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Original Article

Effect of Pre-fermentation Maceration on the Content of Antioxidant Compounds in Grapevine Juice

Mojmir BARON, Michal KUMSTA, Bozena PRUSOVA*, Lenka TOMASKOVA, Jiri SOCHOR

*Mendel University in Brno, Faculty of Horticulture, Department of Viticulture and Enology, Valtická 337, CZ-691 44 Lednice, Czech Republic; MojmirBaron@seznam.cz; michal.kumsta@mendelu.cz; prusova.bozena@email.cz (*corresponding author); lenka.tomaskova@mendelu.cz; jiri.sochor@mendelu.cz*

Abstract

Lower antioxidant capacity of white wines is caused by a lower content of phenolic compounds. In red wine, a higher content of phenolic compounds is the result of maceration. This study deals with effects of pre-fermentation maceration on contents of some selected compounds (above all antioxidants) in juices made of grapes of white varieties 'Welschriesling', 'Green Veltliner' and 'Neuburger'. The grapevine mush was macerated for 0; 2; 4; 8; 12; 24 and 48 hours. The spectrophotometry methods was used to estimate juice antioxidant activity and content of flavanols, anthocyans, catechins, hydroxycinnamic acids and flavonols. Contents of total titratable acids and pH were estimated as well. Results of this study show increasing tendency of all parameters with length of maceration. The highest content of antioxidants were found out in the variety 'Welschriesling'. Important are increasing values of hydroxycinnamic acids. However, the main role of the phenolics acids is to contribute to the anti-oxidant capacity, the tartaric esters of hydroxycinnamic acids (particularly caftaric acid) are centrally involved in oxidation and browning reactions in white wine. Their levels increased during the whole period of maceration. In 'Welschriesling', their content increased by 74 $mg \cdot L^{-1}$. Decreasing tendency show results of titratable acids. Their contents decreased within the first 12 hours of maceration and thereafter they do not change. Longer must maceration could contribute to a decrease in contents of total titratable acids and, thus, to a better harmony of produced wine.

Keywords: hydroxycinnamic acids, juice browning, maceration, phenolics, white wines

Introduction

In the course of alcoholic fermentation, the process of maceration enables to extract phenolic compounds contained in solid parts of grapes. The maceration step, mostly used in technology of red wines, results in the extraction of phenolic substances and production of grape juices and wines rich in these compounds and showing strong antioxidant properties. This maceration step is the most important difference between technology of red and white wine (Paganga *et al.,* 1999).

In red wine, a higher content of phenolic compounds is the result of maceration, in the course of which the phenolic compounds are released (i.e. extracted) from skins, seeds, stalks and pulp of berries (Fuhrman *et al.,* 2001). The extraction of phenolic compounds is further supported by alcohol produced in the course of fermentation and by the increasing temperature. The maceration mostly does not take place in technology of white wines and for that reason the content of phenolic compounds of these wines are lower and they also show a lower antioxidant activity (Lamuela-Raventos and De la Torre-Boronat, 1999; Vinson and Hontz, 1995). The most frequently discussed problem is the increase in contents of phenolic substances, aromatic compounds and organic acids during maceration of white wines. An increase in the content of aromatic compounds is the most desirable phenomenon. Increased levels of phenolic substances are (more or less) not required, mainly due to the fact that they cause the occurrence of bitter, astringent and/or acrid tones in the final product (Peinado *et al.,* 2004). At present, there is an intensive discussion concerning effects of maceration of white grapevine varieties on the quality of final products (Lamuela-Raventos and De la Torre-Boronat, 1999). On the other hand, the maceration of white grapevine varieties is more suitable for white grapevine varieties containing more acids because it shows a positive effect on the extraction of mineral substances (Ribéreau-Gayon *et al.,* 2006).

The duration of the process of maceration (that is one of the most important mechanisms that control the release of these substances into the juice under conditions of a gentle crushing of grapes) is a matter of discussion. In white wine, phenolic substances occur above all as hydroxycinnamic acids that play a decisive antioxidant role prior to the onset of fermentation because they react with polyphenol oxidase and

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inhibit the process of juice browning (Hernanz *et al.,* 2007). However, the phenolics acids contribute to the anti-oxidant capacity of both red and white wines, for example, the tartaric esters of hydroxycinnamic acids (particularly caftaric acid) are centrally involved in oxidation and browning reactions in white wine (Ribereau Gayon *et al*., 2006).

At present, there is an intensive discussion concerning effects of maceration of white grapevine varieties on the quality of final products (Lamuela-Raventos and De la Torre-Boronat, 1999).

The aim of this study was to map and describe which amounts of aforementioned substances were extracted and appeared in grapevine juice within different time intervals of maceration.

Materials and Methods

Biological material

The experiment involved three grapevine varieties: 'Welschriesling', 'Green Veltliner' and 'Neuburger', vintage 2015. Grapes were crushed and destemmed in a stainless crusher-destemmer, macerated, and pressed. The mush was macerated for 0; 2; 4; 8; 12; 24 and 48 hours.

Grapes used in the experimental part of this study originated from 20 years vineyards situated in the locality Velké Bílovice (wine-growing subregion of Velké Pavlovice). In this region, the average annual precipitations and temperature are 550 mm and 9.5 °C, respectively. In the soil of this region, content of clay is 20%, content of free Ca is 12%. Soil pH is in the range 6.1-6.9.

Rootstock 'CR2' typical for Czech Republic was used. The Czech grape training is modificated German training specially Rhone-Hessen, nevertheless fruitfull wood is horizontal tying. Clasps planting: 1 × 1.2 m. High of trunk is 0.75 m. Berries were collected random from the top, middle, and bottom of selected clusters. In order to obtain representative sample, colored berries was not favored over greens. Berries were stored in sealed plastic bags in the refrigerator until processing.

Estimation of antioxidant activity

The determination procedure was described earlier (Sochor *et al.,* 2010a). When doing this, a 150 µl volume of the reagent (0.095 mM 2,2-diphenyl-1-picrylhydrazyl - DPPH•) was incubated with 15 µl of wine sample. The absorbance was measured at 505 nm for 10 minutes. Results were converted to equivalents of gallic acid.

Estimation of total flavanols

Concentrations of total flavanols were estimated by means of method based on the reaction of juice with p-dimethylaminocinnamaldehyde (DMACA). The difference between this method and a widely used reaction with vanillin consists in the fact that in case of DMACA use there is no interference with anthocyans. Besides, it is also more sensitive and selective. A sample of 20 µl wine was added into a 1.5 ml Eppendorf micro test tube containing 980 µl of the reagent (0.1% DMACA and 300 mM HCl in MeOH), vortexed and allowed to react at room temperature for 12 minutes. After this time interval, the absorbance value was measured at 640 nm and the result was compared with a blank sample. Using catechin as a standard (10-200 mg.L^{-1}), concentrations of total flavanols were calculated from the calibration curve. Results are presented as catechin equivalents in mg.L^{-1} (Li *et al.,* 1996).

Estimation of total contents of hydroxycinnamic acids and anthocyanins

These measurements were performed using well-established spectrophotometric methods (Zoecklein, 1990). The wine sample was placed into a 0.2 cm path-length quartz cuvette, Thereafter, 200 µl of the sample and 1.8 ml of 1.1 M HCL were added and the resulting solution was thoroughly mixed and kept for a period of 180 minutes at the room temperature.

A 0.22 M solution of $K_2S_2O_5$ was used as a blank. When estimating anthocyanins, the absorbances were read at 320 nm (A_{320}^{HCl}) and at 520 nm (A_{520}^{HCl}). Concentrations of total anthocyanins (mg.L^{-1}) were calculated as follows:

Total content of anthocyanins (mg.L^{-1}) = 4 × dilution × /A_{520}^{HCl} - (5/3) × A_{520}^{SO2}.

Total content of hydroxycinnamic acids (mg.L^{-1}) = 10 × dilution × 12.387 × A_{320}^{HCl}.

Estimation of total flavanols and catechins

Total flavanols were estimated using the p-dimethylaminocinnamaldehyde (DMACA) method (Li *et al.,* 1996). As compared with a widely used vanillin method, a great advantage of this method is that there is no interference with anthocyanins. Furthermore, it provides a higher sensitivity and a better specificity. Wine sample (20 µl) was poured into a 1.5 ml Eppendorf micro test tube and 980 µl of DMACA solution (0.1% in 1 M HCl in MeOH) was added. The mixture was vortexed and allowed to react at room temperature for 12 min. The absorbances at 640 nm (flavanols) and at 360 nm (catechins) were then read against a blank sample prepared in a similar way but without DMACA. The concentration of total flavanols was then estimated from a calibration curve and constructed by plotting known solutions of catechin (1-16 mg.L^{-1}) against A_{640} (r^2 = 0.998). Results were expressed as mg.L^{-1} of catechin equivalents.

Estimation of pH

Values of pH were estimated in non-diluted wine samples using a WTW pH-meter 526 in combination with the SenTix 21 pH-electrode (manufacturer the WTW Company, Germany).

Estimation of total titratable acids

Contents of total titratable acids were estimated in an automatic titrator TITROLINE EASY (manufacturer SI Analytics GmbH, Germany). A 0.1 mol.L^{-1} solution of NaOH was used as a titration reagent. For analyses, 10 ml wine samples diluted with 10 ml of distilled water was used. Individual samples were thereafter titrated up to pH 8.1. For the pH detection again the SenTix 21 pH-electrode was used. After the end of titration, the consumption of NaOH solution was read on the titrator display. This consumption was multiplied with the factor of NaOH solution used for the titration and with the coefficient 0.75. The result of this multiplication was equal to the content of titratable acids in the wine sample (mg.L^{-1}).

Statistical analyses

Statistical analyses, tabular outputs and graphs were generated using programme packages Excel 2007 (manufacturer Microsoft Office, USA) and the statistical software Statistica 10 (Copyright © StatSoft).

Correlations existing between individual pairs of antioxidants are expressed as the Pearson's correlation coefficient and characterize the tightness of individual relationships. Values between 0.1 and 0.3 indicate a weak correlation while those between 0.4 and 0.6 and between 0.7-0.8 indicate medium and strong correlations, respectively. Values above 0.9 mean that the correlation is very strong. Significant correlations ($p < 0.05000$).

Results and Discussion

Estimation of antioxidant activity

The antioxidant activity expresses the capability of the biological matrix (i.e. of the grapevine juice) to quench and/or trap free radicals. The most frequent methods of its estimation are methods of spectrophotometry. Advantages of these methods consist in their simplicity, good reproducibility, cheapness and, last but not least, unpretentiousness (as far as the human resources are concerned). The estimation of antioxidant activity by means of DPPH˙ radicals represents one of the most frequent methods of this type (Sochor *et al.,* 2010b).

Liang *et al.* (2014) studied phenolic profiles and antioxidant activities in 24 grapes of varieties of *V. vinifera* and observed a great variation in contents of total phenolic compounds (95.3-686.5 mg.100 g^{-1}) and flavonoids (94.7-1,055.0 mg.100 g^{-1}). They mentioned also great differences in antioxidant effects (the capacity to absorb oxygen radicals ranged from 378 to 3,386 mg of Trolox equivalents in 100 g. The total antioxidant activity was significantly correlated with levels of total phenolic substances and flavonoids. Must and red grapes showed much higher contents of photochemicals and antioxidant effects than table and white or yellow ones (Liang *et al.,* 2014).

De la Cerda-Carrasco *et al.* (2014) mentioned that both the antioxidant activity and phenolic composition of wine were dependent on the differential extraction of chemical compounds from grapes during the process of wine making and that it was also critically associated with its quality (de la Cerda-Carrasco *et al.,* 2015).

The antioxidant activity, as expressed by means of the DPPH equivalent, is presented in Fig. 1. Its increase in the course of maceration was observed in all three varieties under study. In 'Welschriesling' its initial and final (i.e. after 48 hours) values were 81.6 mg.L^{-1} and 143 mg.L^{-1}, respectively. In 'Green Veltliner' and 'Neuburger', these increases were nearly identical (i.e. about 60 mg.L^{-1}) at the beginning of maceration and increased to 108 mg.L^{-1} to its end.

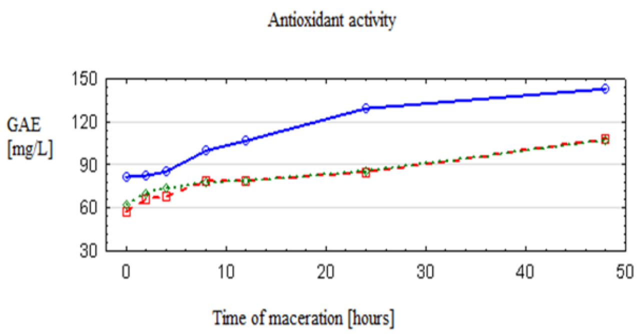

Fig. 1. Values of the antioxidant activity as determine with the DPPH test ('Welschriesling' – blue continuous line; 'Green Veltliner' – red dashed line; 'Neuburger' – green dotted line, GAE-gallic acid)

Estimation of flavanols content

Flavanols are those plant compounds, the name of which is derived from the Latin word *flavus* that means 'yellow'. The most important of them are catechin, epicatechin, gallocatechin, and epogallocatechin (da Silva *et al.,* 2014).

In contents of flavanols, however, there were certain differences among these three varieties (Fig. 2). At the beginning of maceration, the lowest content of flavanols was observed in 'Green Veltliner' (21.3 mg.L^{-1}). After 48 hours it was more than doubled and reached the value of 50.6 mg.L^{-1}. The variety 'Neuburger' showed the highest initial level of flavanols (32.5 mg.L^{-1}). This value increased to as much as 60.6 mg.L^{-1}; this was the second highest value recorded. Although the 'Welschriesling' began at the level of 26.3 mg.L^{-1}, it showed the highest dynamics and the value of DPPH was nearly tripled (to 76.7 mg.L^{-1}) after 48 hours of maceration.

Estimation of the content of anthocyans

Anthocyans are water-soluble pigments that belong to the group of flavonoids. Their colour is changing in dependence on pH. Acid, neutral and alkaline solutions of anthocyans are usually red, violet and blue, respectively. They are present above all in skins of blue grapes. They are released from these skins in the course of maceration and cause the red coloration of wine. Anthocyanins readily polymerize with tannins; they also play an important role in tannin retention in and ageing of wine (Holton and Cornish, 1995; Prior *et al.,* 1998).

Alvarez *et al.* (2006) studied the effect of pre-fermentation maceration of red grapes on contents of polyphenolic compounds, anthocyans and color intensity of wine and concluded that a prolonged maceration resulted in increased contents of these compounds in the fermented mush (Alvarez *et al.,* 2006).

Ivanova-Petropulos *et al.* (2015) studied phenolic compounds in 22 samples of red wine originating from different wine-growing regions. All of them showed a relatively high content of total phenols and also a high antioxidant activity. When using HPLC, altogether 19 phenolic compounds were identified. Their contents were influenced above all by the variety and, to a smaller extent, also by yeasts and methods of fermentation. As compared with juices fermented using commercial (i.e. cultural) yeasts, especially the application of locally isolated (i.e. autochthonous) yeasts increased levels of anthocyans and phenolic acids (Ivanova-Petropulos *et al.,* 2015).

Contents of anthocyans are presented in Fig. 3. The lowest levels were found out in the variety 'Neuburger'; in this case the content of anthocyans (1.4 mg.L^{-1}) was not higher than in other two varieties even after 48 hours of maceration. In 'Welschriesling' and 'Green Veltliner', initial values were very similar (1.4 and 1.6 mg.L^{-1}, respectively) and a similar trend was observed also after 8 hours of maceration. After 48 hours, however, the highest level of anthocyans (3.8 mg.L^{-1}) was recorded in the variety 'Welschriesling'.

Estimation of the content of catechins

Catechins react with tannins to make the primary flavour component in red wine. They belong to the group of flavanols and their taste is tannic to bitter. In general, the smaller the catechin polymers, the stronger this sensation tends to be. The concentration of catechins in wine informs the winemaker

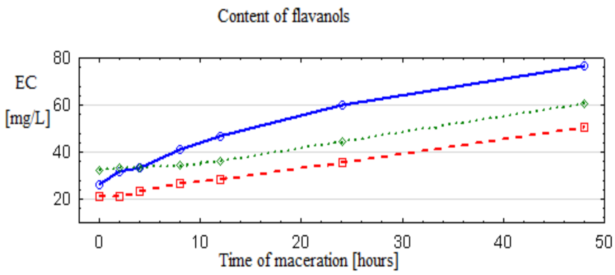

Fig. 2. Changes in contents of flavanols in the course of maceration ('Welschriesling'– blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line, EC-catechinin equivalents)

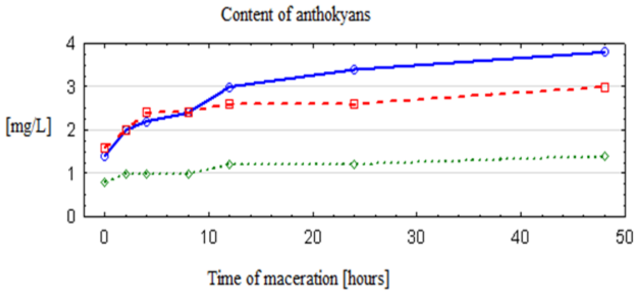

Fig. 3. Changes in contents of anthocyans in the course of maceration ('Welschriesling' – blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line)

about the intensity of extraction of these compounds from seeds (Arts *et al.,* 2000).

Contents of catechins are illustrated in Fig. 4. After 48 hours, it was nearly doubled in all three varieties under study. In 'Welschriesling', the initial and final concentrations were 854.1 and 1,478.4 mg.L^{-1}, respectively while in 'Green Veltliner' and 'Neuburger' they were 645 and 1,109.8 mg.L^{-1} and 679.3 and 1,028.2 mg.L^{-1}, respectively.

Estimation of the content of hydroxycinnamic acid

Hydroxycinnamic acids are classified as non-flavonoids. The group of hydroxycinnamic acids involves gallic acid, coumaric acid, caffeic acid, ferrulic acid, coutaric acid, caftaric acid and fertaric acid. In white wine, these are the most important phenolic compounds and participate in the determination of its color. They occur in the pulp of berries (Adams, 2006).

Hydroxycinnamic acids play a decisive antioxidant role prior to the onset of fermentation because they react with polyphenol oxidase and inhibit the process of juice browning (Hernanz *et al.,* 2007). However, the main role of the phenolic acids is to contribute to the anti-oxidant capacity of both red and white wines. For example, the tartaric esters of hydroxycinnamic acids (particularly caftaric acid) are centrally involved in oxidation and browning reactions in white wine (Ribereau Gayon *et al.,* 2006).

Contents of hydroxycinnamic acids are presented in Fig. 5. Their levels increased during the whole period of maceration. In 'Welschriesling', their content increased by 74 mg.L^{-1} while in 'Green Veltliner' and 'Neuburger' they rose by 57 and 34 mg.L^{-1}, respectively.

Estimation of the content of flavonols

Flavonols represent a group of secondary metabolites that involves many soluble phenolic compounds occurring in grapevine berries. These compounds play different physiological roles and for that reason they often participate in mechanism protecting plants against both biotic and abiotic stresses (Braidot *et al.,* 2008). The most important flavonols are quercetin, myricetin, kaempferol, isorhamnetin and rutin. Flavonols are mostly strong antioxidants and show anti-inflammatory effects (Kennedy *et al.,* 2002).

Contents of flavonols increased relatively slowly (Fig.6). The initial and final values of the variety 'Neuburger' were 28.4 and 37.2 mg.L^{-1}, respectively. In the variety 'Green Veltliner', the corresponding values were 33.7 and 50.4 mg.L^{-1}. The highest levels found out in the variety 'Welschriesling' were 44.7 and 68.5 mg.L^{-1}, respectively.

Estimation of pH values

It was found out that pH values were dependent on the rate of dissociation of acids, concentrations of free acids, and amounts of basic substances that were capable to bound hydrogen ions. This was an important parameter that enabled to determine the degree of ripeness of grapes. Its optimum fluctuates between 3.1 and 3.3. In case that pH value is higher than 3.5, the risk of a high activity of microorganisms is increased. This concerns above all different bacteria and wild yeasts. The susceptibility of juice and also of wine to show the presence of volatile acids, mousiness and other diseases and faults of wine is increased. At higher pH values the efficiency of sulphur dioxide decreases and the oxidation of both juice and wine is accelerated (Ribéreau-Gayon *et al.,* 2006).

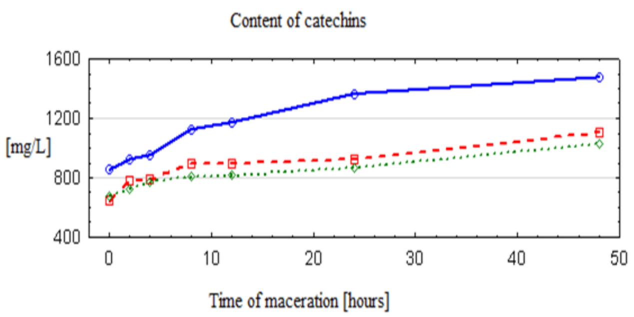

Fig. 4. Changes in contents of catechins in the course of maceration ('Welschriesling'– blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line)

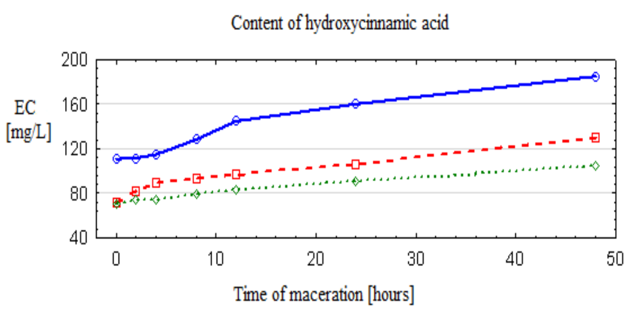

Fig. 5. Changes in contents of hydroxycinnamic acid in the course of maceration ('Welschriesling' – blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line, EC-catechinin equivalents)

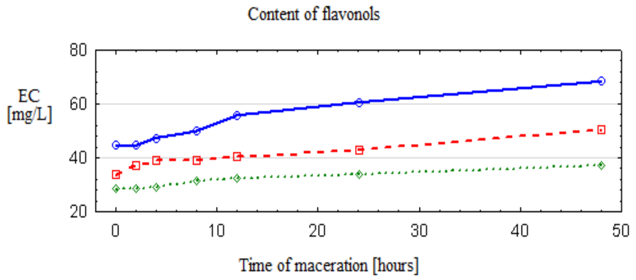

Fig. 6. Changes in contents of flavonols in the course of maceration ('Welschriesling' – blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line, EC-catechinin equivalents)

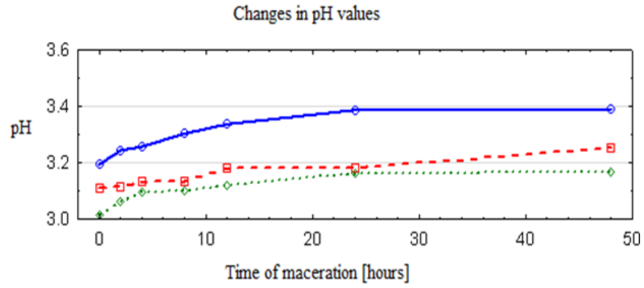

Fig. 7. Changes in pH values of pH in the course of maceration ('Welschriesling'– blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line)

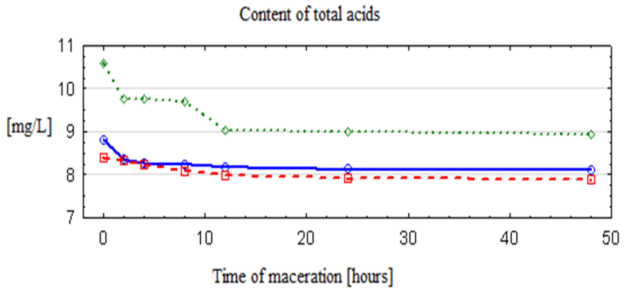

Fig. 8. Changes in contents of total acids in the course of maceration ('Welschriesling' – blue continuous line, 'Green Veltliner' – red dashed line, 'Neuburger' – green dotted line)

Table 1. Results of correlation analysis (A - time of maceration; B – flavanols; C – antioxidant activity (DPPH); D – anthocyans; E - catechins; F - hydrocinnamic acids; G – flavonols; H – pH; I - total acids)

	A	B	C	D	E	F	G	H	I
A	1	0.83	0.78	0.54	0.72	0.57	0.45	0.52	-0.51
B	0.83	1	0.85	0.32	0.73	0.60	0.42	0.54	-0.17
C	0.78	0.85	1	0.61	**0.95**	0.90	0.78	0.86	-0.48
D	0.54	0.32	0.61	1	0.77	0.81	0.89	0.82	**-0.94**
E	0.72	0.73	**0.95**	0.77	1	**0.96**	0.89	**0.93**	-0.65
F	0.57	0.60	**0.90**	0.81	**0.96**	1	**0.97**	**0.98**	-0.69
G	0.45	0.42	0.78	0.89	0.89	**0.97**	1	**0.97**	-0.78
H	0.52	0.54	0.86	0.82	**0.93**	0.98	0.97	1	-0.69

The most significant correlations existed between hydrocinnamic acids and pH (r = 0.98), between flavonols and hydroxycinnamic acid (r = 0.97) and between flavonols and pH (r = 0.97). Significant correlations ($p < 0.05000$)

Values of pH are compared in Fig.7. This parameter increased only by tenths; in 'Welschriesling' it increased from 3.19 to 3.39, in 'Green Veltliner' from 3.11 to 3.25 and 'Neuburger' from 3.02 to 3.17. It is also necessary to say that after 24 and 48 hours the values of pH were nearly the same and that their increase did not continued.

Estimation of the content of total titratable acids

Tartaric acid, lactic acid and smaller amounts of succinic acid, citric acid and acetic acid are the most important organic acids occurring in grapes. In grapes, the total content of acids usually ranges from 7 to 10 $g.L^{-1}$.

The maceration of white grapevine varieties is more suitable for white grapevine varieties containing more acids because it shows a positive effect on the extraction of mineral substances (Ribéreau-Gayon *et al.,* 2006).

Contents of titratable acids are presented in Fig. 8. In all varieties under study, contents of these acids decreased within the first 12 hours of maceration and thereafter they do not change. The highest initial content of acids was found out in the variety 'Neuburger', viz. 10.59 $mg.L^{-1}$ at the beginning 9.02 $mg.L^{-1}$ after 12 hours and 8.95 $mg.L^{-1}$ after 48 hours. The juice of the variety 'Welschriesling' contained at the beginning of maceration 8.82 $mg.L^{-1}$; after 12 and 48h, these values were 8.19 and 8.12 $mg.L^{-1}$, respectively. Their lowest concentrations of total titratable acids were recorded in the variety 'Green Veltliner' (8.40 $mg.L^{-1}$ at the beginning and 7.88 $mg.L^{-1}$ after 48h).

Statistic evaluation

Correlations existing between individual pairs of antioxidants are presented in Table 1. In this table, significant correlations are highlighted in bold. They are expressed as the Pearson's correlation coefficient and characterize the tightness of individual relationships. Values between 0.1 and 0.3 indicate a weak correlation while those between 0.4 and 0.6 and between 0.7-0.8 indicate medium and strong correlations, respectively. Values above 0.9 mean that the correlation is very strong.

Conclusions

Previous studies observed content of phenolic compounds and antioxidation activity in red wines depending on the length of maceration. This study confirms the expected increasing of antioxidant activity, flavanols, anthocyans, catechins, hydroxycinnamic acids and flavonols in white varieties of wines. The most interesting fact is questionable quality of wine influenced undesirable increasing content of phenolic compounds in white wines. On the other hand, longer must maceration could contribute to a decreasing in contents of total titratable acids and, thus, to a better harmony of produced wine. This can be used in individual vintages with a lower pH of grapes (and with a high content of acids). The pH value and the "buffering capacity" of juice increases probably due to a release of potassium ions and N-substances from the solid particles present in the mush; this thereafter resulted in a better harmony and more definite sensory character of produced wine. However, it is quite clear that an "ideal period of maceration" still remains to be the most important factor that influences properties of produced wine and its desirable sensory character.

Acknowledgements

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