermentation temperature (30°C) and a longer fermentation (15 days) are recommended for this variety in the Miniș wine region. At this temperature and duration during fermentation on marc, the colouring materials and aroma and fragrance materials are dissolved in the appropriate amount from the skin cells of the Cabernet Sauvignon grape.

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