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Phenolic Compounds from the Fermentation of Cultivars Cabernet Sauvignon and Merlot from the Slovenian Coastal Region

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Summary

Large scale fermentation of the cultivars Cabernet Sauvignon and Merlot from the Slovenian coastal region was performed in Bücher-Vaslin roto tank vinificators. Six different areas, Prade, Kortina, Škocijan, Ankaran, Labor and Hrvatini, were selected for this study to investigate total phenols, anthocyanins, tannins and colour density, as well as pH, titratable acidity, sugar content and ethanol. Anthocyanins and phenolic compounds were found to be in generally higher concentrations (up to 4240 mg/L) in Cabernet Sauvignon. Prolongation of the fermentation phase with Merlot gave even reduced concentrations of anthocyanins, a phenomenon which was not observed in Cabernet Sauvignon. The highest concentration of anthocyanins was found in Merlot from the Kortina location (735 mg/L), and in Cabernet Sauvignon from the Labor location (998 mg/L). The highest concentration of tannins (1828 mg/L) was found in the grapes of Cabernet Sauvignon from the Ankaran location, while in Merlot 1280 mg/L was detected in a sample from the Prade area. The tone of colour and its intensity reached full maturity in Cabernet Sauvignon. Merlot from the Kortina location reached the highest colour density of 1.57, while in a sample of Cabernet Sauvignon from Hrvatini colour density was 2.89. Only small differences were detected in colour quality between Merlot and Cabernet Sauvignon.

Key words: fermentation, phenols, anthocyanins, tone and colour intensity

Introduction

Vinification process is one of the most important operations in enology and results in the type of wine desired by the consumer. The grape has to be carefully cared for and monitored during its growth cycle until the harvest, so it is not surprising that every good winemaker pays great attention to grape quality as the starting point in the production of wine and cellaring. Successful vinification of grape must to wine means the

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elimination of all negative factors and undesired side effects. This process is a complex of various physico-chemical phenomena and various spontaneous biochemical reactions. The quantity of grapes, the speed of harvesting operations, the capacity of the fermentation tanks, and the replacement of manual operation by automation are some of the most relevant factors in the technology of vinification. According to the results of recent research in this field, great progress in process automation and simplification of some vinification phases is evident (*1*).

Lately fermentation of the constituents of red grapes, as well as of white grapes, has been recognised to be of essential importance for the quality and stability of wine. Red wine is the joint result of red grape must fermentation and the parallel extraction of various compounds from the grape berry and skin. Grape skin fermentation results in the extraction of all the colour compounds relevant for wine structure, its body, colour, bouquet and aroma perception, and of various substances from polyphenolic to nitrogen compounds, polysaccharides, pectins, mineral substances, pyrazine, terpenes, *etc.* (*2*).

Solid-liquid phase contact between the grape skin and must during the fermentation period is the most vital phase for future wine quality. Ethanol fermentation produces carbon dioxide, which increases the fermentation broth volume up to 15–20 %. The up-flow of gas phase also induces the mixing of the liquid phase in the fermentation tank, causing flotation of the husks that conglomerate in the cap or the »floating hat«. Further extraction of the most relevant compounds has to proceed in the direction from the »floating hat« towards the surface of the fermentation broth (*3*).

In the production of red wines various vinification technologies may be used (*4*). They are mostly distinguished by their different treatment of the »floating hat.« In fermentation technology 3 of the most relevant phases may be distinguished (*1*): (*i*) prefermentative phase, which takes place for only a few hours. In this phase anthocyanins and various aromatic compounds are extracted. In this process mixing of the biomass volume and the following parameters should be measured: SO_2, temperature and the activity of various enzymes (*1*); (*ii*) ethanol production, which lasts 4 to 6 days. In this phase the extraction of tannins starts while the extraction of anthocyanins continues. The most relevant parameters that should be measured in this phase are SO_2, temperature and the ratio of grape must to grape skin. Mixing of the biomass volume is important as well (*2*); (*iii*) post-fermentative phase, where the extraction of polysaccharides takes place and which plays a very important role in the establishment of the colloidal structure of the wine, most relevant for the oak barrel or barrique type of wine (*5*).

In the case of grapes of acceptable technological maturity, but with unsatisfactory phenol maturity, the presence of SO_2 at higher process temperatures of up to 30 °C in the pre-fermentative phase could also increase the extraction of colouring substances.

Materials and Methods

Grapes

Grapes from the cultivars Cabernet Sauvignon and Merlot, vintage 1999, were used in this study. The average growth was 7.0 to 7.5 t/ha. Undamaged grapes of best quality were harvested and transferred in small, 20-kg baskets to the cellar. Both technological and phenolic maturation of grapes were monitored and several process parameters were measured: (*i*) parameters before harvest: ratio of total reductive sugars to total acid content, anthocyanin potential, extractability of anthocyanins, phenol maturity of the kernels, husk/juice ratio; (*ii*) the day of the harvest was determined in relation to total acid content; (*iii*) prefermentative phase, alcohol fermentation and post-fermentative phase; (*iv*) must and wine tasting and sensorial analysis during the process of fermentation and husk separation; (*v*) analysis of wine must and wine samples after overflow (soutirage), and skin separation (l'ecoulage), lactic acid fermentation control, sulphurisation and total phenol analysis after weeks of stabilisation.

Location of the vineyards

Ankaran:	Peninsula, 50 m above sea level, sandy-clay soil; total vineyard area 59 ha*
	Vineyard: Cabernet Sauvignon, 17.23 ha, sylvo training system, 3 x 0.9 m
Hrvatini:	Central part of the peninsula, 100 m above sea level, sandy-clay soil; total vineyard area 53 ha
	Vineyard: Cabernet Sauvignon, 3.5 ha, single Guyot training system, 3 x 1 m
Labor:	Continental part, 350 m above sea level, continental influence, sandy-clay soil; total vineyard area 45 ha
	Vineyard: Cabernet Sauvignon, 8.4 ha, cordon training system, 3 x 1 m
Prade:	South-west hilly ground, 50 m above sea level, sandy-clay soil; total vineyard area 43 ha
	Vineyard: Merlot, 14 ha, cordon training system, 3 x 1 m
Škocjan:	South hilly ground, 50 m above sea level, sandy-clay soil; total vineyard
	area 81 ha
	Vineyard: Merlot, 13 ha, cordon training system, 3 x 1 m
Kortina:	South hilly ground, 200 m above sea level, combination of sandy-clay soils and clays, total vineyard area 35 ha
	Vineyard: Merlot, 17 ha, single Guyot training system, 2.8 x 0.8 m

*1 ha = 10 000 m^2

Fermentation

The fermentation process proceeded at an average temperature of 30 °C (maximum oscillation 4 °C) in Bücher-Vaslin Vinimatic roto tanks (France) of total volume of 410 hL. The working volume of each run was 350 hL. Six vinificators per vineyard were used. The results from the representative vinificators are presented in this paper. In all the experiments the following mix-

ing regime was used: 1st to 5th day: 2 rpm/day; 5th to 10th day: 1 rpm/day; and 11th to 18th day: 1 rpm/day.

pH and titratable acidity

pH of the fermentation broth was measured by a Metrel pH meter, model MA 5736 (Italy). For titratable acidity potentiometric titration by Amerine and Ough (*6*) was used.

Ethanol

For ethanol measurement Dujardin-Salleron ebulliioscope (France) was used. Ethanol was measured in volume fraction (%) by Daničič (*2*).

Total phenols

Total phenols were determined by a spectroscopic method, using Folin-Ciocalteu reagent and sodium carbonate. Anthocyanins, tanins, colour density as well as total phenols were measured using a Jenway Visible Recording Spectrophotometer, model 6100 (USA), with glass cell (d=1 mm), Brand QS 1000. Buffer pH=3.5 was used. Cabernet Sauvignon samples were diluted by a factor of two. Absorbance at 765 nm corresponds to the concentration of total phenols in the sample. The final mass concentration was determined from a calibration curve according to Amerine and Ough (*6*).

Anthocyanins

For determination of total anthocyanins the spectrometric method of Ribereau-Gayon and Stonestreet (*7*) was used. Absorbance at 520 nm was measured.

Tannins

The concentration of total tannins was determined according to Amerine and Ough (*6*). Absorbance of 550 nm was applied.

Colour density

Colour density was determined by spectrometry according to Amerine and Ough (*6*). The absorbances at 420, 520 and 620 nm were measured.

Sugar content

Grape must sugar content in °Brix was measured by refractometry using an Atago refractometer, model PR-101 (Japan) (*8*).

Sensory analysis

Sensory analysis of grape and grape must quality in the vineyard as well as in the cellar was applied. The head enologist was the supervisor of the quality; no notes were produced.

Results and Discussion

Flavonoids are the dominant polyphenols and can represent up to 80 or 90 % of the total phenols in red wines. This group also includes anthocyanins and flavonols, which are extracted from the grape berry skins on the 4th or 5th day of fermentation, as well as catechins and leucoanthocyanins extracted from the kernels in the further phase of fermentation (*6,8*).

In extended fermentation the most pronounced nonflavonoids extracted into wine musts are mostly hydroxycinnamic and hydroxybenzoic acid derivatives from the kernels, and stilbenes, *e.g.* resveratrol with its pronounced antioxidative activity (*8,9*).

The results of the analysis of the 1999 vintage show differences in the amounts of phenolic substances in the cultivars Merlot and Cabernet Sauvignon (Figs. 1–6). They are more pronounced in Cabernet Sauvignon. Samples of Cabernet Sauvignon usually exceed the convenient upper limit for phenolic substances. The content of phenolics can reach even 4109 to 4240 mg/L (*9*).

With Merlot the period of the increase in the content of phenolic substances lasted until the beginning of ethanolic fermentation, the second phase of fermentation, and was lower than expected. This observation was most pronounced at the microlocations of Prade and Kortina. With the cultivar Cabernet Sauvignon this trend was observed only in the samples from the Labor location.

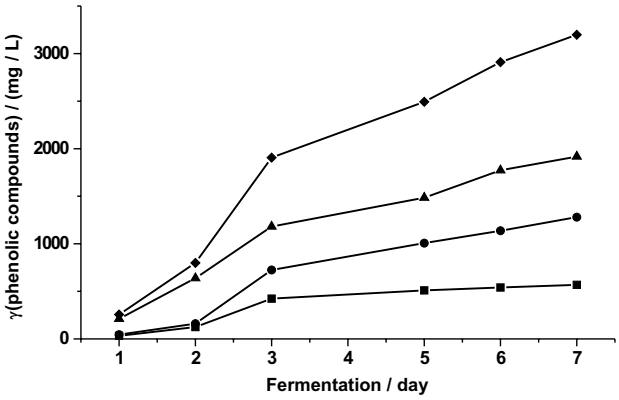

Fig. 1. Increase of phenolic compounds during fermentation of Merlot. Microlocation Prade; the average sugar grade at harvest was 22.0 °Brix
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

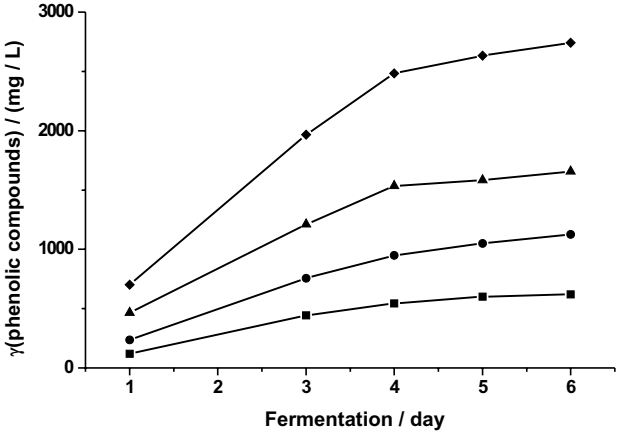

Fig. 2. Increase of phenolic compounds during fermentation of Merlot. Microlocation Škocjan; the average sugar grade at harvest was 22.5 °Brix.
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

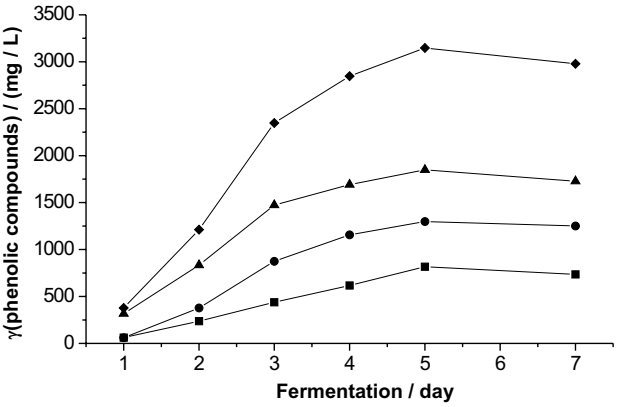

Fig. 3. Increase of phenolic compounds during fermentation of Merlot. Microlocation Kortina; the average sugar grade at harvest was 21.0 °Brix
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

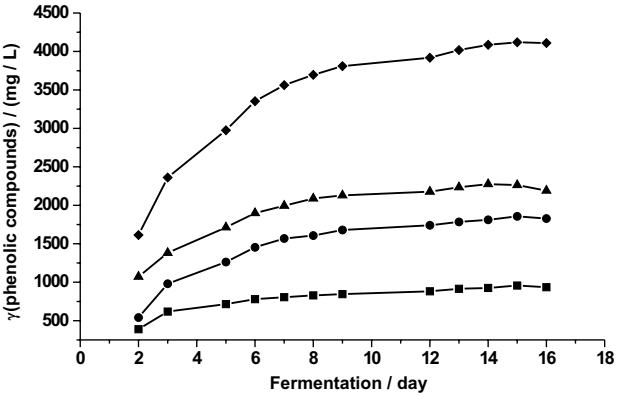

Fig.4. Increase of phenolic compounds during fermentation of Cabernet Sauvignon. Microlocation Ankaran; the average sugar grade at harvest was 23.0 °Brix
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

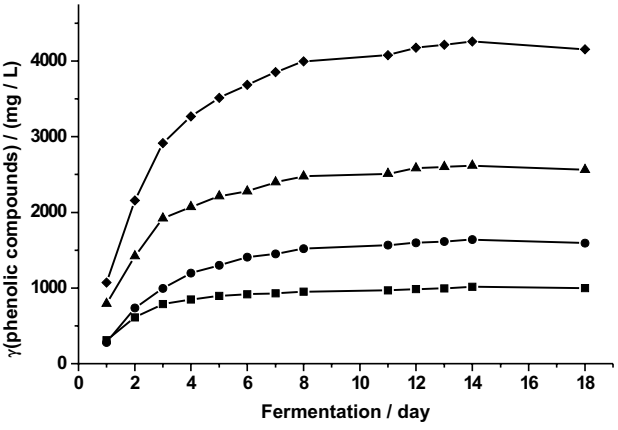

Fig. 5. Increase of phenolic compounds during fermentation of Cabernet Sauvignon. Microlocation Hrvatini; the average sugar grade at harvest was 22.0 °Brix.
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

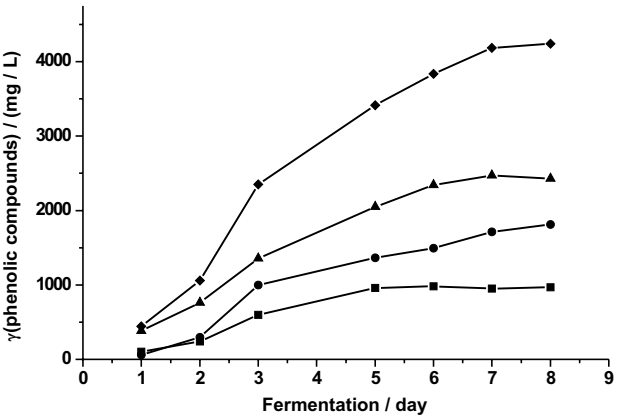

Fig. 6. Increase of phenolic compounds during fermentation of Cabernet Sauvignon. Microlocation Labor; the average sugar grade at harvest was 22.0 °Brix.
◆ Phenols; ■ Anthocyanins; ▲ Nontannins; ● Tannins

From the comparison of the amount of anthocyanins it could be concluded that the cultivar Cabernet Sauvignon has a much higher potential for anthocyanins than the cultivar Merlot. It is interesting that the prolongation of the fermentation phase of Merlot would even reduce a production of anthocyanins. In contrast, prolongation of fermentation with Cabernet Sauvignon did not result in a reduction of anthocyanins.

The extraction of colouring substances in red wines was most pronounced in the prefermentation phase. In the post-fermentation phase of Cabernet Sauvignon, only a slow increase in anthocyanin concentration was observed (*6*). For Merlot the highest content of anthocyanins of 735 mg/L was found at the Kortina location, while for Cabernet Sauvignon 998 mg/L were recorded at the Labor location.

Fermentation of the samples of Cabernet Sauvignon gives more tannic phenols than that of Merlot. Analysis of tannins in Cabernet Sauvignon showed that the highest amount of 1828 mg/L was obtained from the Ankaran location, while in Merlot 1280 mg/L was found in samples from the Prade area. A high level of phenols was observed at full ripeness of the grapes of Cabernet Sauvignon. Merlot from the Kortina location reached the highest colour density of 1.57, while in the sample of Cabernet Sauvignon from Hrvatini colour density of 2.89 was detected.

From the comparison of the results of three differently positioned vineyards growing Merlot (Tables 1–3), the following conclusions could be drawn: at the lower microlocation of Prade (50 m above sea level), higher amounts of sugars (18.1 °Brix) but nearly the same amount of final alcohol (11.15–11.92 %) as at the other locations were observed. From the results of chemical analysis it is obvious that with the increase in the height of the location of vineyards above sea level the content of anthocyanins, tannins and colour density increase, the level of phenols and the tone are not influenced, while the level of non-tannins decreases (Figs. 1–3).

From the comparison of the results of three differently positioned vineyards growing Cabernet Sauvignon

Table 1. The results of physical and chemical analyses for cultivar Merlot for separate days of fermentation. Harvest from the location Prade. The average sugar grade at harvest was 22.0 °Brix

t(fermentation)	ρ	*φ*(ethanol)	*γ*(total acidity)	*γ*(phenols)	*γ*(anthocyanins)	*γ*(nontannins)	*γ*(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
1	18.1	1.38	5.78	256	33	211	45	0.24	1.3	3.25
2	18.3	1.18	6.83	799	125	638	161	0.27	0.76	3.37
3	12.5	6.76	7.20	1906	424	1181	725	1.00	0.49	3.37
5	7.3	11.02	7.55	2493	510	1485	1007	1.45	0.31	3.22
6	7.9	11.92	7.73	2910	541	1772	1138	1.48	0.39	3.23
7	7.5	12.30	7.73	3198	569	1918	1280	1.46	0.40	3.25

Table 2. The results of physical and chemical analyses for cultivar Merlot for separate days of fermentation, from the location Škocjan. The average sugar grade at harvest was 22.5 °Brix

t(fermentation)	ρ	*φ*(ethanol)	*γ*(total acidity)	*γ*(phenols)	*γ*(anthocyanins)	*γ*(nontannins)	*γ*(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
1				701	121	465	237	0.39	0.70	3.33
3	12.0	7.11	6.83	1967	443	1211	756	1.44	0.53	3.35
4	9.3	8.31	6.90	2482	544	1534	948	1.38	0.41	3.36
5	7.8	10.94	6.75	2634	602	1583	1051	1.37	0.40	3.36
6	7.3	11.50	6.75	2742	621	1656	1127	1.38	0.40	3.36

Table 3. The results of physical and chemical analysis for cultivar Merlot for separate days of fermentation from the location Kortina. The average sugar grade at harvest was 21.0 °Brix

t(fermentation)	ρ	*φ*(ethanol)	*γ*(total acidity)	*γ*(phenols)	*γ*(anthocyanins)	*γ*(nontannins)	*γ*(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
1	16.9	2.01	5.55	378	62	317	61	0.41	1.05	3.28
2	15.1	3.91	6.23	1212	237	834	378	0.82	0.69	3.33
3	11.5	7.30	6.68	2348	438	1473	874	0.57	0.47	3.45
5	6.6	11.26	7.65	2847	617	1691	1156	1.59	0.39	3.21
6	6.9	11.15	6.98	3147	817	1849	1298	1.91	0.41	3.49
7	7.2	12.81	7.58	2979	735	1728	1251	1.56	0.39	3.25

Table 4. The results of physical and chemical analysis for cultivar Cabernet Sauvignon for separate days of fermentation from the location Ankaran. The average sugar grade at harvest was 23.0 °Brix

t(fermentation)	ρ	*φ*(ethanol)	*γ*(total acidity)	*γ*(phenols)	*γ*(anthocyanins)	*γ*(nontannins)	*γ*(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
2	15.4	3.99	7.20	1613	389	1072	541	0.54	0.50	3.38
3	11.5	7.84	7.13	2362	619	1382	980	0.65	0.42	3.46
5	8.9	10.62	8.18	2976	715	1713	1263	1.97	0.39	3.29
6	7.7	11.59	8.10	3353	779	1899	1454	2.01	0.39	3.33
7	7.4	11.79	7.95	3562	805	1994	1568	2.13	0.38	3.35
8	7.4	12.14	7.65	3696	828	2089	1607	2.32	0.38	3.36
9	8.6	12.47	7.43	3809	845	2130	1679	2.59	0.37	3.53
12	8.3	13.08	7.13	3918	882	2177	1741	2.60	0.37	3.37
13	8.3	13.06	6.90	4018	914	2234	1784	2.75	0.37	3.37
14	8.2	13.08	6.68	4087	925	2275	1812	2.84	0.35	3.40
15	8.0	13.00	6.30	4119	957	2263	1856	2.89	0.34	3.40
16	8.0	13.00	6.23	4109	935	2191	1828	2.82	0.35	3.41

(Tables 4–6), with the increase of the height of the location of vineyards above sea level, the most pronounced effect was an increase of the amounts of phenols at the microlocation of Labor, while the content of tannins, anthocyanins and non-tannins, colour density and tone remained the same. The effects of prolonged fermentation are well expressed in the musts of all the vineyards and are the most pronounced in the increase of the amounts of phenols and anthocyanins (Figs. 4–6).

Conclusions

From the comparison of the two cultivars, Merlot and Cabernet Sauvignon, the following conclusions

Table 5. The results of physical and chemical analyses for cultivar Cabernet Sauvignon for separate days of fermentation from the location Hrvatini. The average sugar grade at harvest was 22.0 °Brix

t(fermentation)	ρ	φ(ethanol)	γ(total acidity)	γ(phenols)	γ(anthocyanins)	γ(nontannins)	γ(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
1	18.0	1.23	6.38	0.63	0.57	1071	309	280	791	3.43
2	13.2	5.63	6.98	0.66	0.42	2156	613	736	1420	3.46
4	7.7	10.46	6.38	1.54	0.41	2916	787	994	1922	3.47
5	7.3	11.92	7.65	1.69	0.41	3269	849	1198	2071	3.23
6	7.1	11.18	6.83	1.70	0.40	3515	897	1301	2214	3.52
7	7.1	11.32	6.60	1.80	0.40	3687	918	1409	2278	3.35
8	8.1	11.61	6.30	1.96	0.39	3853	931	1453	2400	3.53
11	7.9	12.45	5.48	2.17	0.38	3997	952	1519	2478	3.59
12	7.9	12.34	5.25	2.32	0.36	4079	969	1567	2512	3.60
13	7.8	12.31	5.40	2.47	0.35	4179	985	1596	2583	3.61
14	7.6	12.36	5.18	2.62	0.34	4216	995	1615	2601	3.61
15	7.6	12.26	5.18	2.91	0.35	4260	1018	1640	2620	3.61
18	7.7	12.53	4.95	2.89	0.35	4154	998	1592	2562	3.63

Table 6. The results of physical and chemical analyses for cultivar Cabernet Sauvignon for separate days of fermentation from the location Labor. The average sugar grade at harvest was 22.0 °Brix

t(fermentation)	ρ	φ(ethanol)	γ(total acidity)	γ(phenols)	γ(anthocyanins)	γ(nontannins)	γ(tannins)	Colour density	Tone	pH
day	°Brix	%	g/L	mg/L	mg/L	mg/L	mg/L			
1	20.5	0.00	6.90	445	104	384	61	0.25	0.55	3.26
2	19.0	1.28	6.75	1060	241	763	296	0.51	0.49	3.33
3	16.2	4.10	7.20	2351	599	1355	997	0.61	0.38	3.39
5	10.9	9.10	7.20	3415	960	2050	1366	2.31	0.42	3.41
6	10.1	10.52	8.33	3839	982	2343	1496	2.75	0.36	3.34
7	8.7	11.74	8.18	4186	950	2473	1713	2.77	0.37	3.38
8	8.0	12.36	8.03	4240	971	2429	1811	2.74	0.36	3.38

could be observed. Regarding the relative amount of phenolic substances, Cabernet Sauvignon could very easily reach high levels of maturation. Comparison of the amounts of anthocyanins and tannic substances showed that for Merlot these levels are nearly 50 % lower than for Cabernet Sauvignon. This result demonstrates the lower phenolic potential of Merlot.

The amount and the quality of tannins in red wines are the most relevant factors that affect the preservation of quality and taste of wine. The results of this research show that prolonged fermentation has its effect, but it depends on the cultivar, while the site of the vineyard is only of minor importance. Differences in pedology, sandy-clay or sandy-clay with clay soil, do not have an influence on the final results.

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Fenolni spojevi nastali fermentacijom sorti Cabernet Sauvignon i Merlot iz slovenskoga primorja

Sažetak

Industrijska fermentacija sorta Cabernet Sauvignon i Merlot iz slovenskoga primorja provedena je u rotacijskom tanku vinifikatora Bücher-Vaslin. U ovom su istraživanju ispitivani ukupni fenoli, antocijanini, tanini, intenzitet boje, pH, ukupna kiselost, količina šećera i etanol sa 6 različitih područja: Prade, Kortina, Škocijan, Ankaran, Labor i Hrvatini. U sorti Cabernet Sauvignon utvrđen je uglavnom veći udjel fenolnih spojeva (4420 mg/L) i antocijanina. Produženom fermentacijom sa sortom Merlot dobivene su čak manje koncentracije antocijanina, što nije uočeno u sorti Cabernet Sauvignon. Najveća je koncentracija antocijanina (735 mg/L) izmjerena u sorti Merlot uzgajanoj u Kortini i u Cabernet Sauvignon s područja Labora (998 mg/L). U grožđu Cabernet Sauvignon iz regije Ankaran ustanovljena je najveća koncentracija tanina (1828 mg/L), dok je u Merlotu s područja Prade izmjereno 1280 mg/L. Nijansa boje i njezin intenzitet postigli su punoću u Cabernet Sauvignon. Merlot s područja Kortine imao je najveći intenzitet boje od 1,57, a u uzorku Cabernet Sauvignon iz Hrvatina 2,89. Intenzitet boje sorta Merlot i Cabernet Sauvignon vrlo se malo razlikovao.