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Influence of the time of maceration on phenolic composition of wines produced from the indigenous variety Prokupac

Original Article

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Abstract:

The influence of the time of maceration on phenolic composition of wine (white, rosé and red wine) produced from the indigenous variety Prokupac was studied in this paper. White wine was produced from free run, without maceration. In the production of rosé wine, cool-temperature extraction prior to fermentation in 2-hour period was used, while for the production of red wine, maceration was carried out at 15-20 °C for 2 weeks. With the increase of the time of maceration, the concentration of total phenolic compounds also increases, whereby, the lowest content was detected in white wine (565.4 mg/l GAE). The phenolic content in red wine was almost 2.5 times higher when the time of maceration was extended to 14 days (1433.8 mg/l GAE). A significant increase in total flavonoids content was detected: 153.4 mg/l CE (white wine), 179.3 mg/l CE (rosé wine), and 1205.0 mg/l CE (red wine). The total flavonoid share in total phenolic content of the wine increases with the increase of the time of maceration and its value is as follows: 27.1% (white wine), 34.3% (rosé wine) and 84.0% (red wine).

Key words:

maceration, parameters of quality, Prokupac, wine

Apstract:

Uticaj vremena maceracije na fenolni sastav vina proizvedenih od autohtone sorte grožđa Prokupac

U ovom radu je proučavan uticaj vremena maceracije na fenolni sastav vina (belo, roze i crveno vino) proizvedeno od autohtone sorte Prokupac. Belo vino je proizvedeno bez maceracije. U proizvodnji roze vina vršena je hladna maceracija pre fermentacije u dvosatnom periodu, dok je za proizvodnju crvenog vina maceracija vršena na 15-20 °C tokom 2 nedelje. Sa povećanjem vremena maceracije povećava se i koncentracija ukupnih fenolnih jedinjenja, pri čemu je najmanji sadržaj otkriven u belom vinu (565,4 mg/l GAE). Sadržaj fenola u crvenom vinu bio je skoro 2,5 puta veći kada je vreme maceracije produženo na 14 dana (1433,8 mg/l GAE). Otkriven je značajan porast ukupnog sadržaja flavonoida: 153,4 mg/l CE (belo vino), 179,3 mg/l CE (vino roze), i 1205,0 mg/l CE (crveno vino). Ukupan udeo flavonoida u ukupnom fenolskom sadržaju vina povećava se sa povećanjem vremena maceracije i njegova vrednost je sledeća: 27,1% (belo vino), 34,3% (vino roze) i 84,0% (crveno vino).

Ključne reči:

maceracija, parametri kvaliteta, Prokupac, vino

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Introduction

Grape varieties, climate factors, soil and vineyard practices are crucial for the composition of the grapes, but the main wine attributes are highly influenced by winemaking procedures. In general, the application of different winemaking practices does not have a great effect on basic wine parameters (ethanol content, pH-value, total and volatile acidity), but significantly enhances the color, polyphenolic and volatile profile of wines (González-Neves et al., 2013). These characteristics are of great importance for the red wines as they are crucial for the sensorial properties, flavor, mouthfeel, aging ability of wines and value in the marketplace.

Also, it is well known that pre-fermentative practices, such as cold maceration or macerating enzymes application, considerably affects the extraction of compounds responsible for the phenolic composition and sensory characteristics of red wines (González-Neves et al., 2013; Casassa et al., 2019). Besides that, red wines with a higher concentration of phenolic compounds or antioxidant activity (Kocabey et al., 2016; Alencar et al., 2018), with better sensory properties (Şener, 2018) can be produced by prolonged maceration stage which occurs simultaneously with alcoholic fermentation. According to some authors, antioxidant activity of different Brazilian (Alencar et al., 2018), Portuguese (Jordão et al., 2012) and Australian (Yoo et al., 2011) red wines increased with maceration time prolongation, but after reaching its maximum value, it remained stable till the end of the process. Many studies showed that the basic quality parameters for Teran (Plavša et al., 2012), Castelão (Spranger et al., 2004), Bombino Nero (Suriano et al., 2015), Plavacmali (Budić-Leto et al., 2008) red wines do not deviate substantially with maceration time prolongation. On the other hand, the duration of maceration significantly affected the content of total anthocyanins, low molecular weight proanthocyanidins (Budić-Leto et al., 2008; Suriano et al., 2015) and tannins (Şener, 2018) in wine. Furthermore, the higher antioxidant capacity of Karaoglan (Kocabey et al., 2016), Merlot and Vranec (Kostadinović et al., 2012) wine was observed in samples produced with longer maceration time. Sensory analysis of Cabernet Sauvignon (Şener and Yildirim, 2013), Monastrell, Syrah (Busse-Valverde et al., 2012), Teran (Plavša et al., 2012), Karaoğlan (Yilmaztekin et al., 2015), Cabernet franc (Cadot et al., 2012) wines showed that maceration time significantly affected sensory characteristics, but some researchers (Barbará et al., 2020; Plavša et al., 2012; Yilmaztekin et al., 2015) highlighted that the wine produced with ten days maceration treatment gives the most harmonious wine, with the best aroma and flavor attributes. It was also shown that wine astringency and color intensity increased with longer maceration time (Cadot et al., 2012; Kocabey et al., 2016; Şener, 2018). All these results indicate the importance of maceration treatment in winemaking, as well as the fact that desirable quality of wine can be produced by optimizing maceration duration.

There are many indigenous grape varieties in Serbia, but Prokupac receives most of the attention in the local and international markets. Thus, the objective of this study was to examine the effect of maceration duration on basic wine parameters and to verify the effects on the total content of anthocyanins, phenolic compounds and flavonoids in order to obtain premium quality wine from these grape varieties.

Material and methods

Wines

Three wines were used for the experiment (white, rosé and red wine) produced from the Prokupac grape variety (*Vitis vinifera,* 2016 vintage), producer from Aleksandrovac (Republic of Serbia). The wines were made from the same grapes, where the key difference was duration of maceration. White wine was produced from wine dripping without maceration, and alcohol fermentation is carried out in the stum, i.e. in unfermented grape juice. For the production of rosé wine, short-term cold maceration was carried out for 2 hours in the press, while for the production of red wine the maceration lasted during the whole fermentation period of 2 weeks at temperature of 15°C to 20°C.

Relative density

Pycnometric analysis was used. The relative density is calculated by the formula:

$$d_{20}^{20} = \frac{m_{vine} - m_0}{m_{water} - m_0}$$

where: d_{20}^{20} - relative density of wine, m_{wine} - mass of pycnometer with wine, m_{water} - mass of pycnometer with water, m_0 - mass of empty pycnometer.

Alcohol content

Pycnometric analysis with distillation was used. From the calculated relative density in the formula below, the value expressing the alcohol content of the wine is read through the tables:

$$d_{20}^{20} = \frac{m_{dist} - m_0}{m_{water} - m_0}$$

where: d_{20}^{20} - relative density of distillate, m_{dist} - mass of pycnometer with distillate, m_{water} - mass of pycnometer with water, m_0 - mass of empty pycnometer.

Total extract

After the wine has been distilled to determine the alcohol content, a residue used to determine the extract content of the wine is left in the distillation balloon. The relative density is determined, where the rinsing, quantitative transfer, refilling, thermostating and measuring are performed as in the previous cases, and the contents of the extract in g/l are read from the tables (Blesić, 2016).

Total acids

Into an erlenmeyer flask 10 ml of wine and 20 ml of distilled water are poured. A few drops of the bromo-thymol blue indicator are added. Titration with a solution of NaOH 0.1N is carried out. Total acids (g/l, calculated on tartaric acid) are calculated as:

$$\text{Total acids} = V_{NaOH} \cdot 0.75$$

Volatile acids

About 300 ml of distilled water is poured into a larger normal vessel and 50 ml of wine into a smaller one. The normal water vessel is heated, and the created water vapor is introduced into the wine. When about 100 ml has been distilled, the wine is heated and at least 200 ml of wine is collected. 3-4 drops of phenolphthalein are added and titrated with a solution of NaOH (0.1N). Volatile acids (g/l, calculated on acetic acid) are calculated as:

$$\text{Volatile acids} = V_{NaOH} \cdot 0.12 \cdot 16.67$$

Sugar free extract

Sugar free extract is the difference between total dry extract and total sugars.

Ash

Into the previously measured porcelain cup 10 ml of wine is poured. Annealing is carried out at the temperature which rises from 200 °C to 500 °C, and when the content is completely charred, 5 ml of distilled water is added and evaporation is carried out in a water bath, as well as re-annealing and cooling in a desiccator. For determination of the ash content (g/l), measuring was performed on an analytical scale, while the mass of the empty cup was subtracted from the obtained mass, and the value obtained was multiplied by 100.

Reducing sugars

A fast French method based on the oxidoreduction processes of Fehling's solution and sugar was used, whereby divalent copper ($CuSO_4$) from Fehling's solution was reduced to monovalent (Cu_2O) and the sugar was oxidized to the corresponding acids. In an erlenmeyer flask 5 ml of Fehling I and Fehling II was poured and 20 ml of distilled water were added. The content of the erlenmeyer flask is heated to boiling and the filtrate is added from the burette. During the titration, the content in the erlenmeyer flask is kept around the boiling point. The filtrate is added until the last traces of blue colour disappear, indicating that the reduction of Fehling's solution has been completed. A red precipitate is formed at the bottom of the erlenmeyer flask and the liquid above the precipitate is colorless. The content of wine sugar (g/l) was calculated as:

$$\text{Sugars} = \frac{50}{a}$$

Free and total sulphur dioxide

The aspiration method was used. Into a flat bottom balloon 10 ml of H_2O_2 is added together with a few drops of methyl red and methylene blue indicator mixture. The colour should be grey, dirty blue, indigo blue or greenish. If a purple color is obtained, it should be neutralized with a solution of NaOH (0.01N). As soon as the purple color disappears, adding of NaOH solution is stopped. Usually, only a few drops are needed for neutralization. Into a round bottom balloon 10 ml of ortho-phosphoric acid and 20 ml of wine are poured. Then cooling by the condenser and aspiration pumps is activated. After 15 minutes, the whole free SO_2 goes into the flat bottom balloon, where the colour changes to purple. Titration with a solution of NaOH 0.01N is performed until the colour changes to green. The consumed volume of NaOH solution can be read on the burette and free SO_2 content (mg/l) is calculated as:

$$\text{Free } SO_2 = V_{NaOH} \cdot 16$$

The same sample of wine, heated for 10 minutes, is used to determine bound SO_2, while H_2O_2 and the mixture of indicators are prepared once again. After 10 minutes, titration with NaOH solution is carried out until the color changes to green. The amount of bound SO_2 is calculated in the same way. Total sulphur is the sum of bound and free sulphur.

pH-Value

The pH-value was measured with a pH-meter, three-point calibration.

Total phenols

A spectrophotometric method using Folin-Ciocalteu reagent was used. The absorbance is measured at 765 nm. Into a normal 10 ml vessel 0.1 ml of wine is poured. Red wines require necessary dilution, usually at a ratio of 1:5. Then 7.9 ml of distilled water is added, 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of Na_2CO_3 sodium-carbonate solution. The same mixture is prepared for the blank assay, only distilled water is used instead of wine. The absorbance was measured with a spectrophotometer and the results were read from a calibration curve.

Index of total phenols

Determination of total phenol index is a spectrophotometric method. The method is simple, but the results obtained do not provide the information on what phenolic compounds are present in wine and at what concentration. Red wine requires necessary dilution of 1:100, rosé wine from 1:10 to 1:50, and white wine 1:10. The absorbance was measured at 280 nm, in quartz cuvettes with an optical path of 10 mm, in relation to distilled water as blank assay. The total phenolic index of TPI is obtained by multiplying the absorbance by the dilution factor:

$$TPI = A_{280} \cdot F$$

Total flavonoids

Spectrophotometric method with aluminum chloride ($AlCl_3$) was used. Into a normal vessel with 4 ml of distilled water, 0.1 ml of wine is added. In case of red wines, a dilution of 1:5 is usually used. At zero moment, 0.3 ml of 5% $NaNO_2$ solution is added to the normal vessel, 0.3 ml of 10% $AlCl_3$ solution is added after 5 minutes, and 2 ml of NaOH 1 mol/dm^3 solution is added in the sixth minute, and the vessel is refilled with the distilled water to the index mark. The absorbance was measured at 510 nm, in comparison to the distilled water as blank assay. On the basis of the measured values from the calibration curve, the concentration of total flavonoids (mg/l) was determined.

Total anthocyanins

The Ribereau-Gayon method was used. One ml of wine was mixed with 1 ml of 0.1% HCl (in 96% ethanol) and 20 ml of 2% HCl solution. The obtained mixture was stirred and 10 ml of the mixture were separated into two test tubes. Into the first tube 4 ml of distilled water is added and into the second tube 20% of a solution of sodium bisulfite $Na_2S_2O_3$. After 20 minutes, the optical density at 520 nm was measured using a spectrophotometer. From the difference in optical density between the two tubes, the anthocyanin content (mg/l) was calculated based on a working curve for which malvidin-3 glucoside was used as a standard.

Results and discussion

The results of testing the basic physicochemical parameters are shown in **Tab. 1**. The alcohol content ranged from 11.43 vol% to 11.85 vol%, meaning that the maceration conditions had no effect on the alcohol content in the wine.

The content of reducing sugars ranged from 1.1 g/l to 1.4 g/l, indicating that the degree of sugar overfermentation was complete, regardless of the maceration conditions.

With the extension of the maceration time in wines, an increase in the content of the sugar-free extract and the ash content were determined. In this respect, the extraction of various compounds from the solid parts of the grapes (skin, seeds) into stum is prolonged, whereby the content of the sugar-free extract and the ash content in wine are increased. The lowest content of sugar-free extract was found in white wine (18.6 g/l), with virtually no maceration. With rosé wine, where the maceration lasted for two hours, the content of sugar-free extract was just slightly higher, and was 18.9 g/l. The highest content of sugar-free extract was found in red wine at 21.3 g/l. The lowest ash content was also found in white wine (1.86 g/l), while the highest content was in red wine (2.34 g/l).

Total acid content ranged from 4.34 g/l to 5.36 g/l and volatile acid content from 4.5 meq/l to 6.5 meq/l. Duration of maceration caused decrease in the content of total acids, so the lowest content was in red wine of 4.34 g/l. The reduction of total acids during maceration is explained by the release of minerals that pass into the wine and react with tartaric acid during maceration period, resulting in

Table 1. Basic physicochemical parameters of quality of white, rosé and red wine

Parameters	White wine	Rosé wine	Red wine
Relative density, 20 °C	0.9922	0.9918	0.9927
Alcohol, % V/V	11.43	11.76	11.85
Total extract, g/l	19.0	19.0	21.6
Reducing sugars, g/l	1.4	1.1	1.3
Sugar free extract, g/l	18.6	18.9	21.3
Total acids, g/l	5.36	5.16	4.34
Volatile acids, meq/l	6.5	4.5	5.7
pH-value	3.24	3.26	3.75
Free SO_2, mg/l	26.4	28.8	12.8
Total SO_2, mg/l	148.0	110.4	28.8
Ash, g/l	1.86	1.99	2.34

Table 2. Chromatic characteristics of white, rosé and red wine

Parameters	White wine	Rosé wine	Red wine
A_{420}	0.098 ± 0.005	0.187 ± 0.002	1.455 ± 0.030
A_{520}	0.030 ± 0.003	0.096 ± 0.002	1.882 ± 0.020
A_{620}	0.001 ± 0.001	0.008 ± 0.002	0.483 ± 0.019
I	0.129± 0.006	0.291± 0.006	3.820±0.013
N	3.337± 0.388	1.955± 0.046	0.773± 0.021
A_{420}%	76.44± 2.70	64.33± 0.88	38.09± 0.66
A_{520}%	23.05± 1.90	32.91± 0.53	49.26± 0.61
A_{620}%	0.51± 0.87	2.75± 0.90	12.65± 0.52
dA%	/	/	48.49± 1.24

the formation of salts.

With reduction of the total acid content, the pH value of the wine increases, so that a maximum value of 3.75 was measured in red wine, while with white wine it was 3.24.

According to the available results (Budić-Leto et al., 2008) the maceration time had no effect on the relative density, alcohol content, total extract of reducing sugars, acidity and ash in red wine.

Within the experimental part of this paper, the intensities and shades of wine colors were analyzed. **Tab. 2** shows the chromatic characteristics of the tested wines. The results are presented as the mean value out of three repetitions, with standard deviation.

The color intensity of the wines (I) is the lowest in white wine; when rosé wine is concerned, short maceration led to a significant increase in colour intensity, while with red wine there is a significant rise in measured value. The shade of colour (N), which is mostly used as a parameter of aging wine, is highest in white and lowest in red wine.

The colour of rosé wine is non-specific. According to Glories (1984), the optimum ratio of the colour components to the type of rosé wine is 35:55:10, and in the case of the tested rosé wine is 64:33:3. It is characterized by an extremely light colour with a predominant yellow-orange tone.

Maceration duration changed some chemical constituents and color of red wines (Kocabey et al., 2016). Extended maceration duration resulted in increase in the concentration of phenolic compounds, reflecting the antioxidant activities of wine. The highest concentrations of total and individual phenolic compounds as well as antioxidant activities were found in wines macerated for 15 days.

The maceration process plays an important role in the composition of the colour and sensory properties of red wine (Şener, 2013, 2018). Macerations carried out at low temperature ranges (10 °C to 15 °C) resulted in red wines with the highest levels of total phenolic content, anthocyanin and colour intensity, and richer fruity, flowery and spicy aroma. Prolonged maceration leads to a stable red colour, too.

Chromatic characteristics of red wines were observed four times during the year (Babincev et al., 2016). Intensity, hue and brilliance of color of these wines were determined. The highest value of color intensity was observed in young wines (Merlot 2.26, Vranac 2.24) and the lowest value was recorded for Prokupac, aged 12 months (1.18). With wine ageing the color intensity slightly decreased. The maximum value for hue in a very young wine was found in Merlot (0.86) and in 12-month-old wines Cabernet (0.99). Brilliance of wine also decreased with wine ageing and it was most pronounced in young Vranac (47.1) and the lowest value was found in 12-month old Prokupac (27.7).

Test results for total phenols, total phenol index (TPI), total flavonoids, and total anthocyanins are shown in **Tab. 3** as the mean value out of three repetitions with standard deviation.

Table 3. Total flavonoids and anthocyanins in white, rosé and red wine

Parameters	White wine	Rosé wine	Red wine
Total phenols, mg/l GAE	565.4 ± 17.0	523.4 ± 3.5	1433.8 ± 90.9
TPI (A_{280})	12.29 ± 0.49	13.83 ± 0.41	31.77 ± 4.19
Total flavonoids, mg/l CE	153.4±1.8	179.3±2.1	761.7±15.9
Total anthocyanins, mg/l	/	2.8	154.0±2.9

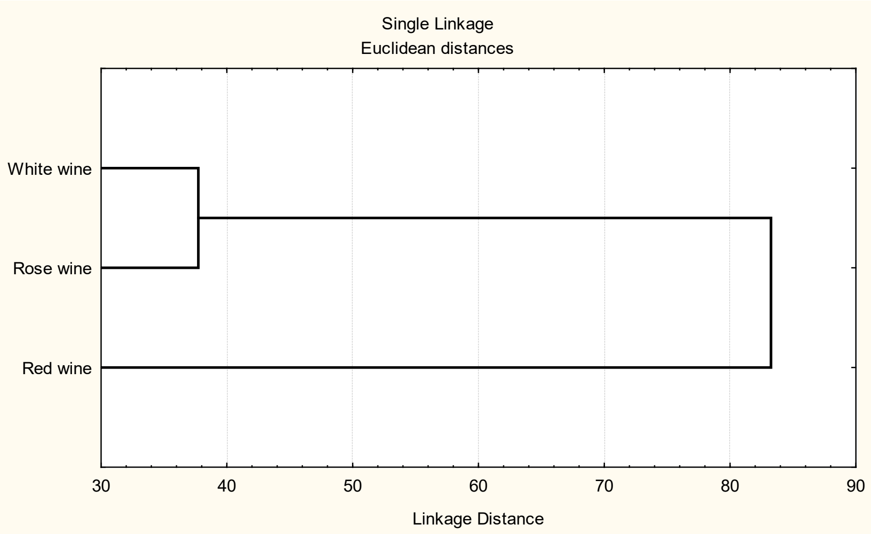

Fig. 1. Dendrogram obtained by cluster analysis using means of basic physicochemical parameters of white, rosé and red wine quality

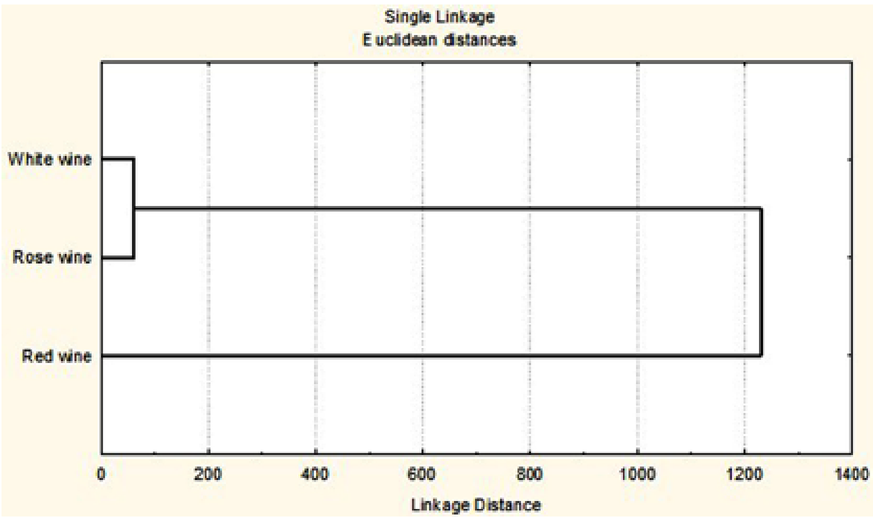

Fig. 2. Dendrogram obtained by cluster analysis using means of chromatic characteristics and total flavonoids and anthocyanins of white, rosé and red wine

Total phenols, in contrast from expected, are higher in white wine than in rosé wine. Most are found in red wine and their value is 1433.8 mg/l.

On the other hand, the index of total phenols in rosé wine (13.83) is slightly higher than in white wine (12.29), while it is the highest in red wine (31.77).

Total flavonoids are the lowest in white wine (153.4 mg/l) and the highest in red wine (761.7 mg/l). Anthocyanins are below the lower detection limit in white wine, 2.8 mg/l, and in red wines they are 154 mg/l. Here the influence of maceration becomes obvious.

According to the results of Alencar et al. (2018), the content of phenolic compounds increased up to 15th day during the process of maceration, and anthocyanins up to 20 days. Extending the maceration time thus also improved the antioxidant activity of the wine.

Chemometric analysis of wine samples is shown in **Fig. 1** and **2**, which were made in the program Statistica v.8.0 (StatSoft Inc., Tulsa, OK, USA).

Conclusion

Maceration has a very large influence on the physical and chemical parameters of wine quality. During the maceration process, many substances are extracted, and transferred from the solid parts into the wine. As a result, red wines have a much more complex chemical composition than white and rosé wines.

In our samples, the duration of maceration had no significant effect on the relative density and alcohol content. The effect is significant on the content of the sugar-free extract and the ash content due to the higher extraction of nitrogen compounds, minerals and numerous other substances.

Chromatic analysis showed that the extraction of coloured substances was much higher with red wine, where the maceration lasted for two weeks at temperature ranging from 15 °C to 20 °C, compared to rosé wine, which only used short-term cold maceration. Total phenols in red wine are almost three times higher than in white and rosé wines, and total flavonoids by slightly more than four times. The difference is greatest when it comes to anthocyanins extraction, which is almost nonexistent with white wine, and with rosé wine 55 times less concentrated than in red wine. The tannin concentration is also highest in red wine and lowest in white wine.

The parallel examination of wine produced from the same, autochthonous Prokupac variety, indicates the potential of this variety and the opportunities it offers to producers for the application of different technologies.

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