The Changes of Selected Phenolic Substances in Wine Technology

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Abstract: The effects of the pressing technology and clarification of white grape musts on concentrations of phenolic compounds and their antioxidative capacity were investigated. Four different varieties were processed by hydraulic or pneumatic pressing technologies. In the individual stages of pressing and after the application of different doses of the clarification agent, must samples were analysed for the content of polyphenols and the antioxidative capacity. The highest concentrations of caftaric acid were estimated in the musts made by hydraulic pressing from grapes of Welschriesling variety. On the other hand, musts made from grapes of Grüner Veltliner showed the highest contents of *trans*-piceid in both variants of pressing. The values of antioxidative capacity of the must samples analysed were not significantly different. The influence of clarification on the changes of phenolic substances in young red wines of Saint Laurent variety was also studied. Six various clarifiers were tested as applied in two different doses. Polyvinylpolypyrrolidone caused the highest losses of *trans*-resveratrol in the course of red wine clarification. The concentrations of catechin, epicatechin, and total anthocyanins as well as the colour parameters of red wines were influenced at most by the application of egg white.

Keywords: polyphenols; piceid; resveratrol; caftaric acid; colour; antioxidative capacity; grape must; pressing; red wine; clarifying agents

Vitis vinifera L. fruits show a high concentration and a great variety of phenolic compounds. Grape juice is a rich source of flavonoids and hydroxycinnamate derivatives in the human diet (Rice-Evans *et al*. 1996). The fermented grape fruit product – wine is also rich in polyphenols. Phenolic substances and other components of wine increase the serum antioxidant capacity and protection of LDLs against oxidation and decrease native plasma protein oxidation and reduce platelet aggregation. Resveratrol, 3,4',5-trihydroxystilbene, has been linked to these effects. Resveratrol also exists in its glucosidic form, named piceid (Keevil *et al*. 2000; Chou *et al*. 2001; etc.).

The phenolic compounds in wine range from relatively simple compounds to complex tannin-type substances with antioxidant activity. The antioxidative capacities of German wines ranged from 0.6 to 2.8 mmol/l while the wines from Tokaj showed much higher values, the mean

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being 4.2 mol/l (Nikfardjam *et al.* 2006). The antioxidant activity of wines is determined by different methods based on the elimination of radicals or measurement of the redox properties of the following substances: TEAC, DMPD, ORAC, FRAP (Fogliano *et al.* 1999; Fernández-Pachón *et al.* 2004; Lachman *et al.* 2007). The photochemiluminescence method (PCL) for quantification of antiradical properties and non-enzymic antioxidants of polycomponent systems was developed by Popov and Lewin (1994). It is based on the antioxidant-sensitive inhibition of the photo-induced autoxidation of luminal which is accompanied by chemiluminescence.

White wines contain significantly lower amounts of total polyphenols as compared with red wines, mainly of hydroxycinnamate and flavonoid derivatives (Fernández-Pachón *et al.* 2006). The composition of polyphenols is dependent on the variety, growing conditions, ripeness of grapes, and their infestation by fungal diseases, and wine making (Pezet & Cuenat 1996; Jeandet *et al.* 2006; Mikeš *et al.* 2008). Phenolic composition of white wines can be affected by pressing and grape maceration (Villaño *et al.* 2006). The influence of pre-fermentative maceration variables (time and temperature) on the phenolic profile of white wines has been evaluated by Hernanz *et al.* (2007).

The methods of vinification and applied technological procedures (maceration, fermentation, clarification, aging etc.) can significantly modify both the concentration and composition of phenolic compounds and, therefore, also the colour intensity and hue of red wines (Vritovsek *et al.* 1997; Matějíček *et al.* 2003; Hernandez *et al.* 2006). Wine clarification decreases the content of extractive and volatile compounds and very often also represents a significant intervention into the content of polyphenols. The range of changes and mainly losses of individual phenolic compounds differ in the dependence on the type of the clarifying agent and chemical structure of phenols (Gao *et al.* 1997; Balík 2003; Castillo-Sanchez *et al.* 2006).

MATERIAL AND METHODS

Samples preparation. Grapes of white varieties Neuburger (Neu), Grüner Veltliner (GV), Welschriesling (Wel), and Pálava (Pal) (Tramine × Müller Thurgau) were pressed using two different (hydraulic or pneumatic) pressing systems. Samples were taken in regular intervals, viz. at the beginning of the pressing and after obtaining 15%; 30%; 45%; 60%, and 75% (w/v) of must from the pressed grapes. Immediately after pressing, three doses of must were clarified using mostgelatine in doses of 50, 100, and 200 ml/100 l (Tables 1 and 2). After centrifugation, these experimental musts were stored (for one month at –20°C) until the analyses for polyphenols and total antioxidative capacity.

Young red wines of the Saint Laurent variety were clarified by means of two different doses of six different clarifiers (Tables 3 and 4). After centrifugation, these red wine samples were immediately analysed. All tests were run in triplicates.

Chromatographic methods. The separation of gallic acid, catechin, epicatechin, *trans*-resveratrol, *trans*-piceid, and caftaric acid was carried out using Luna Phenomenex C18(2) columm (2 × 150 mm, 3 µm) (Torrance USA) on Hewlet Packard HPLC model 1050 (Wilmington, USA) with diode-array detector HP 1040A. The injected volume was 5 µl. For chromatographic mobile phases were used (A) acetonitrile:*o*-phosphoric acid:water (5:0.1:94.9), and (B) acetonitrile:*o*-phosphoric acid:water (80:0.1:19.9) at a flow rate of 0.25 ml/min in a gradient from 0 to 45% in 55 minutes.

Spectrophotometric methods. The concentrations of total polyphenols were determined (*i*) by means of a spectrophotometric (Heloisß/Unicam) method with the Folin-Ciocalteu agent "Polyphenols" calculated as gallic acid and designated as "Polyphenols I" calculated as catechin (Singleton & Rossi 1965), and (*ii*) by direct measurement of the wine samples diluted with distilled water at 280 nm calculated as catechin as designated as "Polyphenols II" (Zoecklein *et al.* 1990). Total anthocyanins, the values of colour intensity, colour hue, and "chemical age" of wine were estimated by spectrophotometry (Heloisß/Unicam) according to Somers and Evans (1977). The colour coordinates CIE $L^*a^*b^*$ were measured using the trichromatic method by Chroma-Meter CT 210/Minolta.

Determination of antioxidative capacity. The antioxidative capacity was determined by photochemiluminescence method (PCL) using KIT ACW (400.801) and the instrument Photochem (Analytik Jena AG, Germany). Free radicals (superoxide anion radicals) were produced by optical excitation (irradiation) of a photosensitiser (dye). These radicals were partially eliminated from the sample by reaction with the antioxidants present in the sample. The remaining radicals cause the luminescence in the measuring cell, thereby allowing determination of the antioxidant capacity of the sample. The samples of grape musts were diluted to give signals lying within the range of the calibration curve and within the linear range of the instrument. The total antioxidative capacity (TAC) of the grape musts was quantified by comparison with the standard (constructing a calibration curve with ascorbic acid) and was given in equivalent units of the standard (Popov & Lewin 1994).

Reagents and solvents. Standards: *trans*-resveratrol, *trans*-piceid, catechin, epicatechin, gallic acid, and caftaric acid were obtained from Sigma-Aldrich (Prague, Czech Republic), and ł-ascorbic acid as part of KIT ACW (400.801) from Analytik Jena AG (Germany). Acetonitrile was purchased from Merck (Prague, Czech Republic), and *o*-phosphoric acid from Fluka (Sigma-Aldrich, Prague, Czech Republic).

The essential information on limits of detection and repeatability of the used analytical methods was described in a paper of Mikeš *et al.* (2008).

Statistical methods. The data were evaluated using the method of variance analysis with the Tukey test ($P = 0.05$) and correlation analysis of the program Statgraphics.

RESULTS AND DISCUSSION

Seventy two samples of must were taken in the course of pressing grapes coming from the grapevine varieties under the study. At the beginning of pressing, only a low overpressure (0.05MPa) was used in both experimental variants. To the end of pressing, when 75 % (w/v) of must were obtained from the pressed grapes, the pressures in hydraulic and pneumatic variants of this experiment were 2.0 MPa and 0.22 MPa, respectively. In the majority of the experimental variants, the content of total polyphenols increased on 23–125 mg/l regarding original values; phenolic compounds were detected either in very low concentrations (catechin max. 5.38 mg/l and gallic acid max. 0.58 mg/l) or near to the detection limit (*trans*-resveratrol 0.05 mg/l) (Table 1). Nikfardjam *et al.* (2006) found only very low concentrations of *trans*-piceid and *trans*-resveratrol as well, and according to them the total content of polyphenols in German white wines ranged from 248 to 747 mg/l.

In our experiments, higher concentrations of caftaric acid (0.24–59.1 mg/l) were found out in all musts under study. The changes recorded in the content of this compound during the pressing process are presented in Figure 1. There was a non-unequivocal tendency for its increasing

Table 1. Contents of selected phenolic compounds and antioxidative capacity on start and at the end of grape pressing

Variety	Press/Phase	Polyphenols	Gallic acid	Catechin	Caftaric acid	Piceid	TAC (mmol/l)
		(mg/l)					
Neuburger	hydraulic/start	253	0.11	2.05	2.98	0.26	1.47
	hydraulic/finish	270	nd	nd	2.69	0.05	0.86
	pneumatic/start	268	0.26	5.38	33.12	0.74	2.24
	pneumatic/finish	348	nd	1.35	nd	nd	2.09
Grüner Veltliner	hydraulic/start	398	nd	2.90	34.59	0.80	2.36
	hydraulic/finish	368	0.58	0.94	nd	nd	2.99
	pneumatic/start	348	0.34	3.49	10.42	0.66	0.83
	pneumatic/finish	473	nd	nd	nd	nd	2.73
Welschriesling	hydraulic/start	340	0.15	2.10	50.86	0.43	2.98
	hydraulic/finish	370	nd	0.88	32.28	0.45	2.06
Pálava	pneumatic/start	340	nd	nd	1.36	nd	1.83
	pneumatic/finish	368	0.20	nd	0.76	nd	1.50

nd = not detected

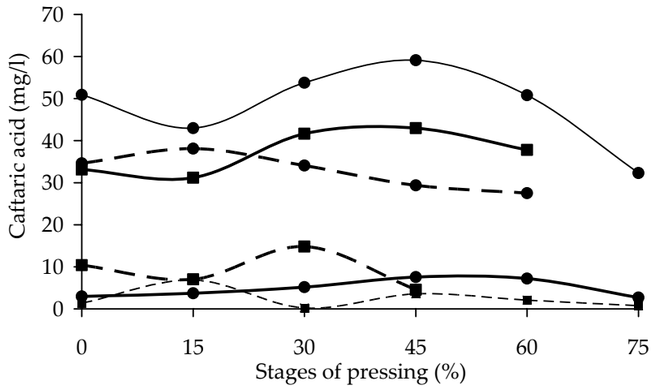


Figure 1. Changes of caftaric acid in the course of pressing grapes

concentrations due to the increasing pressure, and a tendency for its decrease towards the end of the pressing process. A similar trend was observed also for *trans*-piceid (Figure 2) but its concentrations were lower (0.05–1.03 mg/l). According to Fernández-Pachón *et al*. (2006) the content of caftaric acid in white wines varied from 3.86 to 53.5 mg/l and contributed significantly (together with gallic and caffeic acids) to the antioxidative activity of the analysed wine. Determined values of TAC are presented in Table 1. They ranged from 0.83 to 2.99 with no explicit differences between the must samples analysed. Otreba *et al*. (2006) found out that the average antioxidative capacity of Austrian white wines was 2.11 mmol/l, also without a uniform course of values. However, Villaño *et al*. (2006) observed that the antioxidative activity of must increased in the dependence on the increasing pressure.

The analysis of variance of the data obtained (Figures 3 and 4) demonstrated that the content of caftaric acid in must of the variety Welschriesling (mean 48.3 mg/l) differed significantly from those of other cultivars under study, i.e. Grüner Veltliner (mean 22.3 mg/l) and Neuburger (mean 19.64 mg/l). The lowest, but statistically non-significant concentration was detected in the course of pressing of grapes of the variety Pálava (mean 2.48 mg/l). The significantly highest contents of *trans*-piceid were found out in the course of Grüner Veltliner grapes pressing (mean 0.75 mg/l). However, statistical analysis did not show that the observed differences in the concentrations of polyphenols under study and in TAC values might have been caused by different methods of pressing.

Subsequently, musts of three cultivars were clarified using three different doses of mostgelatine (Table 2). The contents of total polyphenols ranged from 120 to 300 mg/l. The estimated concentrations of phenolic compounds (caftaric acid, catechin, gallic acid, and *trans*-piceid) and TAC values varied and no explicit trend could be found. The effect of clarification on the contents of these substances, as compared with those of non-clarified control

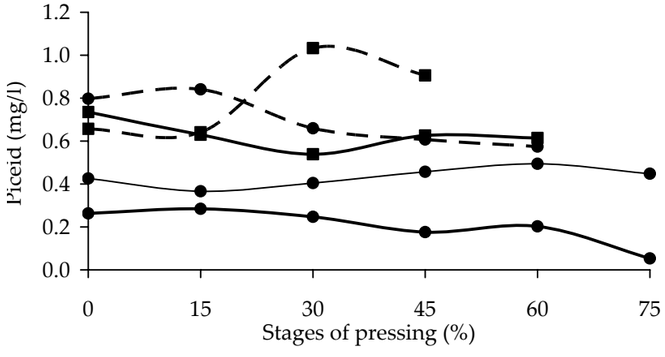


Figure 2. Changes of piceid in the course of pressing grapes

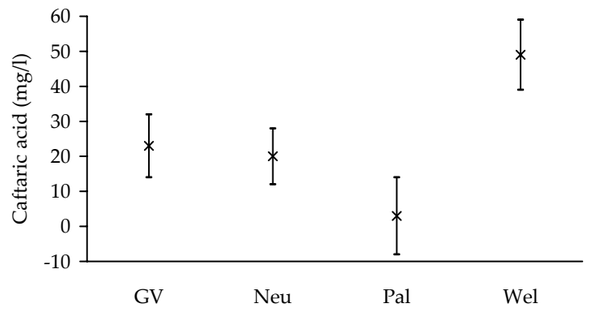
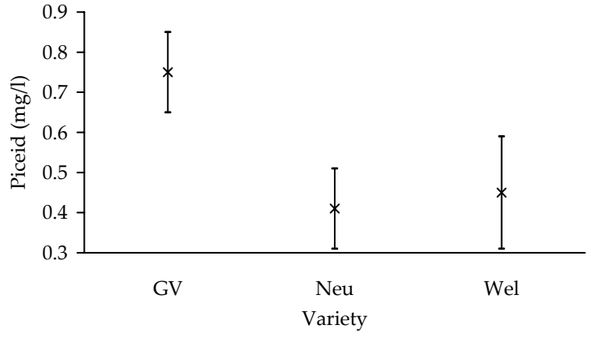


Figure 3. Variance analysis of caftaric acid as dependent on grape variety (Tukey, $P = 0.05$)

GV – Grüner Veltliner, Neu – Neuburger, Pal – Pálava, Wel – Welschriesling



GV – Grüner Veltliner, Neu – Neuburger, Wel – Welschriesling

Figure 4. Variance analysis of piceid as dependent on grape variety (Tukey, $P = 0.05$)

(100 %), is illustrated in Figure 5. The individual columns and line segments represent the means and confidence intervals of means, respectively. Only the content of total polyphenols decreased with increasing doses of the clarifying agent. The highest losses of polyphenols (28–44 %) occurred after its application in the dose of 200 ml/100 l. As reported Villaño *et al*. (2006), the clarification treatments did not significantly affect the phenolic composition or the antioxidant activity of wines.

The correlations between the values of antioxidative capacity (TAC) as measured in the course

Table 2. Dependence of selected phenolic compounds and antioxidative capacity on grape must clarification

Variety	Most gelatine (ml/100 l)	Polyphenols	Gallic acid	Catechin	Caftaric acid	Piceid	TAC
		(mg/l)					(mmol/l)
Neuburger	0	170	0.48	2.32	9.24	0.29	0.86
	50	165	0.53	2.70	16.10	0.33	0.66
	100	160	0.27	2.64	14.12	0.29	0.63
	200	120	0.24	2.49	10.23	0.24	0.77
Grüner Veltliner	0	263	0.18	2.51	25.29	0.60	1.35
	50	245	0.17	2.67	22.14	0.66	0.82
	100	223	0.17	2.73	17.38	0.69	1.03
	200	190	0.15	2.71	17.88	0.63	1.51
Welschriesling	0	300	0.54	3.03	46.34	0.51	1.78
	50	245	0.21	2.51	49.48	0.46	1.86
	100	190	0.24	2.99	60.08	0.57	1.22
	200	168	0.37	2.90	60.40	0.56	1.30

Table 3. Concentrations of selected phenolic compounds and total polyphenols as a function of the red wines clarification methods

Clarifying agent	Dose (g/100 l)	Notation	Gallic acid	Catechin	Epicatechin	Resveratrol	Polyphenols I	Polyphenols II
			(mg/l)					
Non-clarification		NON	28.6	92.2	34.6	1.93	1171	2210
Egg white	10	EG10	28.4	26.0	9.3	1.27	1075	2117
	20	EG20	28.0	26.6	9.8	1.44	1160	2141
Casein	50	CA50	28.4	88.2	32.2	1.86	1091	2113
	200	CA200	27.9	91.8	34.6	1.69	1021	2093
Gelatine	5	GE5	27.7	92.1	33.2	1.90	1155	2181
	10	GE10	28.2	81.4	29.6	1.55	1107	2149
Gelatine/ Siligel	5/50	ES5/50	27.2	91.4	34.0	1.72	1155	2169
	10/100	ES10/100	27.5	68.2	27.6	1.60	1167	2161
Bentonite	25	BE25	28.1	67.0	23.4	1.71	1112	2165
	100	BE100	27.7	86.1	30.6	1.80	1123	2060
PVPP	40	PP40	27.7	76.5	29.6	0.53	989	1976
	80	PP80	25.7	61.0	27.7	0.12	845	1782

of must pressing and clarification on one hand, and the concentrations of total polyphenols and caftaric acid (other phenols were present only in very low concentrations) on the other, are presented in Figure 6. As can be seen, the antioxidative capacity increased in dependence on polyphenols and caftaric acid concentrations; however, this correlation was statistically insignificant (R^2 = 0.1416–0.4278) and, apparently, the TAC value was significantly influenced by some other components of grapes and/or musts. Similarly, Otreba *et al.* (2006) concluded that the correlation between the TAC values and total polyphenols content was very weak.

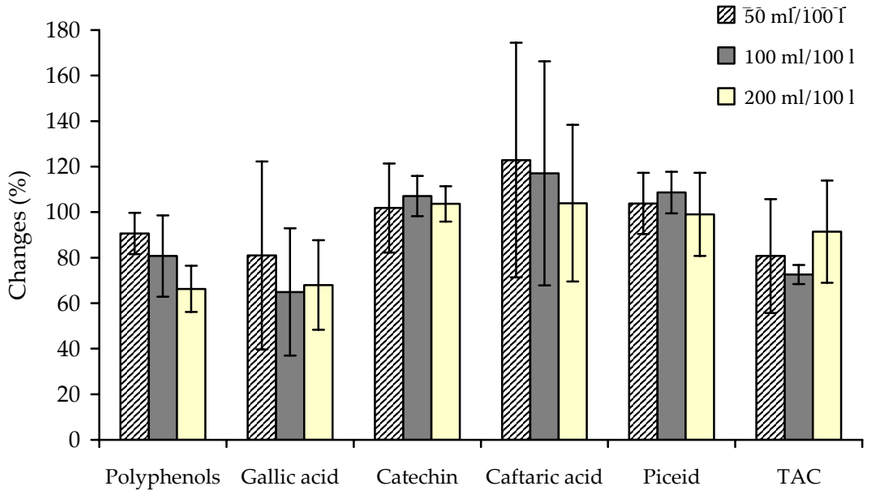


Figure 5. Changes in concentration of selected phenolic compounds and antioxidative capacity as a function of the grape must clarification (100% = non clarified control)

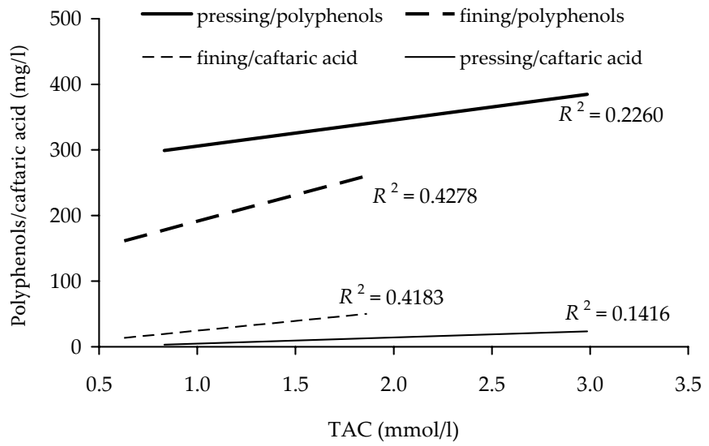


Figure 6. Correlation analysis between total polyphenols (caftaric acid) and antioxidative capacity as a function of the grape pressing and must fining

The total content of polyphenols ranged in young, non-clarified red wines (Saint Laurent), from 1171 to 2210 mg/l depending on the method used. All clarifiers showed only minimum effects on the content of gallic acid. The highest content of phenolic compounds under the study was recorded in the case of catechin (92.2 mg/l); the content of resveratrol was nearly 2 mg/l (Table 3). The concentration of total anthocyanins (213 mg/l) corresponded with the estimated colour intensity (3.06) and with the values of colour coordinates ($L^*a^*b^*$) presented in Table 4. The value of "Chemical age" indicated the proportion of polymeric forms of anthocyanins in relation to the total colouration of red wine (Somers & Evans 1977).

Concentrations of total polyphenols ranged, in dependence on the type and dose of clarifying agent and the method used, from 845 mg/l to 2210 mg/l. The highest total loss of polyphenols was recorded in the treatment with the clarifier PVPP (polyvinylpolypyrrolidone) using the methods "Polyphenols I" and "Polyphenols II", the losses ranged from 15% to 20% and from 10% to 20%, respectively. The highest losses were recorded in

Table 4. Concentrations of total anthocyanins and colour parameters as a function of the red wines clarification methods

Clarifying agent	Dose (g/100 l)	Notation	Anthocyanins (mg/l)	Chemical age	Colour intensity	Colour hue	Colour coordinates		
							L^*	a^*	b^*
Non-clarification		NON	213	27.2	3.06	0.81	46.2	56.0	27.9
Egg white	10	EG10	139	56.2	5.89	0.61	20.6	59.3	27.3
	20	EG20	144	52.3	5.83	0.61	21.4	59.8	28.5
Casein	50	CA50	170	31.6	3.98	0.65	37.1	62.9	31.5
	200	CA200	186	23.6	2.28	0.76	54.6	51.7	21.4
Gelatine	5	GE5	195	29.0	3.40	0.72	42.6	59.9	29.3
	10	GE10	184	30.0	3.69	0.68	40.2	61.7	30.3
Gelatine/Siligel	5/50	ES5/50	195	27.7	3.24	0.74	44.2	59.1	28.5
	10/100	ES10/100	173	32.1	4.14	0.65	36.1	62.9	32.5
Bentonite	25	BE25	168	34.0	4.28	0.65	34.9	63.0	33.5
	100	BE100	178	27.9	2.98	0.76	46.7	57.6	28.1
PVPP	40	PP40	189	26.3	2.91	0.71	47.4	57.7	25.9
	80	PP80	181	23.5	2.56	0.69	51.2	55.8	22.9

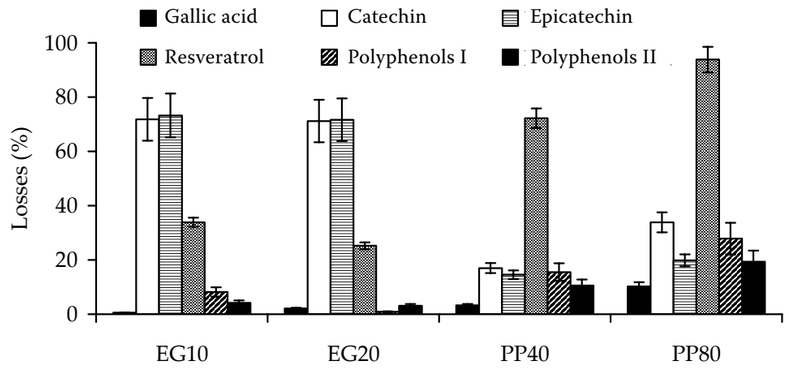


Figure 7. Losses of selected phenolic compounds and total polyphenols as a function of the red wines clarification methods (for key to abbreviation, see Table 3) (0% = non clarified control)

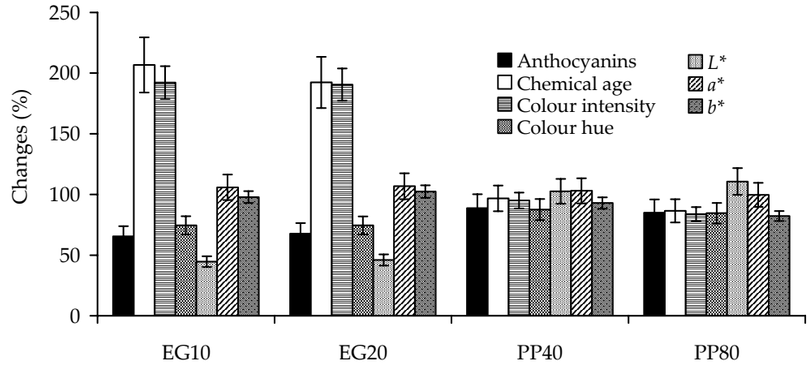


Figure 8. Changes in concentrations of total anthocyanins and colour parameters as a function of the red wines clarification methods (for key to abbreviation, see Table 3) (100% = non clarified control)

the case of resveratrol (between 72% and 94%) in the samples treated with PVPP. The concentration of resveratrol was reduced also by egg white (ca by 30%). On the other hand, however, the clarification with egg white decreased the contents of catechin and epicatechin at the most (by 71–73%). The use of PVPP decreased their concentrations to a smaller extent (17–34%), similarly as the application of bentonite (7–33%) (Figure 7). Similar results concerning the fining losses of resveratrol were published by Vritovsek *et al.* (1997). Fining with PVPP greatly reduced (as much as by 90%) resveratrol concentration while gelatine fining had no effect on it.

The concentration of total anthocyanins pigments was reduced at the most after the application of egg white (by 32–34%), however, the overall colour intensity of red wine increased. The use of bentonite (Blancobent) showed a smaller effect on the colour properties of red wine. In all the treatments used, an increase was observed in the proportion of polymeric forms of anthocyanins in the final colouration of red wine (the value of "Chemical age") as well as an increase in the intensity of red tone as measured by the coordinate a^* of the colour system CIE (Figure 8). In contrast to Castillo-Sanchez *et al.* (2006), we did not find out significant changes in the colour parameters of the wines PVPP-treated in regard to the other fining agents.

CONCLUSIONS

The content of total polyphenols increased in the course of wine grape pressing. The concentrations of catechin, gallic acid, and *trans*-resveratrol were very low. The contents of caftaric acid in the variety Welschriesling (mean 48.3 mg/l) and *trans*-piceid in the variety Grüner Veltliner (mean 0.75 mg/l) differed significantly from those of all other cultivars under the study. The highest losses of polyphenols (28–44%) were recorded after the must clarification with mostgelatine in the dose of 200 ml/100 l. The statistical analysis did not demonstrate that the differences in the concentrations of polyphenols and TAC values might have been caused by different pressing technologies and/or by clarification with mostgelatine. The PVPP clarification of red wine Saint Laurent caused the highest total losses of polyphenols. The highest losses caused by this clarifying agent were recorded in case of resveratrol (between 72% and 94%). An opposite tendency, however, was observed for catechin and epicatechin. The clarification showed only minimum effect on the concentration of gallic acid. The concentrations of catechin, epicatechin, and total anthocyanins, as well as the colour parameters of red wines, were influenced at the most by the application of egg white.

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