



INFLUENCE OF FERMENTATION CONDITIONS ON THE CONTENT OF PHENOLIC COMPOUNDS, ANTHOCYANINS AND THE SPECTRAL CHARACTERISTICS OF CABERNET SAUVIGNON WINES

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The wine characteristics have been determined by the variety potential and specificity, but also by the yeast strain carrying out the alcoholic fermentation. Phenolic components and anthocyanins play a significant role in the formation of the organoleptic wine qualities, especially of red wines. The yeast affects the colour of the wine mainly with the extracellular β -glucosidase they produce. The objective of the study was to investigate the influence of the technological factors yeast strain, temperature and inoculum amount of yeast culture on the variation of the total phenolic compounds and anthocyanins during the alcoholic fermentation and on the spectral characteristics of Cabernet Sauvignon wines. The experiment was carried out with grapes of Cabernet Sauvignon variety with sugars content of 23.10% and titratable acids of 6.15 g/dm³. The alcoholic fermentation was conducted with inoculum amount of yeast culture 2, 3 and 4% of the strains *Bordeaux* and *8-11* of *Saccharomyces cerevisiae* species. The fermentation temperature was 20, 24 and 28 °C. The course of the process was followed by the variation in the dry matter, measured daily with the Abbe refractometer. The dynamics of the total phenolic compounds and the anthocyanins during the fermentation was determined by measuring on 1st, 5th, 10th and 20th day. After the alcoholic and the malolactic fermentations in the experimental wines were also analyzed the content of flavonoid phenolic compounds, non-flavonoid phenolic compounds and their spectral characteristics. The results demonstrated that under the experimental conditions both strains *Bordeaux* and *8-11* exhibited the best fermentation activity at 28 °C. The temperature factor had a stronger impact on the variations of total phenolic compounds and anthocyanins in the course of the alcoholic fermentation. The quantitative maximum of both indicators was recorded on the 5th day of the process. In the experimental wines, the variants fermented under the conditions of 28 °C/4% contained more total phenolic compounds, anthocyanins, flavonoid phenolic compounds and non-flavonoid phenolic compounds. The red colour ratio also was the highest. The wines of *8-11* strain were distinguished by a higher content of total phenolic compounds, anthocyanins and, respectively a more intense colour.

Keywords: wine, yeast, alcoholic fermentation, phenolic compounds, spectral characteristics.

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Introduction

The wine characteristics have been determined by the variety potential and specificity, but also by the yeast strain carrying out the alcoholic fermentation. It has a significant influence on its composition with its metabolism and physiological features.

Phenolic components play a significant role in the formation of the organoleptic wine qualities, especially of red wines. The amount of phenolic compounds in grapes depended on number of factors, as the main of them being the variety, the soil and weather conditions in the growing area, the applied agricultural and technical practices, the degree of maturity, etc. Most of the phenolic compounds are contained in the seeds and the skins, from where they pass into the must and the wine (Abrasheva et al., 2003).

The wine anthocyanin profile has also been determined by the variety specifics (Burns et al., 2003; Otteneder and Marx, 2004). The anthocyanins are localized mainly in the grapes skin, in free or bound state in the form of glycosides (Singleton and Trousdale, 1992; Fisher and Strasser, 1999). The fermentation conditions and certain technological factors such as the time of contact with the solids, the temperature, the yeast strain, also influence the amount of phenols and anthocyanins in wine (Stoyanov et al., 2004).

In the production of red wines, with the increase of the alcoholic content during the fermentation, the extraction of phenolic and colouring substances is enhanced as the optimal temperature for this being 28–30 °C (Getov, 2002). The yeast affects the colour of the wine mainly with the extracellular β -glucosidase they produce. Strains with low β -glucosidase activity produce wines with higher anthocyanin content (Tsvetanov et al., 1994; Tsvetanov et al., 1995). The higher activity does not result in a direct decrease in their amount but it is directly related to their colouring ability, converting them into copolymerization aglycons (Spasov et al., 1998; Rodrigues et al., 2004). Part of the anthocyanins during the fermentation are adsorbed by the yeast cells, as well as in the oxidation, variations in the medium pH, interacting with other substances and pass into insoluble form and precipitate (Chobanova, 2012). As a result, both quantitative and qualitative variations in the phenolic and anthocyanin composition of the wine are observed at the end of the process.

The objective of the study was to investigate the influence of the technological factors yeast strain, temperature and inoculum amount of yeast culture on the variation of the total phenolic compounds and anthocyanins during the alcoholic fermentation and on the spectral characteristics of Cabernet Sauvignon wines.

Material and methodology

Plant material

The experiment was carried out with grapes of Cabernet Sauvignon variety. Each technological variant was crumbled and crushed separately and by equal distribution of the grapes, it was ensured the uniformity of the raw material.

Alcoholic fermentation

Conditions

The alcoholic fermentation was carried out under the following conditions:

- ▶ fermentation substrate – 4.0 kg of grape pulp, sulphuring with 50 mg/kg SO₂, with sugars content of 23.10% and titratable acids of 6.15 g/dm³;
- ▶ 48-hour active yeast culture of the *Bordeaux* and *8-11* collection strains of *Saccharomyces cerevisiae* species in quantity of 2%, 3%, 4% (the strains were provided by the yeast collection of the University of Food Technologies, Plovdiv, Bulgaria);
- ▶ fermentation temperature – 20 °C, 24 °C, 28 °C.

The course of the alcoholic fermentation was followed by the variation in the dry matter, measured daily by means of an Abbe refractometer at a constant value.

Determining the variations in total phenolic compounds and anthocyanins

The content of the total phenolic compounds and anthocyanins during the fermentation was determined, taking the following steps: start (day 1), vigorous fermentation (day 5), silent fermentation (day 10) and after the malolactic fermentation (day 20). Their amount was analyzed in the clear part, after centrifugation of the samples, using a UV-Vis spectrophotometer Cary 50 Varian, respectively by the methods:

- ▶ total phenolic compounds (TPC), g/dm³ gallic acid – method of Singleton and Rossi with a Folin-Ciocalteu reagent and measurement of sample absorption spectrophotometrically at λ 750 nm (Ivanov et al., 1979);
- ▶ anthocyanins, mg/dm³ – method of Gayon and Stonestreet by pH changing and using of buffer solutions with pH 0.6 and pH 3.5 and the absorption of both samples was measured spectrophotometrically at λ 520 nm (Ivanov et al., 1979).

Determination of the content of phenolic compounds, anthocyanins and spectral characteristics of the experimental wines

After the end of the alcoholic fermentation (found by chemical analysis of the sugars) and the spontaneous malolactic fermentation (established by paper chromatography), the experimental wines were decanted and by UV-Vis spectrophotometer Cary 50 Varian, they were further analyzed for the content of:

- ▶ flavonoid phenolic compounds (FPC), mg/dm³ catechin equivalent and non-flavonoid phenolic compounds (NPC), mg/dm³ caffeic equivalent – Somers method with analytical and calculation part – 0.2 cm³ of the test sample was added to 10 cm³ 1n HCl and between 3rd and 4th hour the absorbance of the solution was measured at 280 and 320 nm (Chobanova, 2007)
- ▶ spectral characteristics – determined by measuring the sample absorbance in a 0.1 cm cuvette at λ 420, 520 and 620 nm and equated to a 1 cm cuvette (Chobanova, 2007):
 - colour intensity IC' [abs. unit] – the Glories method, was the sum of the measurements at the three wavelengths:

$$IC' = A_{420} + A_{520} + A_{620}$$

- colour tint T [abs. unit] – the Sudraud method represented the absorbance at 420 nm and 520 nm ratio:

$$T = A_{420}/A_{520}$$

- $dA\%$ – the Glories method, the indicator expressed the participation of free and bound flavylum forms of anthocyanins in the total wine colour:

$$dA\% = \left\{ 1 - [(A_{420} + A_{620})/2 \times A_{520}] \right\} \times 100$$

- yellow colour ratio in the total wine colour – the Glories method, absorbance measurement at 420 nm:

$$\% \text{ yellow colour} = (A_{420}/IC') \times 100$$

- red colour ratio in the total wine colour – the Glories method, absorbance measurement at 520 nm:

$$\% \text{ red colour} = (A_{520}/IC') \times 100$$

- blue colour ratio in the total wine colour – the Glories method, absorbance measurement at 620 nm:

$$\% \text{ blue colour} = (A_{620}/IC') \times 100$$

Results and discussion

The investigation of the studied technological factors impact on the time of the alcoholic fermentation found that *Bordeaux* and *8-11* strains exhibited good fermentation activity. With the temperature and the inoculum amount of the yeast culture increase, within one temperature range, the process began and ended earlier. The dynamics of the dry matter in the fermentation of the grape pulp with both strains were similar. At 20 °C, the process started and ran more slowly, due to the slower cell multiplication. The yeasts exhibited the best activity at 28 °C and the duration of the process was shorter by 3–4 days compared to that at 20 °C. With the increase of the inoculum quantity of yeast culture (2%, 3%, 4%) the fermentation occurred faster because of the higher number of active yeast cells in the medium.

The results of the dry matter variation during the fermentation of Cabernet Sauvignon grape pulp with the studied strains are presented in Figure 1.

In the course of the alcoholic fermentation, the variation of TPC and anthocyanins in the grape pulp was monitored. The results of their dynamics have confirmed the findings of other authors (Sims and Bates, 1994; Spranger et al., 1998), namely reaching the maximum during the exponential phase of yeast development followed by a decrease in their concentration due to the absorption by the cells or the involvement in the condensation processes. The effect of the inoculum amount of the yeast culture of the studied strains was similar and relatively weaker. The influence of the factor temperature was stronger as an indicator of the fermentation process. The higher temperature benefited the colouring matter extraction from

the solids during the fermentation. The studied indicators from the grape pulp composition showed a tendency of going up with the increase of the yeast culture amount and temperature, reaching the maximum rates at 28 °C/4%.

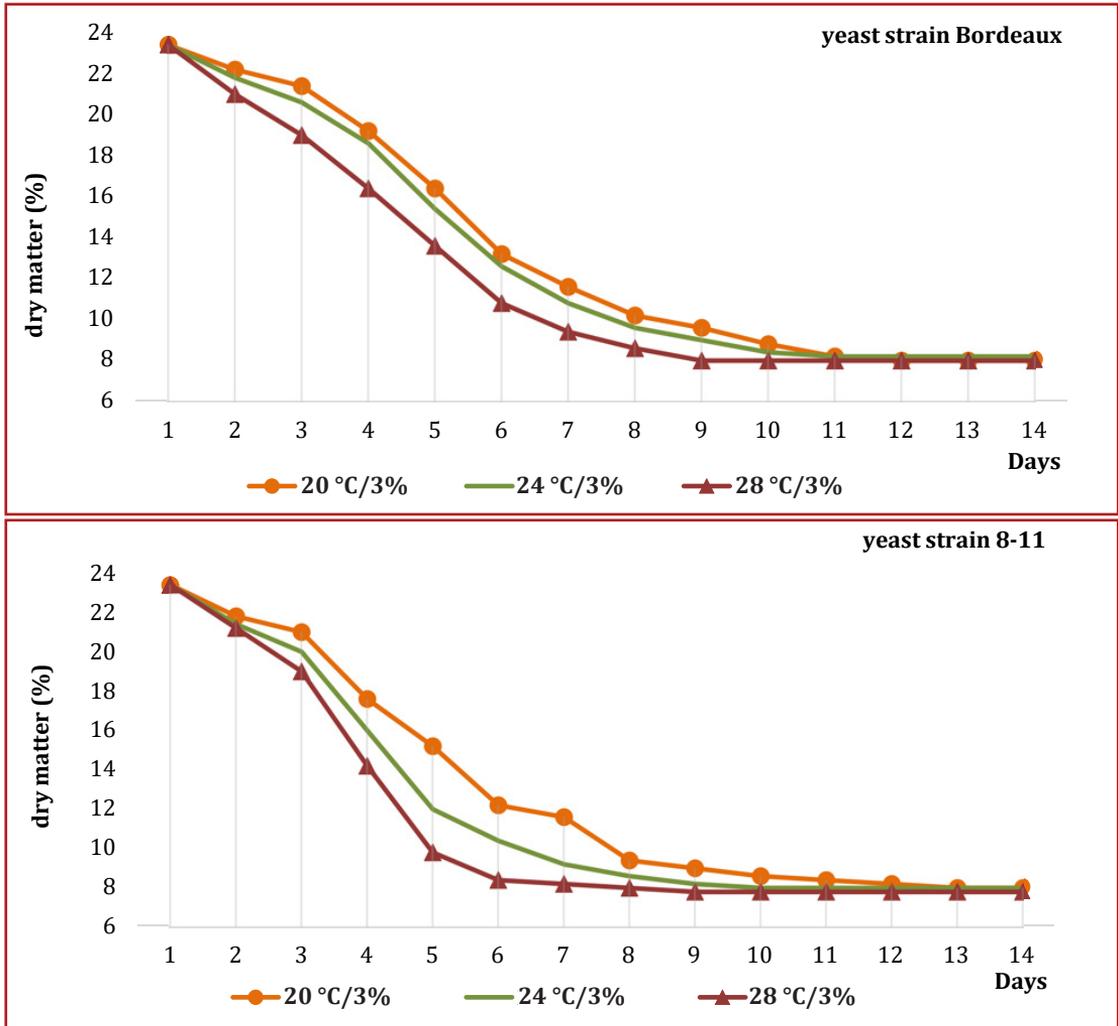


Figure 1 Dynamics of dry matter during the alcoholic fermentation of Cabernet Sauvignon grape pulp with the studied strains

Figures 2 and Figure 3 represent the variations in the TPC concentration and anthocyanins during the fermentation with *Bordeaux* and *8-11* strains at 3% inoculum and temperature 20, 24, 28 °C. The results of the process with 2 and 4% inoculum at the same temperature intervals were similar.

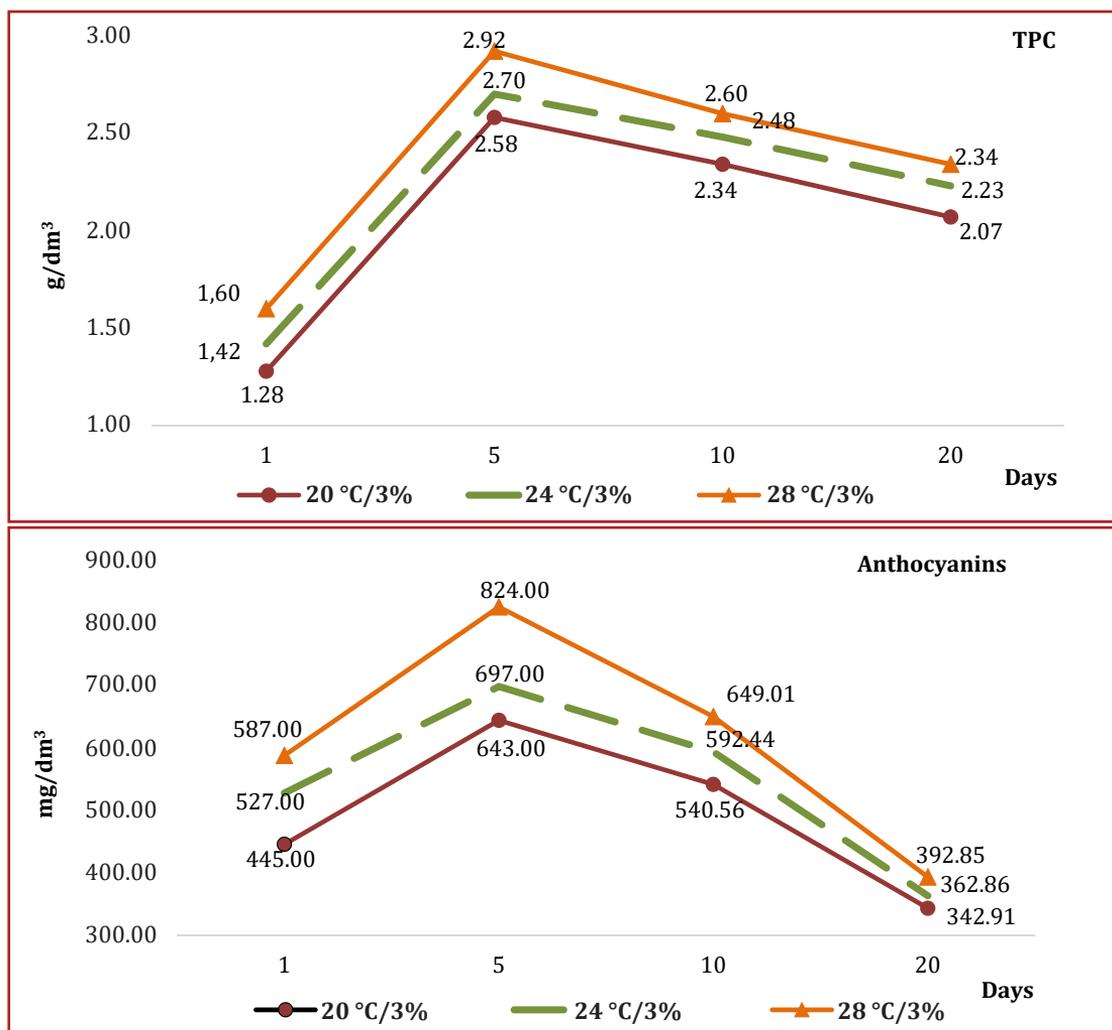


Figure 2 Variations in TPC and anthocyanins during the alcoholic fermentation with *Bordeaux* strain

The yeast effect appeared to be mainly on the amount of anthocyanins through the activity of the produced β -glucosidase. The *Bordeaux* strain had a higher β -glucosidase activity compared to the *8-11* strain (Yoncheva et al., 2007). Anthocyanins reduction during the fermentation was associated with the active cell development during the logarithmic phase, when cells released a significant amount of enzymes, including glucosidases, attacking their molecules (Kanev and Patokova, 2004).

The TPC and anthocyanins ratio was the highest for both strains, in wines fermented at 28 °C. These results have confirmed that it was the optimal temperature for obtaining intensely coloured red wines with a high content of phenolic substances. The quantitative variation of anthocyanins during the fermentation indicated that the maximum was on the fifth day. In the conditions of the experiment at the same fermentation temperature, the amount of anthocyanins in the wines obtained with an inoculum of yeast culture 3 and 4% was higher

for both strains *Bordeaux* and *8-11*. Due to the lower β -glucosidase activity of *8-11* strain, the wines produced with it contained more anthocyanins.

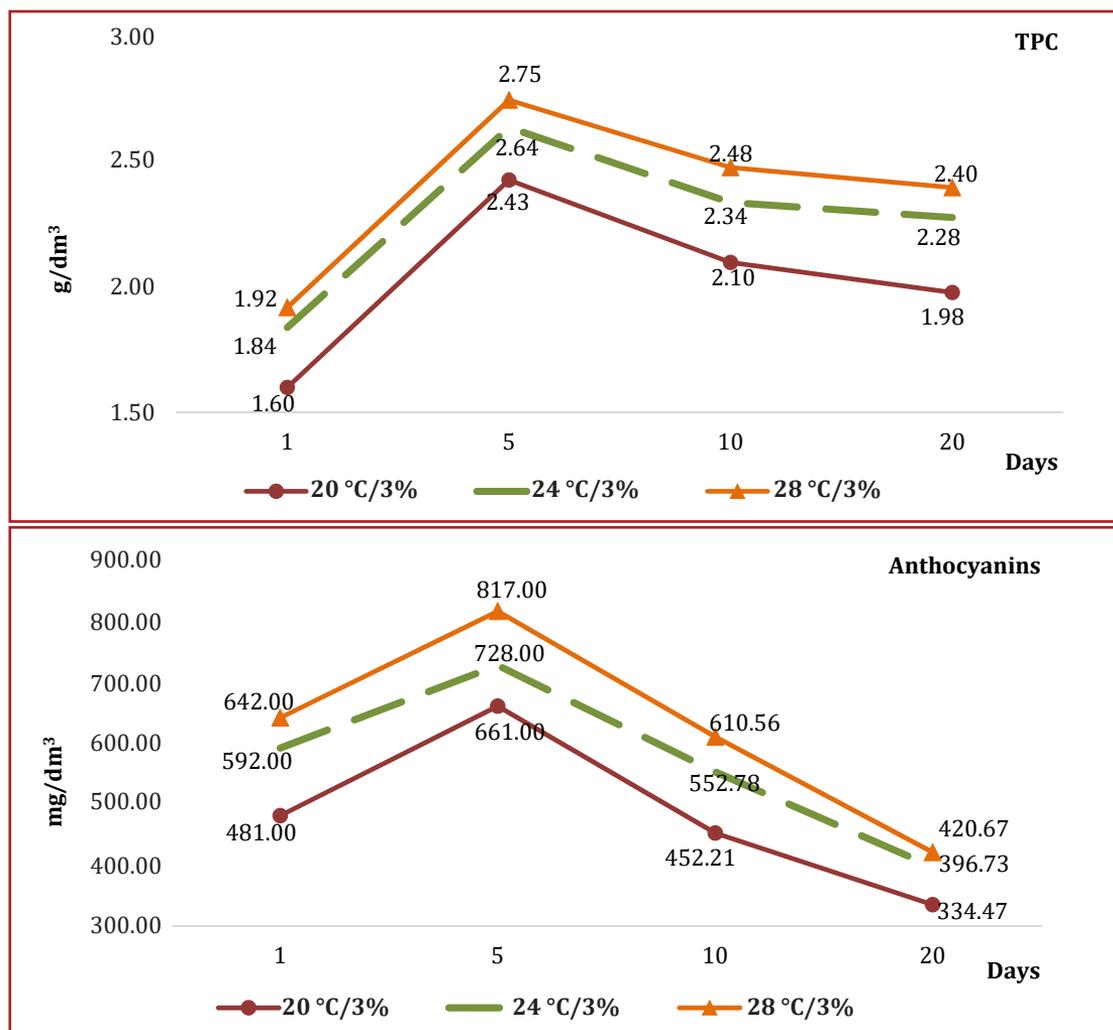


Figure 3 Variation in TPC and anthocyanins during the alcoholic fermentation with *8-11* strain

The rate of TPC in the experimental samples is proportional to that of the anthocyanins, so their variation in the course of the alcoholic fermentation was similar. In wines made with *Bordeaux* strain they ranged from 1.88 (20 °C/2%) to 2.38 g/dm³ (28 °C/4%), while in the samples obtained with *8-11* strain – from 1.88 (20 °C/2%) to 2.44 g/dm³ (28 °C/4%).

This trend was similar for the obtained young wines – the variants fermented under the conditions of 28 °C/4% contained more TPC, anthocyanins, as well as FPC and NPC (Table 1). That was due to the more intense extraction processes. The increase of FPC during the fermentation could be explained by the extraction from the solids and the rise in the amount of anthocyanins that were related to this group of compounds, while the higher rates of NPC

were probably due to the formation of new phenolic acids during the sugars decomposition. Their concentration, however, did not reach such rates to affect negatively the wine quality.

Table 1 Phenolic compounds and spectral characteristics of the experimental Cabernet Sauvignon wines

Variants	20 °C			24 °C			28 °C		
	2%	3%	4%	2%	3%	4%	2%	3%	4%
Indicators	<i>Saccharomyces cerevisiae Bordeaux</i>								
TPC (g/dm ³)	1.88	2.07	1.94	2.14	2.23	2.22	2.27	2.34	2.38
NPC (mg/dm ³ caffeic equivalent)	157.18	164.61	173.83	173.27	175.21	174.27	177.49	184.93	205.57
FPC (mg/dm ³ catechin equivalent)	2,935.77	3,148.63	3,270.06	3,299.35	3,559.36	3,522.93	3,672.24	3,777.20	3,790.79
Anthocyanins (mg/dm ³)	313.60	342.91	326.92	348.23	362.86	350.28	380.43	392.85	422.80
Colour intensity IC' [abs. unit]	9.12	9.25	9.37	9.43	9.47	9.52	9.65	9.78	10.22
Colour tint T [abs. unit]	0.476	0.465	0.473	0.470	0.389	0.477	0.516	0.545	0.512
dA %	70.10	70.40	69.60	69.80	68.20	69.70	67.00	66.10	66.70
% Yellow colour	29.80	29.20	29.40	29.30	29.90	29.70	31.10	30.80	30.70
% Red colour	62.60	62.80	62.20	62.30	61.10	62.20	60.30	60.00	60.00
% Blue colour	7.60	8.00	8.40	8.40	9.00	8.10	8.60	9.20	9.30
	<i>Saccharomyces cerevisiae 8-11</i>								
TPC (g/dm ³)	1.88	1.98	1.91	2.10	2.28	2.25	2.22	2.40	2.44
NPC (mg/dm ³ caffeic equivalent)	164.50	173.16	172.50	181.26	179.15	196.69	195.58	194.93	208.90
FPC (mg/dm ³ catechin equivalent)	3,100.78	3,047.92	3,297.21	3,295.06	3,548.64	3,609.35	3,691.50	3,711.50	3,852.93
Anthocyanins (mg/dm ³)	310.36	334.47	328.28	372.48	396.73	388.15	385.68	420.67	424.94
Colour intensity IC' [abs. unit]	9.48	9.50	9.54	9.78	10.20	10.85	11.12	11.65	11.86
Colour tint T [abs. unit]	0.496	0.500	0.551	0.562	0.562	0.494	0.492	0.504	0.463
dA %	68.80	68.50	65.20	63.10	64.40	67.90	68.60	67.30	71.30
% Yellow colour	30.60	30.70	30.50	30.30	30.90	30.10	30.20	30.50	29.50
% Red colour	61.60	61.30	60.90	60.50	60.40	60.90	61.50	60.40	61.20
% Blue colour	7.80	8.00	8.60	9.20	8.70	9.00	8.30	9.10	9.30

Note: TPC total phenolic compounds, FPC flavonoid phenolic compounds, NPC flavonoid phenolic compounds

The wine composition rates and its spectral characteristics were within the typical ranges of Cabernet Sauvignon wines. With increasing the temperature and ratio of yeast culture, their amount went up due to the better extraction conditions. The highest rates were recorded in the variants fermented at 28 °C with 4% inoculum (Table 1). The wines of 8-11 strain were characterized by a slightly higher content of TPC, anthocyanins and, respectively a more intense colour. The values of dA% indicator, determining the brightness of the red colour were directly correlated with them. That affected positively the colour characteristics of the samples. The ratio of the three main colours was within the typical ranges for young red wines. The red colour share was the greatest in the variants obtained at 28 °C. The higher rate of the yellow colour in the samples of both strains resulted in a slight brick-coloured tinge, which was also confirmed by the tint values. That could be assumed as a prerequisite for the faster positive colour development during the wine aging.

Conclusions

Based on the study results it might be summarized:

- ▶ *Bordeaux* and 8-11 strains showed the best fermentation activity at 28 °C. When increasing the inoculum amount of the yeast culture the fermentation also took place faster.
- ▶ The impact of the temperature on the variation of TPC and anthocyanins in the course of the alcoholic fermentation was more pronounced than the inoculum quantity of the yeast culture.
- ▶ The maximum concentration rate of TPC and the anthocyanins during the fermentation was recorded on the fifth day.
- ▶ In the experimental wines, the variants fermented at 28 °C/4% contained more TPC, anthocyanins, FPC, NPC and the share of the red colour was the highest.
- ▶ The wines of 8-11 strain had higher TPC content and because of the lower β -glucosidase activity of the strain they contained more anthocyanins and therefore had a more intense colour.

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