STUDY OF PHENOLIC COMPOSITION AND ANTIOXIDANT CAPACITY OF CROATIAN MACERATED WHITE WINES

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The objective of the present study was to determine the phenolic content and the antioxidant capacity of white wines produced with different types and durations of the maceration process. Seventeen macerated and non-macerated wines produced in two Croatian winegrowing sub-regions were analyzed. The main physicochemical parameters were determined according to the methods recommended by the O.I.V. Total phenolics were analyzed by Folin-Ciocalteu method and concentrations of twenty-three individual polyphenols were determined by high-performance liquid chromatography (HPLC)-diode array detection (DAD) technique. Antioxidant capacity was evaluated by ABTS free radical method. The study shows that a long maceration process provides highest polyphenol contents and antioxidant capacity of white wine in comparison to other applied types of maceration. Good correlation between total phenolic content and related antioxidant capacity was found in all tested wines. According to maceration type (no maceration, cold maceration, 10 days maceration, 30 days maceration and 6 months maceration time) pronounced differences were found in the concentrations of some individual phenolic acids (gallic, syringic, caftaric) and flavan-3-ols (epicatechin-gallate, epicatechin, procyanidin B1, B2). Discriminant statistical technique applied allowed classification of the wines into four consistent groups.
Keywords: polyphenols, antioxidant capacity, maceration, Croatian white wines, HPLC, ABTS

Studie über die Polyphenolzusammensetzung und über die antioxidative Kapazität kroatischer mazerierter Weißweine. Das Ziel der vorliegenden Studie war es, den Phenolgehalt und die antioxidative Kapazität der Weißweine zu bestimmen, die durch verschiedene Arten und Dauer der Mazeration hergestellt werden. Siebzehn mazerierte und nichtmazerierte Weine aus zwei kroatischen Weinbauuntergebieten wurden analysiert. Die wichtigsten physikalisch-chemischen Parameter wurden nach den vom OIV empfohlenen Methoden bestimmt. Der Gesamtphenolgehalt wurde mit der Folin- Ciocalteu-Methode analysiert, die Konzentrationen von dreiundzwanzig einzelnen Polyphenolen wurden durch Hochleistungs-Flüssigkeitschromatographie (HPLC)-Diodenarray-Detektion (DAD)-Technik bestimmt. Die antioxidative Kapazität wurde durch die ABTS Freie Radikale-Methode bewertet. Die Studie zeigt, dass lange Mazeration den höchsten Polyphenolgehalt und die höchste antioxidative Wirkung im Vergleich zu anderen Arten der angewandten Mazeration bietet. Eine gute Korrelation zwischen Gesamtphenolgehalt und der damit verbundenen antioxidativen Kapazität wurde in allen getesteten Weinen gefunden. Zwischen den Mazerationstypen (keine Mazeration, kalte Mazeration, 10 Tage Mazeration, 30 Tage Mazeration sowie 6 Monate Mazeration) gab es deutliche Unterschiede in den Konzentrationen einiger Phenolsäuren (Gallussäure, Syringasäure, Caftarsäure) und der Flavan-3-ole (Epicatechingallat, Epicatechin, Procyanidin B1, B2). Eine Diskriminanzanalyse erlaubte die Klassifizierung der Weine in vier konsistente Gruppen.
Schlagwörter: Polyphenole, antioxidative Kapazität, Mazeration, kroatische Weißweine, HPLC, ABTS

Polyphenolic compounds are secondary plant metabolites, present in fruit and vegetables and integral part of the human diet. These compounds are always present in wine in higher or lower amounts and they contribute to the sensory and chemical quality of the final product (Mitič et al., 2010). Polyphenolic compounds have the ability to act as antioxidants by a free radical scavenging and metal ion chelation (Lodovici et al., 2001) and there is a positive correlation between total phenolic content in wine and related antioxidant capacity (Frankel et al., 1995; Burns et al., 2000; Vinkovič Vrček et al., 2011).

Growing awareness of their role in health and ageing resulted in an increasing interest among researchers that evidenced a wide range of beneficial health effects (Struch, 2000; Sun et al., 2002; Yao et al., 2004; Babu and Liu, 2009). While the majority of red wines are very well studied and known for their high polyphenolic content and antioxidant activity, white wines are usually characterized by lower polyphenolic content and antioxidant properties (Makris et al., 2003). The main factors that contribute to these differences are grape variety and vinification procedures during the ageing of wine (Ramos et al., 1999; Zafrilla et al., 2003).

White wines usually contain 100 to 400 mg/l of total polyphenols (Margalit, 1997) on the contrary to red wines which contain on average 1800 mg/l (190 to 3800 mg/l) of total polyphenols (Amerine and Ough, 1988).

Phenolic compounds, both flavonoids and non-flavonoids, in red wine are derived from the solid parts of grape (skins, seeds, stems or pulp) that are extracted during pomace contact together with the grape juice at the first stage of alcoholic fermentation (Fuhrman et al., 2001). This process, called maceration, is not standard in the production of white wines which are usually made from the free running juice. However, there are white wines produced with the maceration step resulting in polyphenol-enriched wines with antioxidant characteristics similar to those of red wines and a concentration range of total polyphenols of 867 to 1859 mg/l (Darias-Martín et al., 2000; Ružič et al., 2011). There are considerable variations due to the grape variety employed, temperature and contact time (Darias-Martín et al., 2000). White wines, produced without maceration, contain lower amounts of total phenols compared with red wines, mainly non-flavonoids which are concentrated in the berry flesh. The hydroxycinnamic acids (HCAs) and their derivates are their major components (Mozetič et al., 2006). Their antioxidant properties, which are being enhanced by conjugation with tartaric acid, may exert a positive health effect. Tartaric esters of HCAs (e.g. trans-caftaric, caffeic, p-coumaric, coutaric and trans-fertaric acid) represent 80 % of all polyphenols of white grapes juice (Betes-Saura et al., 1996). Caftaric acid plays an important role in phenol oxidation and oxidative browning in must. The oxidized derivates of coutaric and caftaric acid provide the yellowish-gold color in white wine (Mitič et al., 2010). Skin contact greatly increases total HCAs and flavanol content that are directly correlated to the antioxidant capacity of white wines (Makris et al., 2003). Flavonols and their glycosides, localized in the grapes skin, are extracted to a larger extent during maceration. The presences of flavonols in white wines, mainly quercetin-like flavonols, affect their color and antioxidant capacity (Williamson and Manach, 2005; De Beer et al., 2005; Montoro et al., 2005). Although there have been studies on the polyphenolic composition and antioxidant activities on several white wine samples (Vinkovič Vrček et al., 2011; Katalinič et al., 2010; Kovačevič Ganič et al., 2006) there were no researches on the differences in these properties between macerated and non-macerated white wines produced in Croatia. Due to the lack of scientific data about the effects of different types of maceration, the aim of our study was to analyze white wines produced with cold maceration, prolonged (till 30 days) and long maceration (6 months) for the first time. New trends in grape and wine production and consumption of macerated white wines encouraged some of Croatian wine producers, mostly from the two different sub-regions Istria and Plešivica, to accept these technologies. Regarding the winegrowing regions included, the most commonly grape variety used for production is indigenous 'Malvasia Istriana' (*Vitis vinifera L.*). Some of the producers, belonging to the "natural wine" movement, have produced wines with 30 days-long maceration or 6 months-long maceration in amphorae with spontaneous alcoholic fermentation.

The effect of different maceration treatments on commercially available wines was evaluated by comparing the results to non-macerated or cold macerated white wines from the same varieties.

MATERIALS AND METHODS

WINE SAMPLES

Phenolic contents and antioxidant capacity were determined in 17 macerated and non-macerated white wines from the two Croatian sub-regions Istria and Plešivica (vintages 2007 to 2012). All samples were obtained directly from wineries, stored at 10 °C, protected from

light and analyzed shortly after opening. The grape varieties used for wines under study were ‚Malvasia Istriana', ‚Chardonnay', ‚Rhine Riesling', ‚Pinot gris', ‚Sauvignon blanc' and several local varieties in one blended wine. The list of analyzed wines including geographical origin, variety, code and harvest year is given in Table 1.

CHEMICALS AND STANDARDS

Total phenolic content was determined by means of Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate and 96 % ethanol, obtained from Kemika (Zagreb, Croatia) and gallic acid supplied by Sigma-Aldrich (St. Louis, USA). Orto-phosphoric acid 85 %, L(+)-tartaric acid and formic acid 98 - 100 % were obtained from Riedel-de Haën (Seelze, Germany). Vanillin 99 % and sodium metabisulphite were obtained from Sigma (St. Louis, USA). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Trolox were purchased from Sigma-Aldrich (St. Louis, USA).

Methanol and acetonitrile (HPLC grade) were obtained from J.T. Baker (Deventer, Netherlands).

Standards of phenolic compounds (epigallocatechin, procyanidin B1 and B2, rutin, quercetin-3-glucoside) for HPLC wine analysis were purchased from Extrasynthese (Genay, France), phenolic acids, flavan-3-ols and stilbenes were obtained from Sigma-Aldrich (St. Louis, USA). Kaempferol, quercetin and isorhamnetin were supplied by Fluka (Taufkirchen, Germany). Quercetin-3-O-glucoside and hydroxybenzoic acids were purchased from Sigma-Aldrich (St. Louis, USA).

Stock solutions of all standards were prepared in methanol. Working standards were made by dilution of the stock solutions in methanol-water (1:1 v/v). Calibration curves were obtained from triplicate injections of five concentrations.

PHYSICOCHEMICAL ANALYSIS

Standard methods of analyses for general wine composition (alcohol, residual sugar, dry extract, total acidity, volatile acidity, ph, ash, free and total SO_2) were used.

Table 1: List of analysed wine samples

No.	Grape variety	Type of maceration	Region	Year
1	Malvasia Istriana	long – 6 months	Istria	2007
2	Chardonnay	long – 6 months	Plešivica	2008
3	Rhine Riesling	long – 6 months	Plešivica	2009
4	Chardonnay (50 %) + 7 local varieties	long – 6 months	Plešivica	2012
5	Malvasia Istriana	prolonged – 30 days	Istria	2009
6	Malvasia Istriana	prolonged – 30 days	Istria	2010
7	Malvasia, Sauvignon blanc, Pinot gris	prolonged – 30 days	Istria	2010
14	Malvasia Istriana	prolonged – 10 days	Istria	2009
13	Malvasia Istriana	prolonged – 10 days	Istria	2011
12	Malvasia Istriana, Chardonnay	prolonged – 10 days	Istria	2011
8	Malvasia Istriana	cold maceration	Istria	2010
9	Malvasia Istriana	cold maceration	Istria	2011
10	Chardonnay	cold maceration	Istria	2011
11	Malvasia Istriana	cold maceration	Istria	2012
15	Malvasia Istriana	non-macerated	Istria	2010
16	Malvasia Istriana	non-macerated	Istria	2011
17	Rhine Riesling	non-macerated	Zagreb	2011

DETERMINATION OF TOTAL PHENOLIC CONTENT

Total phenolic content (TP) of the wines was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). TP was measured spectro-photometrically (Specord 400, Analytik Jena, Germany) at 765 nm. Results are given as gallic acid equivalents (GAE mg/l). The data represent the average of three measurements.

DETERMINATION OF INDIVIDUAL POLYPHENOLS

HPLC separation, identification and quantification of 23 individual polyphenolic compounds in wine were performed on Agilent 1100 Series system (Agilent, Palo Alto, USA), equipped with DAD and FLD (Agilent 1200) coupled to Agilent Chem Station Software (ver-

sion B.01.03). Wine samples were filtered through 0.45 µm PTFE membrane filters and then injected (20 µl) on a reversed-phase column Luna Phenyl-Hexyl (4.6 × 250 mm; 5 µm particle, Phenomenex, Torrance, USA), thermo stated at 50°C. The solvents were water/phosphoric acid (99.5:0.5, v/v, solvent A) and acetonitrile/water/phosphoric acid; 50:49.5:0.5, v/v/v, solvent B), and the flow rate was 0.9 ml/min. The linear gradient for solvent B was: 0 min, 0 %; 7 min, 20 %; 35 min, 40 %; 40 min, 40 %; 45 min, 80 %; 50 min, 100 %; 60 min, 0 %. Hydroxybenzoic acids were detected at 280 nm, p-hydroxycinnamic at 320 nm, flavonols at 360 nm. Flavanols were detected at ζ_{ex} = 225 nm and ζ_{em} = 320 nm. Phenolic compounds were identified by matching the retention time of each chromatographic peak with external standards and DAD spectrum. Quantification of individual phenolic peaks was performed by the external standard method. The data represent the average of three measurements. Results were expressed in mg/l of wine as mean values ± standard deviations (SD).

Table 2: Concentration (mg/l) of individual HBAs and flavanols determined in the studied white wines. Values are means of triplicate determination (n = 3) with their standard deviations. (GA = gallic acid, PCA = protocatechuic acid, VA = vanillic acid, SA = syringic acid, CAT = catechin, EPICAT = epicatechin, ECG = epicatechin gallate, EGC = epigallocatechin, B1 = procyanidin B1, B2 = procyanidin B2, n.d. = not detected)

No.	GA	PCA	VA	SA	CAT	EPICAT	ECG	EGC	B1	B2
1	20.88±0.25	1.80±0.01	6.94±0.01	3.57±0.10	19.95±0.09	20.46±0.06	0.50±0.01	n.d.	4.39±0.13	9.76±0.09
2	21.96±0.23	5.75±0.03	11.26±0.01	3.90±0.02	26.16±0.38	0.84±0.05	0.91±0.03	15.3±5.42	9.23±0.18	25.42±0.04
3	20.63±0.19	4.15±0.07	9.52±0.07	2.36±0.02	17.63±0.19	n.d.	0.16±0.01	n.d.	n.d.	n.d.
4	18.49±0.42	3.92±0.04	13.46±0.17	3.35±0.02	15.69±0.08	0.87±0.03	0.52±0.03	n.d.	6.81±0.04	6.81±0.03
5	9.33±0.19	1.74±0.07	4.41±0.08	3.56±0.02	10.19±0.02	0.73±0.04	n.d.	n.d.	n.d.	n.d.
6	11.54±0.04	2.68±0.04	5.82±0.01	2.77±0.04	30.87±0.04	1.07±0.00	0.50±0.02	n.d.	n.d.	n.d.
7	2.07±0.06	3.32±0.04	16.00±0.01	5.34±0.03	29.09±0.01	1.60±0.02	1.22±0.06	n.d.	n.d.	n.d.
8	2.01±0.05	n.d.	1.07±0.01	0.25±0.01	0.74±0.04	1.04±0.04	n.d.	n.d.	0.43±0.04	2.02±0.04
9	0.51±0.02	n.d.	1.80±0.05	0.44±0.01	0.45±0.01	0.58±0.12	n.d.	n.d.	1.54±0.06	2.24±0.04
10	4.05±0.06	1.62±0.05	10.68±0.02	5.30±0.02	10.87±0.11	3.85±0.07	0.14±0.01	n.d.	n.d.	n.d.
11	n.d.	2.72±0.04	3.60±0.04	0.86±0.05	6.49±0.06	0.22±0.01	0.19±0.01	n.d.	n.d.	1.63±0.06
12	5.80±0.15	3.27±0.01	10.16±0.06	3.47±0.10	32.53±0.04	1.205±0.02	0.37±0.01	n.d.	3.71±0.05	n.d.
13	9.68±0.01	1.79±0.01	4.31±0.08	3.68±0.06	14.74±0.20	0.26±0.01	n.d.	n.d.	n.d.	n.d.
14	12.43±0.16	2.03±0.01	5.18±0.03	4.95±0.08	9.88±0.04	0.35±0.01	n.d.	7.89±1.53	3.74±0.06	2.72±0.10
15	1.61±0.01	n.d.	0.67±0.04	0.19±0.01	0.60±0.04	1.02±0.03	n.d.	n.d.	0.38±0.03	1.61±0.02
16	0.52±0.01	n.d.	1.24±0.06	0.48±0.02	0.54±0.01	0.71±0.04	n.d.	n.d.	1.49±0.01	2.12±0.04
17	1.24±0.06	n.d.	7.70±0.03	0.24±0.01	1.59±0.01	0.51±0.04	0.20±0.01	n.d.	0.66±0.03	0.48±0.02

FREE RADICAL METHOD

The antioxidant activity of wines was determined using the ABTS-free radical method (Re et al., 1999). Absorbance measurements are transformed to antioxidant activity using Trolox as reference. Absorbance measurements were recorded on Specord 400 spectrophotometer. The cation radical $ABTS^+$ is generated directly by the reaction of an ABTS stock solution (7 mmol/l) with 140 mmol/l potassium persulphate in a 1:0.5 stoichiometric ratio; the mixture was allowed to stand in the dark at room temperature for 12 to 16 h. 5 ml of the formed cation radical $ABTS^+$ were mixed with 50 µl aliquots of wine and the absorbance was measured at 734 nm, 6 min after mixing. A blank control of ethanol/water mixture was run for each assay. Results are expressed as µmol of Trolox equivalents (TEAC) per liter of wine. All determinations were carried out in triplicate.

STATISTICAL ANALYSIS

All analyses of each sample of wine were run in triplicate, and the mean and standard deviations were reported. Analysis of variance ANOVA test was performed using the SAS System for Windows 9.0, 2004 (SAS Institute Inc., Cary, NC, USA). The differences in the content levels were estimated with t-test. P-values of < 0.01 were considered statistically significant. Canonical discriminant analysis was performed to evaluate the utility of individual polyphenols content in wine samples for discrimination between different maceration treatments (SAS System for Windows 9.0, 2004). Based on same traits the first two canonical variables were plotted.

Table 3: Concentration (mg/l) of individual HCAs, flavonols and stilbenes determined in the studied white wines. Values are means of triplicate determination (n = 3) with their standard deviations. (CftA = caftaric acid, CA = caffeic acid, CouA = coumaric acid, FrtA = fertaric acid, FrlA = ferulic acid, SinA = sinapic acid, Q-3glu = quercetin-3-glucoside, Isor = isorhamnetin, Q = quercetin, Kaemp = kaempferol, Trans-R = *trans*-resveratrol, Trans-Rglu = resveratrol-3-*O*-glucoside, Cis-R = *cis*-resveratrol, n.d. = not detected)

No.	CftA	CA	CouA	FrtA	FrlA	SinA	Q-3glu	Isor	Q	Kaemp	Trans-R	Trans-Rglu	Cis-R
1	18.46±0.06	1.30±0.01	0.27±0.01	0.28±0.02	0.62±0.04	n.d.	0.54±0.03	n.d.	1.04±0.06	n.d.	0.69±0.01	0.30±0.05	1.08±0.05
2	24.59±0.01	2.63±0.08	0.93±0.02	0.26±0.01	0.50±0.02	n.d.	0.81±0.01	n.d.	1.31±0.03	n.d.	0.55±0.04	0.38±0.01	2.21±0.04
3	34.81±0.26	2.67±0.04	0.36±0.01	0.54±0.06	0.79±0.03	0.23±0.02	n.d.	n.d.	1.03±0.09	n.d.	0.39±0.01	0.88±0.02	4.43±0.02
4	40.85±0.06	3.52±0.05	0.37±0.02	0.58±0.03	0.63±0.04	0.18±0.01	0.615±0.02	n.d.	1.27±0.38	n.d.	0.46±0.06	0.74±0.01	3.63±0.08
5	6.66±0.08	0.71±0.03	0.66±0.05	0.17±0.01	0.50±0.01	n.d.	0.40±0.01	n.d.	1.51±0.02	n.d.	0.28±0.02	0.33±0.03	0.70±0.01
6	23.42±0.11	1.16±0.03	0.12±0.01	0.24±0.01	0.49±0.01	0.22±0.01	0.48±0.02	n.d.	1.59±0.01	0.30±0.03	0.78±0.02	0.36±0.00	1.22±0.04
7	42.31±0.13	4.30±0.14	1.38±0.02	0.65±0.05	0.57±0.04	0.13±0.02	3.78±0.12	n.d.	1.99±0.01	n.d.	1.05±0.03	0.34±0.01	5.28±0.02
8	6.26±0.08	0.74±0.05	0.83±0.06	2.95±0.06	1.12±0.04	n.d.	0.41±0.01	n.d.	n.d.	n.d.	0.28±0.02	n.d.	n.d.
9	15.52±0.45	0.94±0.01	0.36±0.03	0.78±0.04	0.41±0.01	n.d.	0.49±0.01	n.d.	n.d.	n.d.	0.69±0.01	n.d.	n.d.
10	9.41±0.06	3.08±0.03	2.83±0.08	0.71±0.08	0.44±0.06	n.d.	n.d.	0.40±0.02	3.43±0.05	n.d.	0.30±0.01	0.54±0.06	1.03±0.04
11	3.37±0.04	0.68±0.03	0.35±0.01	0.44±0.01	1.33±0.04	0.29±0.01	n.d.	n.d.	2.22±0.03	n.d.	0.68±0.03	0.24±0.01	0.64±0.06
12	1.87±0.03	2.34±0.06	0.52±0.01	n.d.	0.81±0.04	0.33±0.02	n.d.	n.d.	3.18±0.03	n.d.	1.02±0.04	0.99±0.02	1.24±0.06
13	8.01±0.02	0.76±0.05	0.22±0.02	0.31±0.01	0.16±0.01	n.d.	n.d.	n.d.	1.66±0.05	n.d.	0.38±0.02	0.29±0.03	0.78±0.02
14	13.66±0.16	0.74±0.04	0.67±0.02	0.44±0.07	0.68±0.11	0.12±0.04	n.d.	n.d.	1.74±0.05	n.d.	0.51±0.01	0.32±0.03	1.13±0.02
15	4.64±0.06	0.26±0.01	0.68±0.03	2.86±0.05	0.41±0.05	n.d.	0.38±0.02	n.d.	n.d.	n.d.	0.37±0.04	n.d.	n.d.
16	1.47±0.08	0.74±0.04	0.47±0.03	0.75±0.06	0.43±0.02	n.d.	0.48±0.04	n.d.	n.d.	n.d.	0.67±0.04	n.d.	n.d.
17	3.30±0.05	1.11±0.01	2.83±0.02	n.d.	0.59±0.01	n.d.	0.51±0.00	n.d.	1.68±0.03	0.30±0.01	0.19±0.03	n.d.	n.d.

Table 4: General wine composition determined in the studied white wines. Values are means of triplicate determination (n = 3) with their standard deviations

No.	Alcohol (%vol)	Residual sugar (g/l)	Dry extract (g/l)	Total acidity* (g/l)	Volatile acidity** (g/l)	pH	Ash (g/l)	SO_2 free (mg/l)	SO_2 total (mg/l)
1	14.55±0.03	4.2±0.2	18.2±0.4	5.1±0.1	0.58±0.02	3.72±0.00	2.52±0.04	6.21±0.05	90.38±0.12
2	13.48±0.02	4.7±0.1	16.4±0.2	5.6±0.1	0.86±0.03	3.65±0.01	2.94±0.02	4.66±0.08	111.12±0.15
3	13.13±0.01	3.5±0.2	16.2±0.1	5.9±0.1	0.76±0.02	3.69±0.01	2.69±0.05	3.12±0.06	75.23±0.18
4	13.23±0.01	4.3±0.1	17.2±0.3	5.7±0.0	0.52±0.01	3.73±0.02	2.71±0.02	6.55±0.05	63.69±0.13
5	15.11±0.05	2.5±0.0	19.5±0.3	5.2±0.1	0.65±0.02	3.81±0.02	3.56±0.03	6.21±0.02	98.22±0.19
6	14.11±0.01	2.7±0.1	20.8±0.2	5.3±0.1	0.71±0.03	3.71±0.01	3.68±0.04	7.77±0.09	76.58±0.21
7	13.48±0.01	3.3±0.1	17.6±0.1	4.9±0.2	0.58±0.01	3.78±0.02	3.78±0.05	13.98±0.07	58.84±0.25
8	13.19±0.02	2.8±0.3	18.8±0.2	4.6±0.1	0.55±0.01	3.42±0.01	2.15±0.07	15.99±0.07	67.58±0.18
9	14.61±0.01	3.9±0.1	19.2±0.2	5.2±0.0	0.42±0.02	3.35±0.01	2.19±0.03	21.36±0.05	46.89±0.16
10	14.11±0.03	4.6±0.1	16.4±0.1	4.9±0.0	0.58±0.02	3.48±0.03	2.11±0.04	19.78±0.08	58.72±0.15
11	13.48±0.02	4.2±0.1	18.2±0.1	4.4±0.1	0.55±0.01	3.52±0.02	2.34±0.05	14.87±0.09	91.65±0.17
12	16.31±0.02	3.2±0.2	22.7±0.3	5.1±0.1	0.65±0.01	3.51±0.01	2.28±0.02	15.54±0.06	79.23±0.18
13	15.84±0.01	3.1±0.1	20.4±0.2	5.8±0.1	0.64±0.03	3.48±0.02	2.18±0.02	17.77±0.08	97.87±0.15
14	13.84±0.01	4.7±0.1	19.1±0.1	6.1±0.2	0.59±0.01	3.59±0.01	2.39±0.03	12.43±0.04	124.28±0.21
15	12.73±0.01	2.5±0.3	18.4±0.2	6.5±0.1	0.41±0.02	3.35±0.02	1.95±0.02	26.88±0.07	96.75±0.24
16	14.41±0.04	2.1±0.1	17.2±0.2	6.8±0.1	0.39±0.02	3.33±0.01	1.98±0.03	31.15±0.06	99.45±0.19
17	13.89±0.02	1.5±0.2	18.5±0.1	7.2±0.0	0.42±0.01	3.29±0.01	2.08±0.05	25.51±0.09	115.12±0.18

** as tartaric acid; **as acetic acid*

RESULTS AND DISCUSSION

PHYSICOCHEMICAL ANALYSIS

All parameters were in the normal range for good quality wine (Tab. 4). The lowest value for pH and highest value for total titratable acidity were determined in wine No. 15 made without maceration that is in accordance with previous studies (Darias-Martín et al., 2000). The highest values for alcohol, extract without sugar and ash were determined in wine No. 12, maceration of which lasted for 10 days.

DETERMINATION OF TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY

The results of the determination of total phenolic (TP) content (as mg/l gallic acid) in macerated and non-macerated wines by Folin-Ciocalteu method (Fig. 1) showed variations in content, ranging from 145.95 ± 0.32 in cold-macerated wine No. 10 to highest value 330.50 ± 1.94 mg/l found in wine No. 2, macerated for six months and made from different varieties. The TP content of wine No. 10 was significantly different from the others ($p < 0.01$) and there was a 2.26-fold difference in TP content between the highest and lowest ranked wine sample. No significant difference was found between wine samples Nos. 5 and 12, 16 and 8, 13 and 14. An average value of TP for all macerated wines analyzed was

227.91 mg/l. Direct comparison of different groups of macerated wines showed the highest content of TP in long-macerated wines, with an average of 275.52 mg/l. All groups of wine except cold-macerated wines showed higher content of TP compared with non-macerated ones. Ružič et al. (2011) published much higher TP values for white macerated wines with an average of 1859 mg/l. Our findings are more in agreement with some TP data for white wines in general. Vinkovič Vrček et al. (2011) and Rastija et al. (2009) reported TP content in Croatian wines ranging from 167 to 347 mg/l and 191 to 652 mg/l, respectively, and Mitič et al., (2010) reported the range of 238.3 to 420.6 mg/l in different varieties of Serbian white wines. The TP value detected in white wine aged for 12 months in different types of amphorae was 247.3 to 279.3 mg/l (Baiano et al., 2014).

Antioxidant activity (AC) results expressed as Trolox equivalents (μM TE/l) of different types of wines showed some variability in the capacity of certain samples. The results were expected considering the observations made on TP content and its positive correlation with the antioxidant capacity in general (Rice-Evans et al., 1996). Higher TP content and antioxidant activity were positively correlated with the length of maceration, as a consequence of better extraction of polyphenols from grape pomace during the process. The long and prolonged macerated wines showed 2-fold increase of AC by comparison with cold-macerated and non-macerated wines. It corresponds with the results of AC in macerated wines determined by DPPH method which showed higher correlation with TP in macerated than in non-macerated wines (Ružič et al., 2011). The relative AC determined for macerated wines in this study correlated significantly with TP of the wines, with high correlation coefficient of 0.8203 ($p < 0.01$) (Fig. 2). It seems that the AC of wines is not a property of individual polyphenols, but is widely distributed among them and it is a consequence of their synergistic activity and TP content (Makris et al., 2003). Some individual flavonols and stilbenes, like quercetin and trans-resveratrol, with low correlation coefficients did not provide a contribution to AC (0.1759 and 0.3383, respectively). Significantly higher values found for catechin (0.7448), caffeic (0.5997), gallic (0.5815) and caftaric acid (0.5777) appeared to have more influence on the AC of white wines. The highest AC found in wine sample No. 2, corresponds with research presented by Ružič et al. (2011) which indicates that mixing of different varieties provides a larger spectrum of phenolics and more combinations for synergistic activity.

The only data available on ABTS activity for Croatian white wines are for 6 organic and conventional white wines obtained from different varieties in a range from 321.52 ± 15.96 to 594.23 ± 4.45 (μM TE/l) (Vinkovič Vrček et al., 2011). According to the review of De Beer et al. (2002) the AC_{ABTS} of white wines from different countries were in the range of 0 to 5 mmol TE/l.

DETERMINATION OF INDIVIDUAL POLYPHENOLS

The content of individual polyphenols in studied white wines determined by HPLC is reported in table 2 and 3. The most abundant phenolic compound in 17 different white wines was caftaric acid (CftA) with an average of 17.74 mg/l and the significantly highest concentration was found in wine No. 7 ($p < 0.01$). The average concentration of caftaric acid of 27.3 mg/l found in wines Nos. 1 to 7 is in agreement with the fact that maceration causes a significant increase in caftaric acid (Ružič et al., 2011; Hernanz et al., 2007). Ružič et al. (2011) reported the highest concentration in macerated wine of 53.10 mg/l and average value of 18.46 mg/l. According to other researches caftaric acid predominates among other hydroxycinnamic acids (HCA) in white wines and its concentration range after maceration process was 18.46 to 116 mg/l (Fernandez-Pachon et al., 2006; Darias-Martín et al., 2000).

Results obtained for caffeic acid (CA) showed the highest concentration in wine No. 7 that is within ranges for CA in white macerated (0.68 to 5.45 mg/l) and Italian organic wines (0.23 to 7.07 mg/l) (Ružič et al., 2011; Lante et al., 2004). The average concentration of CA in macerated wines (1.83 mg/l) is significantly higher than the average in non-macerated ones (0.71 mg/l) ($p < 0.01$). P-coumaric acid (p-CMA) average concentration was 0.71 mg/l in macerated and 1.33 mg/l in non-macerated wines. Ružič et al. (2011) detected p-CMA only in two macerated wines with concentrations of 0.41 and 1.32 mg/l. There was no p-CMA in Croatian white wines and values for red wines ranged from 1.7 to 7.4 mg/l (Rastija et al., 2009). Similar concentrations (0.23 to 7.07 mg/l) were found in Italian organic wines (Lante et al., 2004).

The average concentration of fertaric acid (FrtA) was significantly higher ($p < 0.01$) in non-macerated than in macerated wines (1.81 and 0.60 mg/l, respectively) that is comparable with the quantity (0.79 to 1.9 mg/l) found in other macerated wines (Ružič et al., 2011). Statistically significantly higher values were found in wi-

Fig. 1: Total phenolic (TP) content in macerated and non-macerated white wines

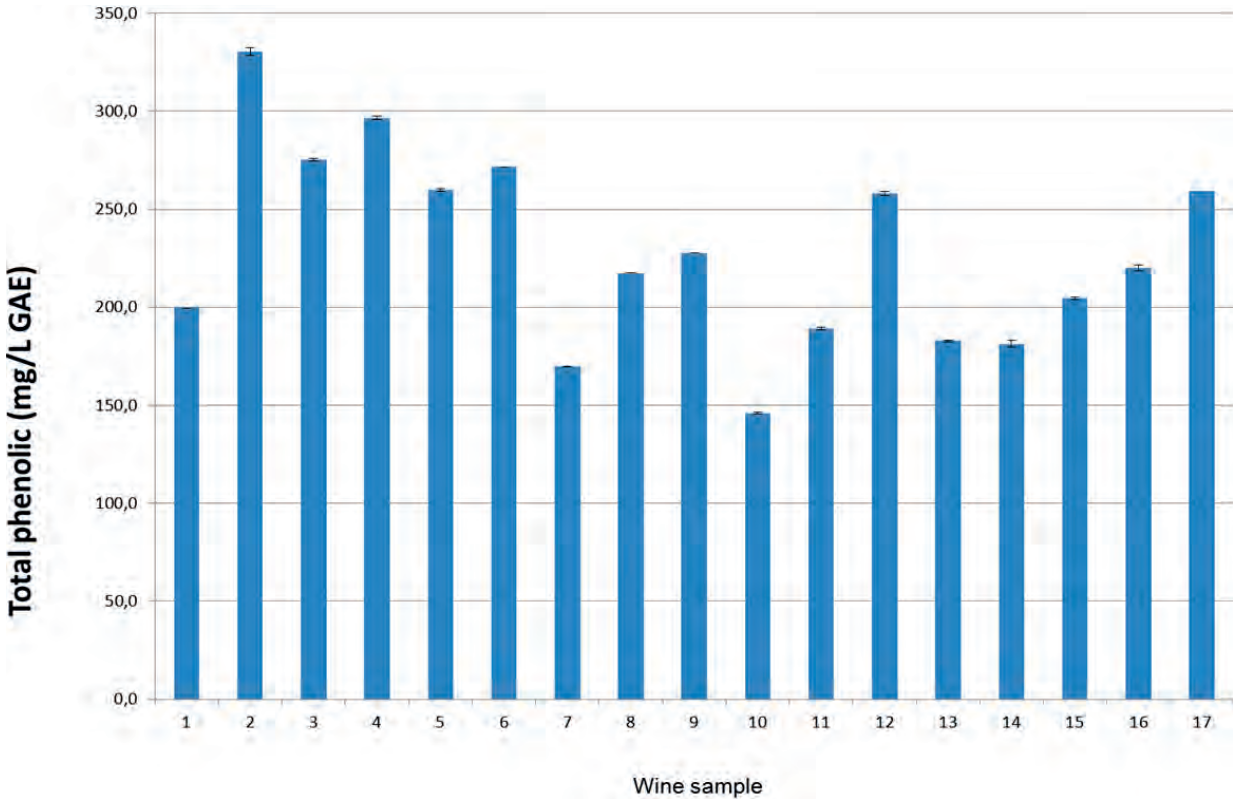


Fig. 2: Correlation between total phenolic (TP) content and related antioxidant capacity

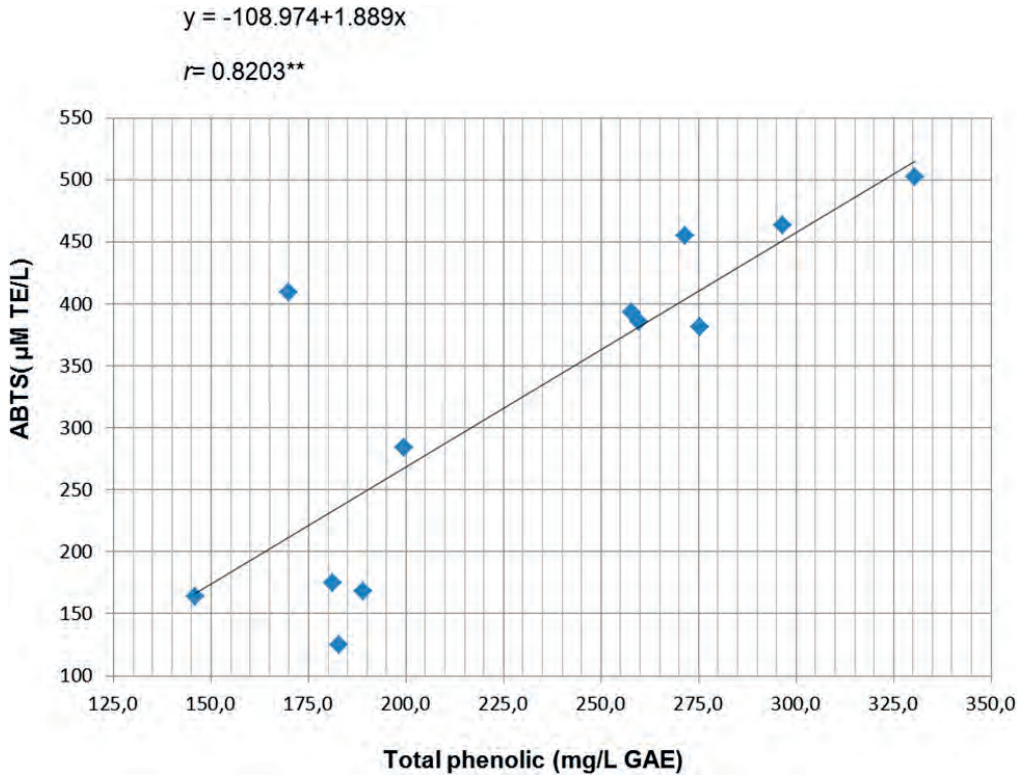
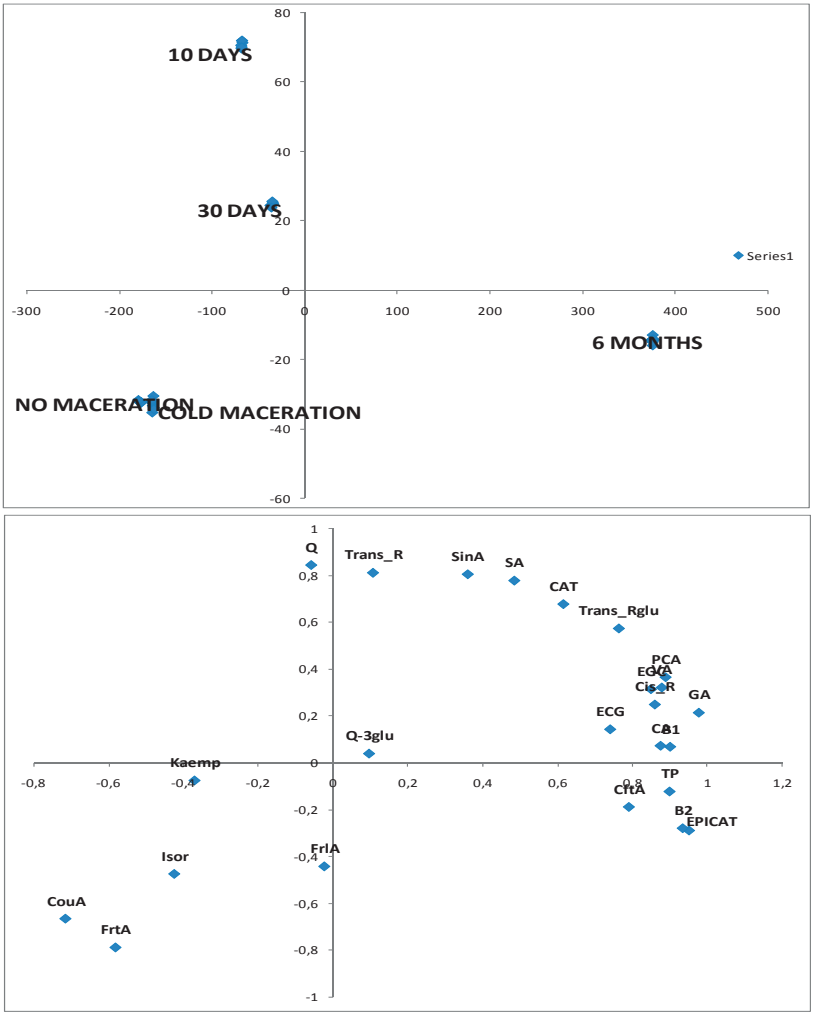


Fig. 3: Canonical discriminant analysis of 17 samples of five different maceration treatments (no maceration, cold maceration, 10 days, 30 days, 6 months) based on the contents of individual polyphenols



nes Nos. 8 and 15 ($p < 0.1$).

Ferulic acid (FrlA) was detected in all wines, with the average value of 0.6 mg/l that corresponds to the mean concentration of 0.7 mg/l in white macerated and Croatian wines (Ružič et al., 2011; Rastija et al., 2009). The significantly different values were found in cold-macerated wines Nos. 11, 8 and 12 ($p < 0.01$), results similar to the concentrations found in macerated white wines (Ružič et al., 2011). Sinapic acid (SinA) was detected in six macerated wines with an average value of 0.22 mg/l while in non-macerated wines it was not present. Gallic acid (GA) was detected as the main hydroxybenzoic acid (HBA) in macerated wines with the average value of 9.96 mg/l. These values are comparable with the quantity of GA ranging from 7.56 to 19.38 mg/l (mean value 11.68 mg/l) in macerated wines (Ružič et al., 2011) and a concentration range of 10.7 to 11.8 mg/l detected in Croatian red wines (Rastija et al., 2009). Significantly higher concentrations of GA ($p < 0.01$) were found in long-macerated types of wines with the highest value of 21.96 ± 0.23 mg/l in wine No. 2. The average concentration of GA in non-macerated wines was considerably lower (1.13 mg/l) which is in agreement with the average concentrations of 2.29 mg/l found in non-macerated wines (Ružič et al., 2011) and 2.4 mg/l found in Croatian white wines (Rastija et al., 2009). The concentration of GA was followed by vanillic acid (VA) with the significantly highest value ($p < 0.01$) detected in wine No. 7 and a mean value of 7.45 mg/l in macerated wines. The content of VA in non-macerated wines was 2-fold lower. VA was the major HBA in blended Croatian white wine with the value of 12.39 mg/l (Komes et al., 2007). Protocatechuic acid (PCA) was present within a range from 1.62 ± 0.05 to 5.75 ± 0.03 mg/l in macerated wines that is lower than the 10.7 mg/l detected in the work of Darias-Martín et al. (2000) and more similar to the values between 0.3 and 1.3 mg/l in the work of Pozo-Bayón et al. (2003) on Spanish sparkling wines. With the mean value of 3.13 in macerated and 0.31 mg/l in non-macerated wines, syringic acid (SA) was the HBA with the lowest concentration in white wines. These findings are in agreement with other studies mainly on red wines, that mostly reported the values of SA in traces. The stilbenes, including trans-resveratrol (trans-R), cis-resveratrol (cis-R) and resveratrol-3-O-glucoside, compounds with multiple health benefits, were found in all macerated wines (0.58, 0.41 and 1.67 mg/l in average, respectively). The average value for trans-R in macerated wines was 0.94 mg/l (Ružič et al., 2011). Total value of 5.18 mg/l for the trans- and cis-resveratrol and their glucosides was the highest value in Spanish wines (Darias-Martín et al., 2000), since their average concentration in general was 0.48 mg/l (Romero-Perez et al., 1996). In comparison to these findings, the mean value of trans-R in white wines from Croatia was 0.45 mg/l (Rastija et al., 2009) and 0.23 mg/l for Greek white wines (Gerogiannaki-Christopoulou et al., 2006). The significantly highest values ($p < 0.01$) for trans-R and cis-R detected in wine No. 7 were considerably lower than the amount of 1.95 mg/l for trans-R found in macerated white wine (Ružič et al., 2011) and higher than the concentration range (0.1 to 0.8 mg/l) for cis-R in white wines and highest value of 1.7 mg/l in Portuguese white wine (Rentzsch et al., 2009; Ribeiro de Lima et al.,1999). Higher concentrations of cis-R in long- and prolonged-macerated wines could be explained by the fact that cis-R derives from trans-R isomer which increasingly extracts during the maceration process and by slow hydrolysis of its glucosides during wine aging (Jeandet et al., 1995; Soleas et al., 1995).

There was only trans-R detected in non-macerated wines with a mean value of 0.41 mg/l that is very similar to the value of 0.48 mg/l found in white wines (Ružič et al., 2011). Considering the conduction of maceration process, non-flavonoids were extracted in two times greater quantity and flavonoids concentration increased 5-fold in the macerated wines. Within the flavonoid compounds, the most abundant group with the most significant difference between non-macerated and macerated wines was the flavanol group. These results are in accordance with previous researches (Darias-Martín et al., 2000; Hernanz et al., 2007; Ružič et al., 2011). The mean concentration of catechin (CAT) in macerated wines was 16.09 mg/l with the highest values detected in long-macerated wines that can be comparable with the values presented in other research on macerated white wines (Ružič et al., 2011). The mean concentration for CAT in non-macerated wines (0.91 mg/l) was lower than the mean values (2.26 and 1.8 mg/l, respectively) for Croatian white wines (Komes et al., 2007; Rastija et al., 2009). The average concentration of epicatechin (EPICAT) in macerated wine was 2.36 mg/l that is in agreement with the mean value for Croatian wine (Komes et al., 2007), with the significantly highest value found in wine No. 1 ($p < 0.01$). The mean value for non-macerated wines (0.75 mg/l) is considerably lower which accords with previous experiments (Ružič et al., 2011; Hernanz et al., 2007). This study confirmed the presence of some flavonols like quercetin (Q) and quercetin-3-glucoside (Q-3glu) in almost all wine samples. The highest concentration of flavonol compounds was found in wine No. 7. Isorham-

netin (Isor) was detected only in wine No. 7 (0.4 ± 0.02 mg/l) and kaempferol (Kaemp) only in wine No. 6 (0.3 ± 0.03 mg/l). The significantly highest concentration of quercetin was found in wines Nos. 10 and 12 ($p < 0.01$). Levels of quercetin and kaempferol were in agreement with the values found in Croatian wines and Australian white wines (Rastija et al., 2009; Jeffery et al., 2008). The concentration range for quercetin-3-glucoside was 0.39 to 3.47 mg/l in macerated wines with a significantly higher value in wine No. 7 ($p < 0.01$). Although the absence or very low concentration of individual flavonols in white wines is expected, since these compounds are located mainly in the grape skins, it can be observed that the process of maceration influenced its concentration as in the previous researches (Hernanz et al., 2007). In order to classify white wines produced by different maceration types (no maceration, cold maceration, 10 days, 30 days and 6 months) canonical discriminant analysis was performed (Fig. 3). A plot of the first two canonical variables shows that Can1 discriminates between three groups: 1) no maceration and cold-maceration; 2) 10 days and 30 days; and 3) 6 months, while Can2 discriminates four groups: 1) 10 days; 2) 30 days; 3) 6 months and 4) no maceration and cold-maceration. No maceration and cold-maceration treatments form one consistent group which is more connected with variables Isor, CouA, FrtA, FrlA and Kaemp. FrlA is strongly correlated with Can2, and Kaemp is strongly correlated with Can1. Group 10 days and 30 days are more influenced with variables Q, Trans-R and Q-3glu while 6 group month is correlated with variables TP, B1, B2, CftA, CA and EPICAT.

CONCLUSIONS

Results achieved in this study showed a strong impact of maceration treatments on the individual polyphenol profiles of analyzed Croatian white wines. This study clearly demonstrates that despite the high impact of other factors (year, variety, region), the polyphenol profiles of white wines depend on the maceration duration time. Among the analyzed wines, 6 months maceration process showed a strong influence on some stilbene, flavonol and flavanol compound concentrations while no maceration and cold-maceration had a stronger impact on phenolic acid compounds.

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