ISSN 1330-9862
(FTB-2860)

original scientific paper

The Influence of Skin Maceration Time on the Phenolic Composition and Antioxidant Activity of Red Wine Teran (*Vitis vinifera* L.)

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Received: June 29, 2011
Accepted: March 20, 2012

Summary

The effect of four different maceration times (5, 10, 15 and 20 days) on anthocyanins, phenolic acids (hydroxybenzoic, gallic, protocatechuic, vanillic, syringic, caffeic and *p*-coumaric acids), flavan-3-ol composition, vanillin index and antioxidant activity of Teran red wine (*Vitis vinifera* L.) has been investigated. Phenolics were determined by HPLC with UV-diode array detection. Vanillin index was determined by UV-VIS spectrophotometry. The total antioxidant capacity of wine was measured with three different methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2′-azinobis(3-ethylbenzothialozine-6-sulphonic acid) radical cation ($ABTS^+$) scavenging methods and ferric reducing antioxidant power (FRAP). Sensory evaluation of wine samples was performed. The obtained results show positive correlation between phenolic acids, flavan-3-ols [(+)-catechin and (–)-epicatechin] and vanillin index and the duration of maceration. Anthocyanin content increased to the maximum within 10 days of skin maceration. The major anthocyanins in Teran wine were malvidin-3-*O*-glucoside, malvidin-3-glucoside acetate and petunidin-3-*O*-glucoside. Antioxidant activity of Teran wines significantly increased with prolonged skin contact as well as total phenolic content ($p<0.001$). Wine produced with maceration time of 10 days had the best sensory properties.

Key words: Teran wine, phenolic composition, antioxidant activity, maceration time

Introduction

Wine phenols are important quality components of red wine that contribute to its colour, flavour, body and structural characteristics. Anthocyanins and polymeric pigments give red wine its colour, while flavanols and their polymers (tannins) are responsible for bitterness and astringency (*1*). Polyphenolic composition of red wine depends on the polyphenolic compounds in the grape the wines are made of, ripening, climatic conditions and winemaking techniques, especially the duration of skin contact (*2*,*3*). Colour in young red wines mostly originates from the anthocyanins extracted from the grape skin during maceration process, with malvidin-3-glucoside as the major component (*2*,*4*). Longer maceration is usually accompanied by increasing concentration of anthocyanins in wine, although some authors found no direct correlation between the length of maceration and anthocyanin content, probably because of possible fixation to the solid parts and reduction in colourless form (*5*).

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Flavan-3-ols, mainly (+)-catechin and (–)-epicatechin, are also extracted from grape skin and seeds during winemaking. They show interaction with anthocyanins through copigmentation process (*6*) and have an important role in defining sensorial characteristics of red wine. The extraction of flavan-3-ols is better when increasing the length of skin contact and percentage of ethanol in model wines, and they continue to be extracted after the anthocyanin extraction has reached maximum (*7,8*). Skin contact period also has a great impact on the extraction of phenolic acids (hydroxycinnamic and hydroxybenzoic acids) (*9*). According to Puškaš *et al*. (*10*), the wine phenolic compounds have high free radical scavenging potential. Free radicals are extremely harmful to living organisms because they attack different constituents of the cell. Antioxidant activity of wines has been determined by different methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (*11*) and 2,2′-azinobis(3-ethylbenzothiazoline-6--sulphonic acid) (ABTS) radical cation (*12*) scavenging methods and ferric reducing antioxidant power (FRAP) (*13*). The protective effect of wine is mainly due to the anthocyanin fraction of the red wine but the results do not exclude the possibility of a synergistic action among the different classes of polyphenols (*14*). Some authors have determined a correlation between the total antioxidant potential of wine and the gallic acid, (–)-epicatechin and (+)-catechin concentrations, total phenolic content and maceration time as well as the grape variety (*15–17*).

Teran (*Vitis vinifera* L.) is the most widespread red autochthonous cultivar on the Istrian peninsula, Croatia, mainly produced with 5–10 days of skin contact, and up until now there has not been a detailed investigation of its phenolic composition and antioxidant activity. Therefore, the aim of this paper is to investigate the influence of skin maceration time on the polyphenolic composition and antioxidant activity of Teran wines.

Materials and Methods

Grapes and winemaking

The grapes of *Vitis vinifera* cv. Teran were harvested at technological maturity (204 g/L of reducing sugars, 10 g/L of total acidity as tartaric acid and pH=2.95) in 2008 vintage year in the Western Istria winegrowing region, Croatia. Grapes were destemmed and crushed, homogenized and transferred into 50-litre tanks for maceration with the addition of 30 mg/L of $K_2S_2O_5$ and 0.3 g/L of selected dry wine yeast BDX (Lallemand Inc, Montreal, Canada). Four skin maceration periods were applied: 5 (M5d), 10 (M10d), 15 (M15d), and 20 (M20d) days at the temperature of 25 °C. Each vinification was performed in three replications. The cap was punched down by hand three times daily. After maceration, the pomace was pressed at hydro press at 0.8 bar. Fermentation temperature and must density were monitored daily. After alcoholic fermentation (malolactic fermentation was not performed), the wines were racked with 15 mg/L of free SO_2. Second racking was done in February 2009, when the wines were bottled into 750-mL bottles and stored until analysis.

Chemicals and phenolic reference standards

The 2,2′-azinobis-(3-ethylbenzothialozine-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tris(2-pyridyl)-*s*-triazin (TPTZ) were obtained from Sigma-Aldrich Chemie (Steinheim, Germany) and HPLC grade acetonitrile and phosphoric acid were obtained from Fluka (Sigma-Aldrich, Buchs, Switzerland). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Sigma-Aldrich (Gillingham, UK). Vanillic, syringic and *p*-coumaric acids were of HPLC grade and obtained from Fluka. Ferulic acid, gallic acid, protocatechuic acid, (+)-catechin and (–)-epicatechin were obtained from Sigma-Aldrich Chemie. HPLC grade caffeic acid and methanol were obtained from Merck (Darmstadt, Germany). Formic acid and Folin-Ciocalteu reagent were of analytical grade and were supplied by Kemika (Zagreb, Croatia). Delphinidin-3-*O*-glucoside, peonidin--3-*O*-glucoside, petunidin-3-*O*-glucoside and malvidin-3--*O*-glucoside were from Polyphenols AS (Sandnes, Norway). All standards used were of HPLC grade. Vanillin (≥97 % FCC, FG grade) and *p*-dimethylaminocinnamaldehyde (*p*-DAC) (HPLC grade) were obtained from Sigma--Aldrich Chemie.

Standard physicochemical analyses

The basic physicochemical parameters were analyzed in the must (reducing sugars, total acidity and pH) and wines (relative density, alcohol, total extract, reducing sugars, total acidity, volatile acidity, free and bound SO_2, pH and ash) according to the International Organization of Vine and Wine (OIV) methods (*18*).

Analyses of phenolic compounds

Total phenols were evaluated as stated by Singleton and Rossi (*19*) using Folin-Ciocalteu reagent. The quantification of total phenols was carried out using a calibration curve prepared with known amounts of gallic acid (GAE). The flavonoid content in the selected wine samples was determined spectrophotometrically according to the method of Lee *et al.* (*20*). Catechins were determined by their reaction with vanillin and *p*-dimethylaminocinnamaldehyde (*p*-DAC), and were expressed as mg/L of (+)-catechin (CE) (*21*). Total anthocyanin content in wines was determined using bisulphite bleaching method (*22*).

Determination of anthocyanins

The free anthocyanin content was determined with HPLC according to the method of Berente *et al*. (*23*). The wine samples were filtered through a 0.45-μm filter (Nylon Membranes, Supelco, Bellefonte, PA, USA) before the HPLC analysis. A volume of 20 μL of each sample was injected using a Varian ProStar Solvent Delivery System 230 (Varian, Walnut Creek, CA, USA) and a photodiode array detector Varian ProStar 330 using a reversed-phase Pinnacle II C-18 column (Restek, Bellefonte, PA, USA) (250×4.6 mm, 5 μm i.d.). The following mobile phases were used: buffer: 10 mM KH_2PO_4 and H_3PO_4 to pH= 1.6, solvent A: acetonitrile/buffer (5:95), solvent B: acetonitrile/buffer (50:50). The oven temperature was 50 °C. Gradient elution was applied at 1 mL/min flow rate according to the program described by Berente *et al*. (*23*). Chromatograms were recorded at 518 nm. Detection was

performed with a photodiode array detector by scanning between 200 and 600 nm, with a resolution of 1.2 nm. Individual anthocyanins were identified by comparing their retention times and visible spectra with those of authentic standards. Quantitative determinations were performed using standard curves of delphinidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (Polyphenols AS). The data acquisition and treatment were conducted using the Star Chromatography Workstation v. 5 software. All analyses were repeated three times, and the results were expressed as mean values in mg/L of wine±standard deviations (S.D.).

Phenolic acids and flavan-3-ols

The wine samples were filtered through a 0.45-μm filter (Nylon Membranes, Supelco) before HPLC analysis. A volume of 20 μL of each sample was injected and analysed applying a Varian ProStar Solvent Delivery System 230 and a Varian ProStar 330 photodiode array detector using a Pinnacle II C-18 reversed-phase column (Restek) (250×4.6 mm, 5 μm i.d.). The solvents consisted of water, 3 % formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL/min. The elution was performed with a gradient starting at 2 % B to reach 32 % B at 20 min, 40 % B at 30 min and 95 % B at 40 min, and became isocratic for 5 min. Detection was performed with a photodiode array detector by scanning between 200 and 400 nm, with a resolution of 1.2 nm. Phenolic compounds were identified by comparing the retention times and spectral data with those of authentic standards. Quantitative determinations were performed using standard curves of determined hydroxycinnamic acids, hydroxybenzoic acids and flavan-3-ols. The data acquisition and treatment were conducted using the Star Chromatography Workstation v. 5 software. All analyses were repeated three times, and the results were expressed as mean values in mg/L of wine±S.D.

Determination of antioxidant activity

Free radical scavenging ability determination by the use of DPPH radical

The samples were analyzed according to the method reported by Brand-Williams *et al.* (*11*). This method is based on the reduction of a stable DPPH radical by methanolic solution of antioxidants, which alters the purple colour of the DPPH radical solution to a bright yellow. In brief, a volume of 3.8 mL of methanolic DPPH solution, c(DPPH)=0.094 mmol/L, was added to 200 μL of diluted sample. Free radical scavenging capacity of the sample was evaluated by measuring the absorbance at 517 nm immediately after the addition of DPPH (t=0) and at 1 min intervals until the reaction, *i.e.* corresponding absorbance, reached its steady state. Antioxidant capacity was expressed as mmol/L of Trolox equivalents (TE), using the calibration curve of Trolox (0–1000 μM), a water-soluble vitamin E analogue. All determinations were performed in triplicate.

Free radical scavenging ability determination by the use of ABTS radical cation

Flow injection analysis (FIA) with electrochemically generated ABTS radical cation is a method based on the scavenging of stable blue-green ABTS radical cation ($ABTS^{\cdot+}$), formed either by chemical or enzymatic oxidation of ABTS several hours prior to the analysis (*12*). The amount of ABTS radical cation scavenged by antioxidants is measured by monitoring the decrease of absorbance of ABTS radical cation, and compared with the decrease of absorbance produced by the addition of a known amount of Trolox, a water-soluble vitamin E analogue. In order to avoid the time consuming step of ABTS radical cation preparation, FIA modification of TEAC method was employed. FIA is based on the electrochemical production of ABTS radical cation in the electrolysis flow-cell forming part of FIA system. A phosphate buffer solution of 0.1 mol/L (pH=7.40) was used as a carrier stream, and the solution of ABTS radical cation generated electrochemically was employed as a second (reagent) stream. Both streams were pumped at a flow rate of 0.5 mL/min. The carrier and reagent streams were mixed by passing through a mixing coil, and the absorbance at 734 nm was monitored by a detector placed immediately after the coil. For the analysis, an aliquot of 20 μL of diluted sample was injected into the carrier stream. The height of the FIA peak obtained by the sample injection (ΔA_{sample}) was compared with the height of the peak produced by the injection of 0.6 mmol/L of Trolox solution (ΔA_{Trolox}). The TEAC values were then determined according to the formula:

$$TEAC = \Delta A_{sample} \cdot c(\text{Trolox}) / \Delta A_{Trolox} \qquad /1/$$

and expressed as equivalent concentration.

Determination of ferric reducing/antioxidant power (FRAP assay)

The ferric reducing/antioxidant power (FRAP) assay was carried out according to Benzie and Strain (*13*). This assay measures the change in the absorbance at 593 nm owing to the formation of a blue-coloured Fe(II)-tripyridyltriazine compound from the colourless oxidized Fe(III) form by the action of electron-donating antioxidants. FRAP reagent was prepared by mixing acetic buffer, TPTZ and $FeCl_3 \cdot 6H_2O$ (20 mM water solution) at a ratio of 10:1:1. Briefly, to a volume of 200 μL of wine, 3.8 mL of FRAP reagent was added. After 4 min, the absorbance of blue colouration was measured against a blank sample. A standard curve was prepared using different concentrations (100–1200 μmol/L) of Fe^{2+}. All measurements were performed in triplicate.

Statistical analysis

Analysis of variance (ANOVA) was used to compare all significant differences among four maceration treatments. Values were expressed as means (N=3) with standard deviations. For the comparison of the results of total phenols, total flavonoids, total nonflavonoids, total anthocyanins, $DPPH^{\cdot}$, $ABTS^{\cdot+}$ or FRAP assays, the coefficients of correlation were determined.

Sensory evaluation of wine

The wines were subjected to sensory evaluation by the 100-point International Organization of Vine and Wine (OIV)/International Union of Oenologists (UIOE)

method, with a panel of 5 judges who are all members of the Croatian Enological Society.

Results and Discussion

Basic chemical composition of wine

The results of the effect of time of maceration on the basic chemical composition of Teran wine are shown in Table 1, representing the average values of three repetitions. The analyzed wines did not differ significantly according to the basic chemical composition, which is in accordance with the results obtained by other authors (*3*).

Total phenols, total flavonoids, total nonflavonoids, total anthocyanins and vanillin index

The influence of maceration duration on the mass fraction of total phenols (TP), total flavonoids (TF), total nonflavonoids (TNF) and total anthocyanins (TA), including vanillin index, *p*-DAC and antioxidant activity ($DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP) is given in Table 2. TP content in Teran wines varied from 1455 to 2718 mg/L of GAE, increasing significantly ($p<0.001$) with the length of maceration, which is in agreement with previous results (*24*), and it is the same with TF (from 1305 to 2358 mg/L of GAE). The largest mass fraction of total anthocyanins (414 mg/L) was noted on the 10th day of maceration time, with a significant reduction ($p<0.001$) on the 15th and 20th days, probably because of their reaction with proanthocyanins and (+)-catechin that forms condensed anthocyanins (*24*). The mass fraction of TNF was significantly higher ($p<0.001$) on the 10th (the mass fraction maximum), 15th and 20th days of maceration in comparison with the maceration for 5 days. Although the mass fraction of TNF during maceration for 15 and 20 days decreased, significant differences among treatments were not observed. Vanillin index in wines significantly increased ($p<0.001$) with longer skin contact from the 5th to 20th day as well as the values of *p*-DAC assay.

Table 1. Basic chemical composition of Teran wines after different maceration time

Parameter	*t*(maceration at 25 °C)/day				LSD
	5	10	15	20	
Relative density (20/20 °C)	0.9940	0.9935	0.9928	0.9940	n.s.
φ(alcohol)/%	12.00	12.10	12.00	11.9	n.s.
γ(total extract)/(g/L)	24.15	24.35	24.28	24.59	n.s.
γ(reducing sugars)/(g/L)	1.8	1.8	1.5	1.6	n.s.
γ(volatile acidity)[1]/(g/L)	0.52	0.51	0.48	0.55	n.s.
γ(total acidity)[2]/(g/L)	10^{a}	10^{a}	9.9^{ab}	9.8^{b}	0.14 (5 %)
pH	3.05	3.02	3.06	2.98	n.s.
γ(ash)/(g/L)	2.62	2.6	2.71	2.77	n.s.

[1]as acetic acid, [2]as tartaric acid; letters represent significant differences at $p<0.05$ level; n.s.=not significant difference, LSD=least significant difference

Table 2. Phenolic content and antioxidant activity of Teran wines produced with different duration of maceration

Parameter	*t*(maceration at 25 °C)/day				LSD
	5	10	15	20	
Phenolic content					
γ(TP as GAE)/(mg/L)	1455^{d}	1918^{c}	2255^{b}	2718^{a}	255.70 (0.1 %)
γ(TF as GAE)/(mg/L)	1305^{c}	1478^{c}	1845^{b}	2358^{a}	235.62 (0.1 %)
γ(TNF as GAE)/(mg/L)	150^{b}	440^{a}	410^{a}	360^{a}	195.34 (0.1 %)
γ(TA)/(mg/L)	377^{a}	414^{a}	315^{b}	291^{b}	60.38 (0.1 %)
γ(VAN as CE)/(mg/L)	1015^{d}	1217^{c}	1458^{b}	1592^{a}	146.88 (0.1 %)
γ(*p*-DAC as CE)/(mg/L)	145^{c}	258^{bc}	320^{ab}	362^{a}	143.26 (0.1 %)
Antioxidant activity					
$DPPH^{\cdot}$ as TE/(mmol/L)	5.59^{b}	8.13^{ab}	8.87^{a}	9.56^{a}	3.18 (5 %)
$ABTS^{\cdot+}$ as TE/(mmol/L)	26.64^{Bc}	30.45^{ABb}	31.29^{Aab}	33.12^{Aa}	4.33 (1 %)
					2.36 (5 %)
FRAP/(mmol/L)	22.24^{Bb}	25.16^{ABb}	28.24^{Aa}	29.51^{Aa}	5.74 (1 %)
					3.13 (5 %)

Different letters in superscript represent significant differences at $p<0.001$, $p<0.01$ and $p<0.05$ level; LSD=least significant difference, TP=total phenols, TF=total flavonoids, TNF=total nonflavonoids, TA=total anthocyanins, VAN=vanillin index, GAE=gallic acid equivalent, CE=catechin equivalent

Influence of skin maceration time on the composition of anthocyanins, phenolic acids, (+)-catechin and (–)-epicatechin

Anthocyanin content reached maximum on the 10th day of maceration. Anthocyanin monoglucosides are the major anthocyanins in Teran wines. Total amount of monoglucosides was between 135.39 and 192.68 mg/L. Malvidin-3-*O*-glucoside was the most abundant anthocyanin (Table 3) (*5*,*25*), which reached its maximum concentration on the 10th day of maceration (192.68 mg/L). Petunidin-3-*O*-glucoside was the second most abundant pigment in Teran wines. The major acetyl and *p*-coumaroyl derivates present in Teran wines are malvidin-3--glucoside acetate and malvidin-3-glucoside-*p*-coumarate. Acylated anthocyanins reached the maximum concentration on the 10th day of skin maceration, while *p*-coumaroyl anthocyanins reached their maximum on the 15th day of skin maceration. Flavan-3-ols [(+)-catechin ($p<0.01$) and (–)-epicatechin ($p<0.05$)] increased significantly during the process of maceration. Both flavan-3-ols reached their maximum on the 20th day of maceration with a higher value of (+)-catechin (23.15 mg/L) than (–)-epicatechin (17.56 mg/L), which is consistent with the literature data (*6*). In Teran wines the following phenolic acids were identified: gallic, protocatechuic, vanillic and syringic acids as hydroxybenzoic acids; and caffeic, *p*-coumaric and ferulic acids as hydroxycinnamic acids. According to the previously published research, the content of nonflavonoid phenols increased during fermentation of skin (*23*), which was also the case in our study. Hydroxybenzoic acids and hydroxycinnamic acids (except ferulic acid) showed a significant ($p<0.05$) increase with prolonged maceration and reached their maximum on the 20th day of maceration (Fig. 1). The most abundant phenolic acids in all Teran wines were gallic (from 9.15 to 11.89 mg/L) and caffeic acids (from 8.11 to 9.34 mg/L).

Antioxidant capacity

Research (*16*,*17*,*26*,*27*) has shown the influence of the concentration of total phenolics, flavan-3-ols (catechin in particular) and phenolic acid (gallic acid in particular) on the antioxidant activity of red wine. Antioxidant capacity of Teran wines as a result of different skin maceration duration determined by all the methods under study is shown in Table 2. As previously reported, the FRAP assay was defined as the ferric reducing/antioxi-

Table 3. Changes in anthocyanins, phenolic acids, (+)-catechin and (–)-epicacechin content depending on the length of maceration

Parameter	*t*(maceration at 25 °C)/day				LSD
	5	10	15	20	
	γ/(mg/L)				
Anthocyanins					
delphinidin-3-*O*-glucoside	(16.11±2.35)^{b}	(25.03±2.01)^{a}	(20.82±2.44)^{ac}	(15.64±1.89)^{b}	9.13 (1 %)
cyanidin-3-*O*-glucoside	(4.81±0.78)^{ab}	(6.70±0.89)^{a}	(5.71±0.68)^{ab}	(4.10±0.55)^{b}	2.53 (5 %)
petunidin-3-*O*-glucoside	(20.52±2.14)^{ab}	(30.32±2.55)^{a}	(27.42±3.01)^{ab}	(16.11±1.82)^{b}	13.35 (1 %)
peonidin-3-*O*-glucoside	(15.72±1.55)^{ab}	(20.21±2.35)^{a}	(18.11±2.14)^{ab}	(11.12±2.65)^{b}	9.72 (1 %)
malvidin-3-*O*-glucoside	(174.51±9.14)^{ab}	(192.68±8.55)^{a}	(150.98±7.56)^{bc}	(135.39±6.1)5^{c}	39.79 (0.1 %)
delphinidin-3-glucoside acetate	(7.85±2.05)^{ab}	(8.55±2.01)^{a}	(7.85±1.87)^{ab}	(6.23±1.35)^{b}	1.81 (5 %)
cyanidin-3-glucoside acetate	2.14±1.16	3.23±2.01	3.81±2.01	3.20±1.16	n.s.
peonidin-3-glucoside acetate	(4.85±1.01)^{b}	(7.51±0.78)^{a}	(6.22±0.85)^{ab}	(5.32±0.55)^{b}	2.36 (5 %)
malvidin-3-glucoside acetate	(20.25±1.45)^{ab}	(27.88±1.16)^{a}	(18.12±2.11)^{b}	(20.33±3.15)^{ab}	10.64 (0.5 %)
peonidin-3-glucoside-*p*-coumarate	2.21±0.42	3.85±1.16	4.51±1.05	2.95±0.66	n.s.
malvidin-3-glucoside-*p*-coumarate	(11.24±2.05)^{b}	(18.14±3.01)^{ab}	(23.40±2.11)^{a}	(20.51±2.23)^{ab}	12.92 (0.1 %)
Phenolic acids					
Hydroxybenzoic acids					
gallic acid	(9.15±0.93)^{c}	(10.14±0.81)^{bc}	(11.58±0.78)^{ab}	(11.89±0.89)^{a}	1.92 (5 %)
protocatechuic acid	(6.21±1.17)^{b}	(7.15±1.26)^{ab}	(7.89±0.52)^{a}	(7.55±0.44)^{a}	1.33 (5 %)
vanillic acid	(4.85±0.55)^{b}	(5.01±0.11)^{ab}	(5.55±0.26)^{ab}	(5.99±0.36)^{a}	1.05 (5 %)
syringic acid	(7.89±1.14)^{b}	(8.01±1.05)^{b}	(8.55±1.10)^{ab}	(9.12±098)^{a}	0.89 (5 %)
Hydroxycinnamic acids					
caffeic acid	(8.11±1.15)^{b}	(8.55±1.01)^{ab}	(9.05±0.77)^{a}	(9.34±0.80)^{a}	0.87 (5 %)
p-coumaric acid	(5.31±0.55)^{b}	(6.05±0.89)^{ab}	(6.78±0.79)^{a}	(6.65±0.98)^{a}	1.35 (5 %)
ferulic acid	1.56±0.55	2.11±0.45	2.87±0.87	3.01±0.80	n.s.
Flavan-3-ols					
(+)-catechin	(19.21±2.06)^{b}	(20.11±2.55)^{ab}	(22.15±2.01)^{a}	(23.15±2.15)^{a}	3.43 (1 %)
(–)-epicatechin	(13.45±1.66)^{b}	(14.52±2.05)^{b}	(17.12±2.16)^{a}	(17.56±2.35)^{a}	2.49 (5 %)

Different letters in superscript represent significant differences at $p<0.001$, $p<0.005$, $p<0.01$ and $p<0.05$ level; n.s.=not significant difference, LSD=least significant difference

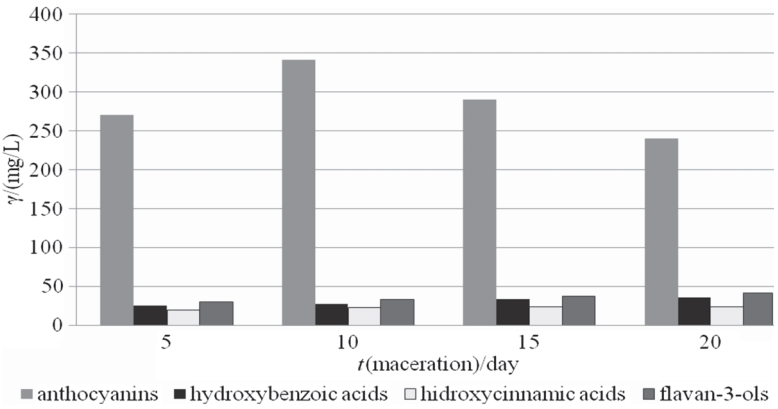

Fig. 1. Impact of the length of maceration on the extraction of some phenolic compounds

dant power, which measures the ability of a compound to reduce Fe^{3+} to Fe^{2+} (*13*,*28*). The consecutive order of wines produced with different skin maceration time, based on the antioxidant capacity evaluated by the FRAP assay, follows the TP observed with the Folin–Ciocalteu assay: M5d<M10d<M15d<M20d. These results are also confirmed by a high correlation coefficient (Table 4) between the TP content and FRAP assay (R=0.980). The DPPH and ABTS radical scavenging capacities of Teran wines expressed as mmol/L of Trolox are also shown in Table 2. In both cases, the results show the increase of antioxidant activity ($ABTS^{\cdot+}$ $p<0.01$ and $DPPH^{\cdot}$ $p<0.05$). These results are in positive correlation with TP, TF and TNF content and in negative correlation with TA content. The antioxidant capacity of Teran wines obtained by $ABTS^{\cdot+}$ assay was higher than the one obtained by $DPPH^{\cdot}$ assay, because DPPH radical reacts only with lipophilic antioxidants, while ABTS radical reacts with both hydrophilic and lipophilic antioxidants (*28*).

Table 4. Correlation between phenolic groups and antioxidant activity

	$DPPH^{\cdot}$	$ABTS^{\cdot+}$	FRAP
TP	0.949	0.969	0.980
TF	0.854	0.891	0.938
TNF	0.827	0.780	0.654
TA	–0.612	–0.630	–0.799

TP=total phenols, TF=total flavonoids, TNF=total nonflavonoids, TA=total anthocyanins

Sensory evaluation of wine

Fig. 2 shows the effect of the duration of maceration on the sensory characteristics and results of evaluation of six-month-old Teran wines. Wine produced with M10d treatment has proved to be the most harmonious in terms of astringency, unlike the wines produced with M15d and M20d treatments. Furthermore, M10d treatment had the greatest impact on the fruitiness of wine. The feel of acidity was the highest at M15d and M20d treatments due to synergistic effect with the tannins. Wines were judged by 100 points as follows: M10d (83 points)>M5d (80 points)>M15d (79 points)>M20d (76 points).

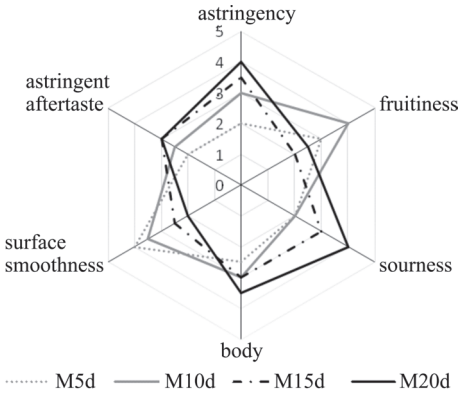

Fig. 2. Influence of different maceration times on sensorial profile of six-month-old Teran wines

Conclusions

It can be concluded from the results of the present investigation that the extensive duration of skin maceration has a significant effect on the increase of phenolic content and antioxidant activity in Teran wines. The content of total anthocyanins including the most abundant anthocyanin malvidin-3-*O*-glucoside reached maximum on the 10th day of maceration and then decreased after further prolongation of maceration (15 and 20 days). Concentration of hydroxybenzoic and hydroxycinnamic acids increased significantly with the duration of skin maceration, so did (+)-catechin and (–)-epicatechin, which were the highest on the 20th day of skin maceration. Total phenols, total flavonoids and total nonflavonoids have a positive correlation, and total anthocyanins a negative correlation with $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and FRAP assays, respectively. Sensory evaluation of Teran wine showed that the most harmonious wine was the one produced with 10-day maceration period, while the wines produced with longer maceration showed a marked astringency, and as such showed a potential for ageing.

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