Journal of Applied Botany and Food Quality 90, 197 - 204 (2017), DOI:10.5073/JABFQ.2017.090.025

University of Perugia, Department of Pharmaceutical Sciences, Section of Food Science and Nutrition, Perugia, Italy

Phenol composition and antioxidant capacity of red wines produced in Central Italy changes after one-year storage

Germana Lombardi, Lina Cossignani*, Laura Giua, Maria S. Simonetti, Angela Maurizi, Giovanni Burini, Roberto Coli, Francesca Blasi

(Received January 31, 2017; Accepted March 27, 2017)

Summary

Much interest is currently concentrated on phenol compounds and antioxidants of wine. The aim of this study was to characterize and evaluate controlled designation of origin (CDO) and typical geographical indications (TGI) red wines from Central Italy and to evaluate possible modifications after one year of storage. The total phenol content and antioxidant activity by ORAC method were determined, while phenolic qualitative and quantitative profiles were evaluated by HRGC-FID or HPLC-DAD. All wines showed a good content of total phenols and an obvious antioxidant effect. After a one-year storage in the bottle, a significant decrease ($P<0.05$) of the ORAC values was observed for TGI wines. Interesting correlations between phenol and ORAC values for CDO wines were found. It can be confirmed that one-year storage in the bottle has not significantly affected the quality of the wines analyzed, in particular the CDO category.

Keywords: chromatography, ORAC, phenol, red wine, storage.

Introduction

Red wine is an important source of phenol compounds, natural antioxidants known for their biological properties and associated with the prevention of oxidative damage (Becker et al., 2004). Several clinical studies and epidemiological studies have shown a correlation between the intake of phenol compounds from red wine and a decrease in the incidence of many diseases (Arranz et al., 2012). Many studies have been carried out over the years and numerous analytical techniques have been developed to analyze phenolic compounds of grapes and wine (Lorrain et al., 2013; Khoddami et al., 2013). Complete analysis of phenolic compounds requires analytical techniques such as HRGC and HPLC associated with UV-Vis and diode array detectors (DAD) often coupled to other detection systems.

A broad bibliography is now available for the study of red or white wines produced in various parts of the world (Granato et al., 2011; Tourtoglou et al., 2014; Ivanova-Petropulos et al., 2015) although few results relate to the wines of Central Italy. Sato et al. (1996) investigated varietal differences in the phenol content of thirty-one wines from various sources, including a Sangiovese Umbrian sample from 1982. Versari et al. (2007), characterized by UV-Vis spectrophotometry, the color components and polymer pigments of commercially available red wines, including a Merlot-Umbrian sample. Recently, Esti et al. (2010) made an explorative sensory study of Grechetto's wine tasting, some of which were produced in Umbria.

The region of Umbria (Central Italy) plays an important role in the production of red wines, including Montefalco Sagrantino known worldwide. To the best of our knowledge, there are no works that focus solely on phenolic composition and antioxidant capacity of Umbrian wines. In this research, twelve Umbrian red wines, six CDO (controlled designation of origin) and six TGI (typical geographical indication) samples were characterized. Total phenol content, antioxidant capacity, HRGC and HPLC phenol profiles were determined. The same analyzes were repeated after one year of storage in the bottle to evaluate possible variations.

* Corresponding author

Materials and methods

Wine samples

Commercial wines, six CDO and six TGI, all collected in 750 ml bottle of cork (eight bottles for each wine) were collected from wineries in the province of Perugia, the capital of Umbria region (Central Italy). All wines were produced with varieties of *Vitis labrusca* L. grape varieties. Wine samples were selected to obtain a broad sampling of vine varieties and production area. All the wine samples were purchased at the market of Umbrian wines in 2010, where the wines are available on the market. It should be noted that the year of release for consumption does not correspond to the same vintage for different wines, on the individual production rules. The samples tested in this work are shown in Tab. 1, while some chemical parameters determined according to the official methods described in Commission Regulation (CEE) No 2676/90 of 17 September 1990 are shown in Tab. 2. Wines were produced according to Italian legislation. The wines were stored in the dark at 10 ± 1 °C until analysis. The samples, representative aliquots from four bottles, were tested shortly after opening. The same wines stored in unopened bottles at 10 ± 1 °C were analyzed in 2011, one year after marketing.

Chemicals and reagents

Rutin, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, kaempferol, rhamnetin and isorhamnetin were obtained from Extrasythese (Genay, France). *cis*-Resveratrol was from Cayman Chemical Company (Ann Arbor, MI, USA). The other phenol compounds were purchased from Sigma-Aldrich (Milan, Italy). They were stored in the dark at temperature recommended by the producer. Folin & Ciocalteu's phenol reagent, BSTFA [*N*,*O*-bis(trimethylsilyl)trifluoracetamide] and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxyl acid) were purchased from Sigma-Aldrich. All analytical grade solvents and reagents were from J.T. Baker (Deventer, Netherlands), Carlo Erba (Milan, Italy), Panreac (Barcellon, Spain) and Supelco (Bellafonte PA, USA). HPLC-grade acetonitrile and water were purchased from J.T. Baker.

Determination of total phenol content

The TP content was determined spectrophotometrically according to the method of Singleton and Rossi (1965), with slight modifications (Blasi et al., 2016). The wine samples were diluted (1:20 or 1:30, v/v) with distilled water. Diluted red wine (1.0 mL), 20% sodium carbonate (4.0 mL) and Folin & Ciocalteu's reagent (1.0 mL) were mixed and brought up to a volume of 20 mL with distilled water.

Tab. 1: Characteristics of Umbrian (Central Italy) red wines

Code	Wine name	Grape variety	Production area	Vintage year
Wines of Controlled Designation of Origin (CDO)				
CDO1	Sangiovese Colli Martani	Sangiovese (85% min.) Ciliegiolo Cannaiolo nero Montepulciano Merlot	Bettona (43°0‘48.96“N-12°29‘11.04“W)	2007
CDO2	Óscano Colli del Trasimeno	Gamay Sangiovese	Colle Umberto (43°07‘59.99“N-12°21‘59.98“W)	2009
CDO3	Rosso Colli Perugini	Sangiovese Merlot Cabernet Sauvignon	Sant’Enea (43°06‘43.56“N-12°23‘19.68“W)	2008
CDO4	Montefalco Rosso	Sangiovese 65% Merlot 20% Sagrantino 15%	Montefalco (42°55‘59.99“N-12°35‘60“W)	2007
CDO5	Baccio del Rosso Colli del Trasimeno	Sangiovese Trasimeno Gamay	Castiglione del Lago (43°06‘59.98“N-12°03‘00“W)	2008
CDO6	Montefalco Sagrantino	Sagrantino 100%	Montefalco (42°55‘17.98“N-12°38‘31.99“W)	2007
Wines of Typical Geographical Indication (TGI)				
TGI1	Sangiovese Umbria	Sangiovese (85% min.) and other red grapes typical of Umbria	Bettona (43°0‘48.96“N-12°29‘11.04“W)	2009
TGI2	Campiglione Rosso	Merlot Cabernet Sauvignon Sangiovese Gamay	Colle Umberto (43°07‘59.99“N-12°21‘59.98“W)	2009
TGI3	Garbino dell’Umbria	Sangiovese Cabernet Sauvignon Merlot	Sant’Enea (43°06‘43.56“N 12°23‘19.68“W)	2009
TGI4	Umbria Rosso	Sangiovese 50% Merlot 50%	Montefalco (42°55‘59.99“ N-12°35‘60“W)	2009
TGI5	Corio Rosso	Sangiovese (80% min.) Gamet Cabernet	Castiglione del Lago (43°06‘59.98“N-12°03‘00“W)	2009
TGI6	Rosso	Sangiovese Cabernet	Marsciano (42°91‘66.67“N-42°55‘12.20“W)	2009

The solution was mixed, kept at 25 °C and incubated at the dark for 90 min and then the absorbance was measured at 750 nm using a Jasco 7850 UV-Vis spectrophotometer (Jasco Inc., Easton, MD, USA). The TP content, expressed as milligrams of gallic acid equivalent per liter of wine (mg GAE/L), was determined using a calibration curve built using 15% ethanol gallic acid as standard solution (2.5-12.5 mg/L) that was analyzed in the same mode of wine samples.

Extraction and derivatization of phenol compounds

Phenols were extracted from red wine according to the method of Minuti et al. (2006). Briefly, sodium metabisulphite (20 mg) and sodium chloride (20 mg) were added to the red wine (1 mL) and then ethyl acetate (3 mL × 3) was used for the extraction. After anhydrification with Na_2SO_4, the extracts were evaporated to dryness under nitrogen stream at 25 °C. The solid residue was dissolved in acetone (50 µL) and derivatized with BSTFA (100 µL). The obtained solution was held in the dark, at room temperature, for 1 h. Then the volume was made up to 1 mL.

High-resolution gas-chromatography analysis

High-resolution GC analyses were carried out using a Perkin-Elmer Autosystem apparatus (Norwalk, CT, USA), equipped with a split/splitless injection port, interfaced to a flame ionization detector (FID). The separation was obtained using the HP1-MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm f.t.). The injector and detector temperature was 300 and 320 °C, respectively. The initial oven temperature was 120 °C, held for 3 min and raised to 320 °C at 5 °C/min; the final temperature was held for 15 min.

Carrier gas (He) flow rate was 1 mL/min; the injection volume was 1 µL with a split ratio of 1:50. A standard solution in acetone, suitably derivatized as reported in the previous paragraph, containing twenty compounds (vanillin, *trans*-cinnamic acid, tyrosol, veratric acid, vanillic acid, homovanillic acid, hydroxytyrosol, 3,4-dihydroxyphenilacetic acid, homogentisic acid, syringic acid, *p*-coumaric acid, gallic acid, ferulic acid, caffeic acid, sinapinic acid, *cis*-resveratrol, *trans*-resveratrol, epicatechin, catechin, fisetin), was used to identify and to quantify the analytes. Calibration curves were obtained by three injections of four different concentrations ranging from 2.5 to

Tab. 2: Some chemical parameters of Umbrian red wines analyzed in 2010 and after one-year storage

Code	Total acidity (g/L)		Volatile acidity (g/L)		Total SO_2 (mg/L)		Free SO_2 (mg/L)		pH		Total dry extract (g/L)		Alcohol content (% vol)	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Wines of Controlled Designation of Origin (CDO)														
CDO1	5.17	5.32	0.35	0.34	86.60	96.00	25.60	32.00	3.41	3.49	25.15	26.30	12.30	12.34
CDO2	5.02	5.10	0.41	0.56	76.80	70.40	25.60	16.00	3.55	3.63	27.50	27375	12.65	12.52
CDO3	5.55	5.17	0.58	0.54	64.00	38.40	38.40	19.20	3.73	3.79	30.60	30.50	14.15	14.11
CDO4	5.62	5.55	0.57	0.60	51.20	57.60	19.20	19.20	3.67	3.71	32.95	32.80	13.83	13.65
CDO5	4.42	4.27	0.42	0.45	89.60	89.60	25.60	44.80	3.91	3.99	31.00	31.00	13.54	13.83
CDO6	4.95	5.02	0.32	0.41	76.80	70.40	25.60	19.20	3.67	3.71	29.70	29.55	13.83	13.79
Wines of Typical Geographical Indication (TGI)														
TGI1	5.10	5.17	0.60	0.63	64.00	76.80	19.20	9.60	3.60	3.65	26.70	28.25	13.65	13.61
TGI2	5.40	5.32	0.53	0.55	70.40	83.20	25.60	32.00	3.63	3.66	31.93	33.10	13.93	14.02
TGI3	5.25	5.55	0.66	0.76	64.00	57.60	25.60	25.60	3.67	3.67	31.50	32.15	14.91	15.59
TGI4	4.95	5.17	0.32	0.40	70.40	108.8	44.80	51.20	3.66	3.67	29.40	29.70	13.56	13.56
TGI5	5.17	5.40	0.28	0.26	102.40	102.40	38.40	51.20	3.55	3.54	29.30	28.70	14.11	14.11
TGI6	5.25	5.40	0.41	0.38	70.40	70.40	38.40	25.60	3.59	3.58	27.75	29.85	12.48	12.73

40 mg/L. The least square method was used to calculate the regression equations.

High-performance liquid chromatography analysis of phenol compounds

Before HPLC-DAD analysis, wine samples (100 mL) were de-alcoholized and dried under vacuum at 40 °C. The residue was diluted in distilled water (50 mL) and then filtered through a 0.20 μm nylon syringe filter (Corning Incorporated, Corning, Germany).

The HPLC analyses were performed using a Shimadzu GT-154 system equipped with a Thermo Spectra Series pump, an Hypersil GOLD column (5 μm particle size, 250 × 4.6 mm i.d., Thermo Scientific, Rockford, IL, USA) and a Spectra System UV6000LP DAD. Detection was performed on line in a range of wavelength between 240 and 390 nm. The chromatograms were acquired and the data handled using Xcalibur software version 1.2 (Finnigan Corporation 1998-2000, San Jose, CA, USA). The solvents were (A) acetonitrile and (B) water/acetic acid (40:1, v/v). The samples were analyzed by gradient elution at a flow rate of 0.8 mL/min. The elution conditions were: 0-15 min at 15% A; 15-30 min from 15 to 35% A; 30-40 min from 35 to 55% B; 40-44 min from 55 to 100% A with re-equilibration of the column at 44-47 min 100% A to 15% A and 47-60 min at 15% A. A standard solution solubilized in water/acetic acid/methanol (8:2:90, v/v/v), containing ten compounds (rutin, ethyl gallate, quercetin-3-β-D-glucoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, myricetin, quercetin, kaempferol, isorhamnetin, rhamnetin), was used to identify and to quantify the analytes. Calibration curves were obtained by three injections of five different concentrations ranging from 0.5 mg/L to 60 mg/L.

Antioxidant capacity

The total antioxidant capacity was assessed using oxygen radical absorbance capacity (ORAC) method as reported by Maurizi et al. (2015). The assay was conducted with FLUOstar Optima fluorescent microplate reader (BMG LABTH GmbH, Germany), provided with a pump, set at wavelengths of excitation and emission at 485 and 520 nm respectively, and interfaced with a computer provided with a MARS Data Analysis software ver. 2.00 for data acquisition and processing. Costar 96 well black opaque plates (Corning Costar Corporation, Cambridge, MA, USA) were used. The values were expressed as micromoles of Trolox Equivalents per liter of wine (μM TE/L). The ORAC values were calculated using the following formula:

$$ORAC = [C_t \times (AUC_s - AUC_0) \times k]/(AUC_t - AUC_0)$$

where:

AUC_s, Area under curve in the presence of wine sample

AUC_0, Area under curve in the presence of blank

AUC_t, Area under curve in the presence of Trolox

C_t, Trolox concentration

k, dilution factor

Statistical analysis

All analytical determinations were carried out in triplicate and the results were expressed as mean value and standard deviation (SD). Student's t test (type: 3, heteroscedastic; tails: 2), calculated using Excel 2003 (Microsoft Corporation, Redmond, WA, USA), was used to evaluate the differences between the results obtained for the different wine categories in different years. The probability of $P<0.05$ was considered statistically significant. Correlation analyses have been performed using Microcal Origin™, version 5.0 (Microcal Software Inc., Northampton, MA, USA).

Results and discussion

Total phenol content and antioxidant capacity

Fig. 1 shows the TP content of red wines analyzed in 2010 and after one-year storage (2011). The present study established that in 2010 the highest concentration of phenol compounds was detected in CDO6 wine (3684 mg GAE/L), even if TGI wines showed TP values (from 1341 to 2436 mg GAE/L) comparable with those of CDO category (from 1968 to 3684 mg GAE/L). In fact there were no significant differences ($P>0.05$) between the TP content of CDO and TGI wine categories. After one-year storage in bottle, a slight decrease of TP content was observed in all samples, with the exception of CDO3

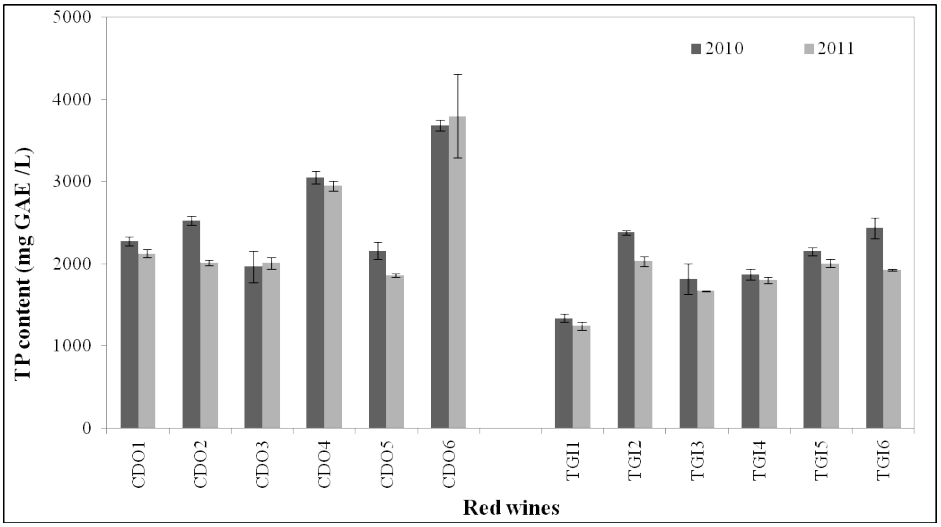

Fig. 1: Total phenol (TP) content (mg GAE/L) of Umbrian red wines analyzed in 2010 and after one-year storage. Error bars represent the SD of the mean values (n = 3).

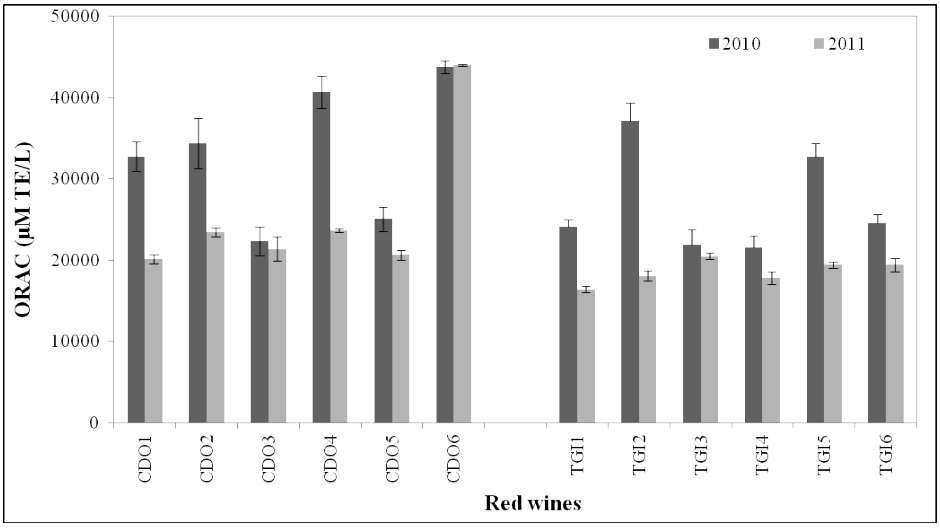

Fig. 2: ORAC values (μM TE/L) of Umbrian red wines analyzed in 2010 and after one-year storage. Error bars represent the SD of the mean values (n = 3).

and CDO6 samples. The decrease could be due to the transformation of phenol compounds into condensed forms, but also to the enzymatic activity from residual microorganisms of wine (MONAGAS et al., 2006). No significant differences ($P>0.05$) in TP content of the two wine categories were also found in 2011. Neither TP content obtained in the two years was different significantly ($P>0.05$). The results obtained in this paper for TP content were in accordance with those reported by other authors. For example, SATO et al. (1996) found a value of 2282.5 ppm for a Sangiovese Umbrian sample, while MILANO et al. (2009) reported a value of 2550 mg GAE/L for an Umbrian red wine (Assisi Rosso 2006, a CDO wine produced in Umbria). All wine samples tested in 2010 showed an evident antioxidant effect (Fig. 2), evaluated by ORAC assay, with values ranging from 22382 μM TE/L to 43801 μM TE/L for CDO and from 21555 μM TE/L to 37111 μM TE/L for TGI wine category. No significant differences ($P>0.05$) between ORAC values of CDO and TGI wines were found. After one year, a significant ($P<0.05$) decrease of ORAC values was observed for all samples (with the exception of CDO6 sample). As regards the correlation between the TP content of red wines and their antioxidant capacity (Fig. 3), positive results have been obtained for all wines and in particular for CDO category, in both years. In literature, conflicting and confused data exist about the correlation between the TP content and antioxidant capacity in wines (ARNOUS et al., 2002; KATALINIĆ et al., 2004).

Phenol profile by chromatographic analyses

In order to determine the polyphenol composition of wine samples, the analysis was initially performed by HRGC-FID (Tab. 3 and 4). The most abundant compounds in both wine categories were tyrosol, gallic acid, epicatechin, and catechin. It was interesting to note that there were no significant differences ($P>0.05$) between the content of the single compounds in CDO and TGI wines analyzed in 2010. After one-year storage only syringic acid content changed significantly ($P<0.05$) between CDO and TGI wines. As regards CDO category, it was verified a significant difference ($P<0.05$) in the content of some phenol acids, but also of *cis*-resveratrol and fisetin. As regards TGI category, it was verified a significant difference ($P<0.05$) also

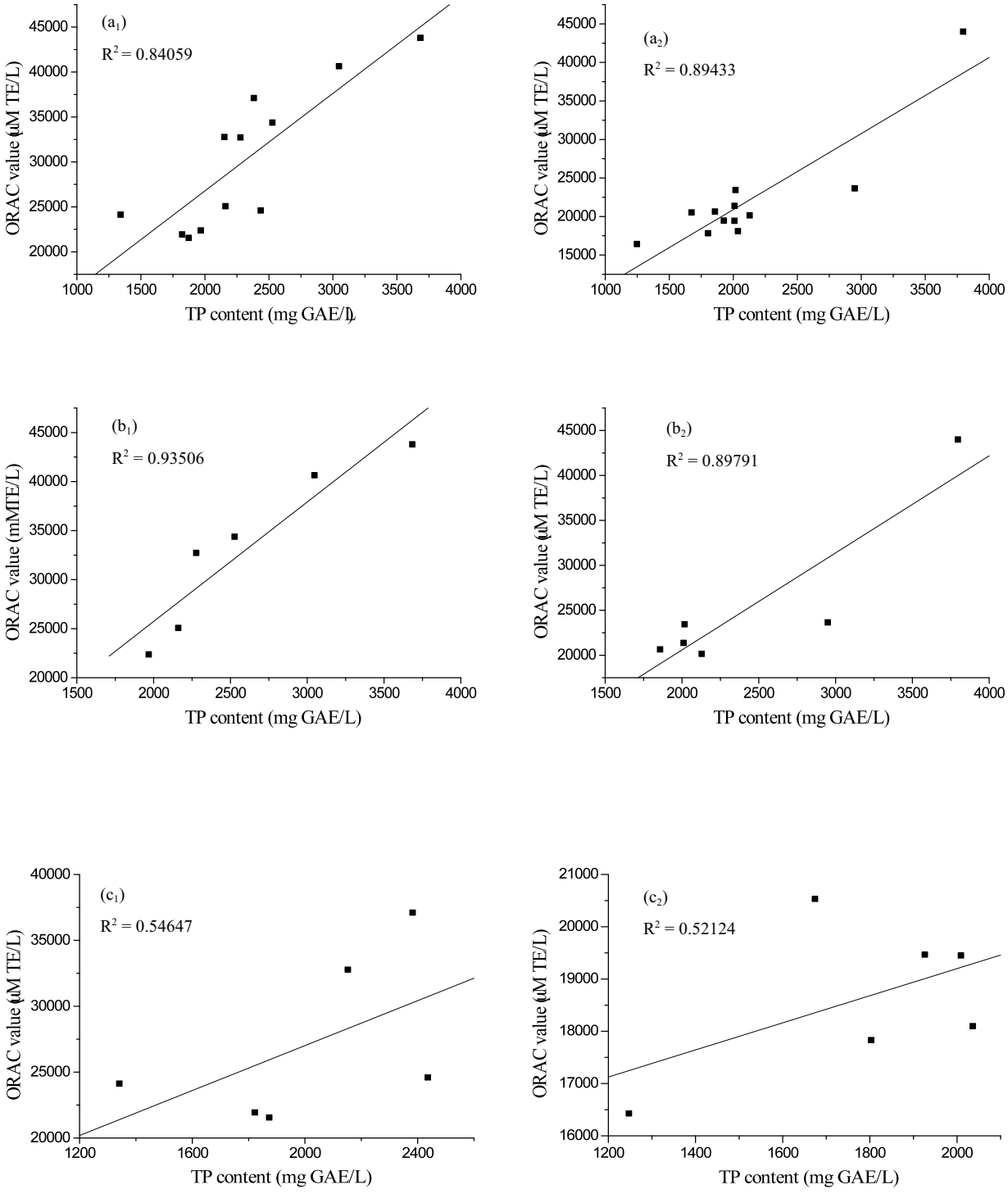

Fig. 3: Correlations between TP content (mg GAE/L) and ORAC value (μM TE/L) obtained for all wines and CDO or TGI categories, in 2010 and after one-year storage. (a_1), 2010 – all wines; (a_2), 2011 – all wines; (b_1), 2010 – CDO wines; (b_2), 2011 – CDO wines; (c_1), 2010 – TGI wines; (c_2), 2011 – TGI wines; TP, total phenol; ORAC, oxygen radical absorbance capacity; CDO, Controlled Designation of Origin; TGI, Typical Geographic Indication.

in the content of homogentisic acid. As regards the comparison with previous literature data, MILANO et al. (2009) found values much lower for ferulic acid (0.16 mg/L), gallic acid (22.71 mg/L) and *trans*-resveratrol (1.24 mg/L), while reported much higher concentrations for caffeic and cinnamic acids, catechin and epicatechin. In order to quantify some flavonols (myricetin, quercetin, kaempferol, rhamnetin/isorhamnetin, rutin), both as aglycones and glycosides, an HPLC-DAD analysis was performed (Tab. 5 and 6). The most abundant compounds were ethyl gallate for CDO wines and quercetin-3-β-D-glucoside for TGI wines, while the most represented flavonol was quercetin, both in CDO (on average 6.78 mg/L) and TGI (on average 4.35 mg/L) wines. In all wines, the glycosidic form of quercetin, kaempferol and isorhamnetin had always a higher concentration (P<0.05) than the respective aglycones, both in wines analyzed in 2010 and in 2011. The results obtained in this study showed a high variability in the phenol composition in wine samples, although

Tab. 3: Content[§] of phenol compounds in red wines analyzed by high-resolution gas chromatography in 2010

Phenols (mg/L)	CDO1	CDO2	CDO3	CDO4	CDO5	CDO6	TGI1	TGI2	TGI3	TGI4	TGI5	TGI6
Vanillin	2.4±1.8	1.6±0.5	1.6±0.1	3.3±0.1	1.8±0.2	1.8±0.4	2.4±0.3	2.0±0.5	1.4±0.0	0.9±0.1	2.2±0.7	3.5±2.0
trans-Cinnamic acid	1.5±0.1	1.3±0.0	9.6±1.4	7.8±5.2	12.4±0.2	10.0±0.7	5.7±0.4	1.5±0.0	1.0±0.1	5.6±0.5	9.7±1.4	6.5±1.0
Tyrosol	21.9±1.8	30.6±2.0	27.1±4.2	21.6±1.2	21.2±6.3	26.6±2.2	10.2±1.0	25.5±3.6	13.0±0.4	20.7±1.9	26.9±3.5	10.7±0.7
Veratric acid	1.7±0.2	1.7±0.0	2.1±0.2	2.1±0.3	1.9±0.2	2.3±0.1	1.7±0.1	1.5±0.1	1.7±0.0	1.1±0.1	2.3±0.3	1.4±0.1
Vanillic acid	2.3±0.1	3.1±0.1	4.7±0.4	4.1±0.1	5.8±0.1	4.0±1.0	3.6±0.2	2.7±0.2	4.4±0.1	3.6±0.3	3.3±0.8	5.1±0.3
Homovanillic acid	2.6±4.6	3.8±0.3	1.2±0.0	4.9±0.3	1.2±0.2	1.4±0.1	1.3±0.0	3.9±0.5	3.0±0.8	1.1±0.1	4.6±0.5	1.2±0.1
Hydroxytyrosol	3.2±0.2	3.8±0.1	3.3±0.4	4.3±0.1	3.8±0.3	4.3±0.4	2.7±0.1	3.3±0.3	2.7±0.1	1.4±0.0	4.0±0.4	3.3±0.2
3,4-DIHY acid	1.6±2.2	1.5±0.0	1.4±0.0	1.8±0.2	1.5±0.1	1.3±0.0	1.3±0.1	1.7±0.1	1.4±0.0	1.5±0.0	2.0±0.1	1.5±0.1
Homogentisic acid	1.6±2.3	1.8±0.1	1.4±0.1	1.4±0.1	2.3±0.3	1.4±0.0	1.2±0.1	1.7±0.1	1.5±0.0	2.0±0.2	1.5±0.0	1.4±0.1
Syiringic acid	3.4±0.2	4.2±0.1	4.4±0.3	4.1±0.2	4.3±0.2	5.3±0.2	3.0±0.0	4.0±0.2	3.9±0.0	3.1±0.4	4.3±0.0	3.8±0.2
p-Coumaric acid	6.5±0.4	6.6±0.1	5.5±0.6	7.7±0.4	8.9±0.6	8.0±0.4	5.6±0.2	5.4±0.8	4.9±0.3	5.8±0.5	7.8±0.4	7.4±0.5
Gallic acid	51.3±4.6	13.9±1.7	40.6±5.5	52.5±3.5	34.5±1.3	53.1±3.6	28.3±2.2	36.5±5.1	18.9±1.8	32.1±3.8	38.9±1.5	29.9±4.2
Ferulic acid	3.3±0.7	1.9±0.2	2.0±0.3	2.2±0.6	1.7±0.1	2.5±0.1	1.8±0.2	1.8±0.0	2.2±0.2	2.0±0.1	2.1±0.7	1.7±0.1
Caffeic acid	6.4±0.4	7.3±0.2	6.0±0.5	12.8±0.8	7.3±0.6	15.7±0.9	5.4±0.3	6.5±0.6	17.0±1.7	9.9±0.9	8.9±4.2	9.1±1.0
Sinapinic acid	2.8±0.0	3.6±0.7	2.2±0.0	2.4±0.2	2.3±0.1	4.4±0.2	2.2±0.1	3.6±0.7	2.5±0.0	2.2±0.1	2.4±0.2	2.6±0.3
cis-Resveratrol	1.7±0.2	2.6±0.1	2.5±0.2	1.5±0.1	3.2±0.1	1.3±0.0	1.5±0.0	2.1±0.2	2.1±0.1	1.2±0.1	2.2±0.6	2.0±0.1
trans-Resveratrol	2.1±0.5	2.9±0.3	2.9±0.3	1.8±0.0	3.7±0.2	1.2±0.0	1.8±0.1	2.1±0.2	2.2±0.1	1.2±0.0	2.0±0.2	3.0±0.3
Epicatechin	25.0±2.2	15.2±1.0	40.5±5.1	37.1±2.3	30.3±1.8	16.3±1.1	26.4±0.9	32.4±4.9	41.9±2.8	40.8±2.6	34.9±4.2	21.5±3.2
Catechin	25.7±2.3	28.7±4.3	33.1±4.9	42.3±2.6	35.5±2.0	33.4±1.0	22.4±1.0	33.7±5.1	35.1±2.4	16.9±1.2	38.5±5.8	29.7±3.2
Fisetin	5.3±2.1	2.9±0.2	6.6±6.0	2.7±0.0	2.9±0.2	3.5±0.8	4.6±1.6	3.2±0.3	3.1±0.6	2.7±0.1	3.0±0.0	3.2±0.1

Tab. 4: Content[§] of phenol compounds in red wines analyzed by high-resolution gas chromatography in 2011

Phenols (mg/L)	CDO1	CDO2	CDO3	CDO4	CDO5	CDO6	TGI1	TGI2	TGI3	TGI4	TGI5	TGI6
Vanillin	3.2±0.1	2.2±0.0	1.9±0.0	2.7±0.1	0.9±0.0	3.3±0.2	3.1±0.1	1.7±0.0	0.9±0.0	2.6±0.1	2.1±0.0	0.6±0.0
trans-Cinnamic acid	1.3±0.0	1.5±0.0	0.9±0.0	1.8±0.1	1.0±0.0	1.7±0.0	1.5±0.0	1.0±0.0	1.1±0.0	1.2±0.1	1.1±0.0	0.9±0.0
Tyrosol	24.9±0.2	30.8±0.9	22.8±0.3	16.4±0.4	28.0±0.3	25.8±0.9	12.5±0.4	19.2±0.0	8.3±0.0	19.5±0.3	28.0±0.2	20.7±0.3
Veratric acid	1.6±0.0	1.1±0.0	1.5±0.0	0.7±0.0	1.4±0.0	0.9±0.0	0.7±0.0	0.8±0.0	1.0±0.0	2.2±0.0	1.7±0.1	1.0±0.0
Vanillic acid	1.1±0.0	4.9±0.6	2.6±0.1	7.1±0.3	0.1±0.0	4.6±0.4	0.4±0.0	12.8±0.2	1.5±0.1	16.0±0.4	1.4±0.2	2.4±0.0
Homovanillic acid	1.7±0.0	1.9±0.0	1.8±0.0	1.9±0.0	2.1±0.0	1.8±0.0	1.6±0.0	1.9±0.0	1.5±0.0	1.6±0.0	1.9±0.0	2.0±0.0
Hydroxytyrosol	3.3±0.0	3.9±0.0	2.7±0.1	3.3±0.1	3.4±0.1	5.9±0.4	2.9±0.1	2.4±0.2	1.7±0.0	2.6±0.1	3.7±0.2	2.7±0.0
3,4-DIHY acid	1.4±0.0	1.3±0.0	1.2±0.0	1.3±0.0	1.3±0.0	1.5±0.0	1.2±0.0	1.2±0.0	1.3±0.0	1.2±0.0	1.3±0.0	1.3±0.0
Homogentisic acid	1.3±0.0	0.8±0.0	2.4±0.0	1.0±0.0	1.4±0.0	1.0±0.1	1.0±0.0	1.9±0.0	1.0±0.0	0.7±0.0	1.3±0.0	1.0±0.0
Syiringic acid	2.3±0.0	3.0±0.0	2.7±0.0	2.0±0.0	2.9±0.0	4.0±0.2	1.5±0.1	1.8±0.0	1.4±0.0	1.4±0.0	2.7±0.1	2.4±0.0
p-Coumaric acid	8.5±0.2	8.9±0.4	5.1±0.2	6.7±0.1	8.9±0.1	7.8±0.2	9.9±0.3	7.8±0.0	4.9±0.0	8.4±0.1	9.9±0.5	4.7±0.1
Gallic acid	67.2±1.0	43.0±0.4	38.1±0.0	43.7±2.0	34.3±0.6	54.8±3.0	41.4±1.0	32.2±0.6	11.4±0.0	37.8±0.4	35.3±0.0	38.0±0.3
Ferulic acid	3.6±0.1	3.7±0.0	3.1±0.1	3.4±0.0	3.1±0.0	3.6±0.0	3.6±0.0	3.5±0.0	6.6±0.0	4.3±0.1	3.5±0.0	2.8±0.0
Caffeic acid	9.3±0.1	10.0±0.3	6.4±0.2	11.2±0.2	8.8±0.1	14.4±1.0	7.2±0.1	17.5±0.0	10.5±0.1	23.3±0.4	8.5±0.1	10.5±0.1
Sinapinic acid	3.9±0.0	4.2±0.1	4.2±0.1	4.4±0.2	4.4±0.1	4.2±0.1	4.1±0.2	5.9±0.1	2.9±0.0	4.1±0.1	4.3±0.2	3.5±0.0
cis-Resveratrol	1.7±0.0	2.6±0.0	0.4±0.0	1.0±0.0	3.0±0.0	0.6±0.0	0.2±0.0	0.3±0.0	0.3±0.0	0.1±0.0	0.4±0.1	0.3±0.0
trans-Resveratrol	2.9±0.0	2.8±0.0	1.2±0.2	1.0±0.0	3.5±0.0	3.2±0.0	1.7±0.1	0.9±0.0	1.5±0.2	0.6±0.0	2.0±0.0	1.5±0.0
Epicatechin	28.7±0.3	31.0±1.3	28.5±0.0	30.3±0.2	33.3±0.4	35.0±1.1	35.0±1.0	21.2±1.0	31.9±0.5	27.3±0.7	32.8±0.1	29.9±0.1
Catechin	25.7±0.4	28.7±0.0	22.9±0.2	42.3±0.0	35.5±0.3	33.4±0.7	26.2±0.7	20.7±0.9	23.9±0.4	27.6±0.8	34.4±2.6	26.0±0.1
Fisetin	12.5±0.1	12.0±0.0	11.3±0.0	11.8±0.1	12.6±0.2	12.0±0.2	11.9±0.0	11.5±0.1	11.2±0.0	11.2±0.0	12.3±3.0	11.5±0.1

[§] mean values ± SD, n=3

3,4-DIHY acid, 3,4-dihydroxyphenilacetic acid

Tab. 5: Content[§] of phenol compounds analyzed by high-performance liquid chromatography in 2010

Phenols (mg/L)	CDO1	CDO2	CDO3	CDO4	CDO5	CDO6	TGI1	TGI2	TGI3	TGI4	TGI5	TGI6
Rutin	3.1±0.4	13.3±0.6	3.0±0.1	3.5±0.3	0.1±0.0	5.7±0.3	1.8±0.2	7.0±0.2	0.1±0.0	1.1±0.0	0.1±0.0	3.8±0.1
Ethyl gallate	14.2±0.7	16.3±0.3	16.7±0.8	20.9±1.1	21.6±1.0	19.6±1.0	7.0±0.3	7.5±0.1	3.8±0.0	8.7±0.9	8.0±0.2	7.9±0.1
Quercetin-3-β-D-glucoside	12.4±0.6	13.5±0.2	10.8±0.7	16.5±0.8	15.9±0.6	14.5±0.5	16.8±0.3	12.3±0.3	6.4±0.2	20.7±1.3	19.0±1.0	15.3±0.1
Kaempferol-3-*O*-glucoside	0.5±0.1	0.8±0.1	2.0±0.1	0.7±0.1	1.8±0.0	0.9±0.0	0.1±0.0	0.3±0.0	0.1±0.0	0.3±0.0	1.0±0.0	0.6±0.2
Isorhamnetin-3-*O*-glucoside	2.1±0.4	3.1±0.1	2.6±0.1	1.1±0.0	1.3±0.0	2.5±0.1	1.4±0.1	0.1±0.0	0.2±0.0	0.1±0.0	2.4±0.1	2.0±0.3
Myricetin	3.2±0.2	7.2±0.1	5.1±0.1	6.2±0.4	4.8±0.4	3.0±0.2	3.6±0.1	3.1±0.1	3.3±0.3	2.3±0.1	5.1±0.1	2.7±0.1
Quercetin	6.0±0.7	10.9±0.1	6.8±0.1	8.5±0.6	5.7±0.6	2.3±0.1	6.2±0.1	6.5±0.0	3.5±0.2	1.8±0.1	4.4±0.0	3.8±0.2
Kaempferol	0.2±0.1	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Isorhamnetin	0.3±0.1	2.3±0.0	1.1±0.0	0.7±0.1	1.4±0.3	0.1±0.0	0.2±0.0	1.1±0.0	0.6±0.0	0.1±0.0	0.8±0.0	0.6±0.1

§ mean values ± SD, n=3

Tab. 6: Content[§] of phenol compounds analyzed by high-performance liquid chromatography in 2011

Phenols (mg/L)	CDO1	CDO2	CDO3	CDO4	CDO5	CDO6	TGI1	TGI2	TGI3	TGI4	TGI5	TGI6
Rutin	2.0±0.0	6.8±0.4	2.2±0.1	3.4±0.3	0.1±0.0	4.8±0.1	0.1±0.0	5.3±0.0	0.1±0.0	1.1±0.0	0.1±0.0	3.8±0.1
Ethyl gallate	11.2±0.1	14.1±0.1	15.6±0.7	19.1±0.8	6.4±0.2	18.0±0.3	8.7±0.1	2.7±0.1	3.1±0.0	7.8±0.1	5.8±0.2	7.8±0.2
Quercetin-3-β-D-glucoside	11.9±0.2	14.8±0.2	9.0±1.2	15.5±0.6	5.6±0.2	15.5±0.5	15.5±0.2	11.5±0.1	6.3±0.0	14.0±0.6	10.4±0.5	13.9±0.6
Kaempferol-3-*O*-glucoside	0.4±0.0	0.7±0.0	1.4±0.1	0.6±0.0	1.2±0.2	0.8±0.0	0.1±0.0	0.2±0.0	0.5±0.0	0.3±0.0	1.0±0.0	0.2±0.0
Isorhamnetin-3-*O*-glucoside	1.5±0.1	3.0±0.1	2.5±0.0	0.7±0.0	1.2±0.0	2.0±0.0	1.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	2.4±0.1	2.4±0.3
Myricetin	2.1±0.3	6.5±0.6	0.5±0.0	2.2±0.2	3.9±0.0	1.4±0.1	2.4±0.0	2.9±0.1	2.8±0.0	0.7±0.0	5.1±0.1	1.4±0.2
Quercetin	2.6±0.0	7.2±0.3	0.4±0.0	1.7±0.3	4.1±0.0	0.7±0.0	4.3±0.1	3.6±0.1	2.6±0.0	0.3±0.0	4.4±0.0	0.5±0.0
Kaempferol	0.1±0.0	0.1 ±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Isorhamnetin	0.1±0.0	1.7±0.2	1.4±0.1	0.1±0.0	1.1±0.0	0.1±0.0	0.1±0.0	0.6±0.0	1.3±0.5	0.1±0.0	0.8±0.0	0.1±0.0

§ mean values ± SD, n=3

coming from the same region. It is known that the quali- and quantitative phenol composition of grape and wine shows a great variability in relation to environmental, viticultural, enological or storage practices (Ivanova-Petropulos et al., 2012). Simonetti et al. (1997) analyzed ten red Italian wines and reported the myricetin content ranging from 0.6 mg/L (Squinzano) to 9.6 mg/L (Cabernet Sauvignon), while in this study the value changed between 3.2 and 7.2 for CDO wines and from 2.3 to 5.1 mg/L for TGI wines, analyzed in 2010. With regard to rutin, it was detected only in some samples as also reported by Simonetti et al. (1997). Rhamnetin was never detected in Umbrian wine samples. After one-year storage, a slight decrease of single phenol content was observed, even if only the concentration of quercetin changed significantly ($P<0.05$) in DOC wine category. In conclusion, this is the first study of phenol content, qualitative composition and antioxidant capacity in wines produced in central Italy. The results confirmed the good quality of CDO and TGI Umbrian red wines, in fact there were no significant differences between wine categories, both as regards TP content, ORAC value and phenol composition. Generally, one-year storage in bottle did not affect significantly the quality of the analyzed wines, in particular for CDO category. The relevance of these results is evident when considering that some Umbrian red wines, as Montefalco Sagrantino, are appreciated all around the word. A broader examination of Umbrian red wine is actually in progress.

Acknowledgments

This work was funded by the Fondazione Cassa di Risparmio di Perugia (Project 2009.020.0004- "I vini umbri: speciazione della frazione polifenolica per via HPLC-MS e/o HRGC-MS ed aspetti salutistici correlati").

References

Arnous, A., Makris, D.P., Kefalas, P., 2002: Correlation of pigment and flavanol content wine antioxidant properties in selected aged regional wines from Greece. J. Food Comp. Anal. 15, 655-665. DOI: 10.1006/jfca.2002.1070

Arranz, S., Chiva-Blanch, G., Valderas-Martínez, P., Medina-Remón, A., Lamuela-Raventós, R.M., Estruch, R., 2012: Wine, beer,

alcohol and polyphenols on cardiovascular disease and cancer. Nutrients 4, 759-781. DOI: 10.3390/nu4070759

Becker, E.M., Nissen, L.R., Skibsted, L.H., 2004: Antioxidant evaluation protocols: food quality or health effects. Eur. Food Res. Technol. 219, 561-571. DOI: 10.1007/s00217-004-1012-4

Blasi, F., Urbani, E., Simonetti, M.S., Chiesi, C., Cossignani, L., 2016: Seasonal variations in antioxidant compounds of *Olea europaea* leaves collected from different Italian cultivars. J. Appl. Bot. Food Qual. 89, 202-207. DOI: 10.5073/JABFQ.2016.089.025

Esti, M., González Airola, R.L., Moneta, E., Paperaio, M., Sinesio, F., 2010: Qualitative data analysis for an exploratory sensory study of *Grechetto* wine. Anal. Chim. Acta 660, 63-67. DOI: 10.1016/j.aca.2009.10.014

Granato, D., Uchida Katayama, F.C., Alves de Castro, I., 2011: Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. Food Chem. 129, 366-373. DOI: 10.1016/j.foodchem.2011.04.085

Ivanova-Petropulos, V., Vojnoski, B., Stefova, M., 2012: Effect of wine-making treatment and wine storage on phenolic content in Vranec wines. J. Food Sci. Technol. 49, 161-172. DOI: 10.1007/s13197-011-0279-2

Ivanova-Petropulos, V., Hermosín-Gutiérrez, I., Boros, B., Stefova, M., Stafilov, T., Vojnoski, B., Dörnyei, Á., Kilár, F., 2015: Phenolic compounds and antioxidant activity of Macedonian red wines. J. Food Comp. Anal. 41, 1-14. DOI: 10.1016/j.jfca.2015.01.002

Katalinić, V., Milos, M., Modun, D., Musić, I., Boban, M., 2004: Antioxidant effectiveness of selected wines in comparison with (+)-catechin. Food Chem. 86, 593-600. DOI: 10.1016/j.foodchem.2003.10.007

Khoddami, A., Wilkes, M.A., Roberts, T.H., 2013: Techniques for analysis of plant phenolic compounds. Molecules 18, 2328-2375. DOI: 10.3390/molecules18022328

Lorrain, B., Ky, I., Pechamat, L., Teissedre, P.L., 2013: Evolution of analysis of polyhenols from grapes, wines, and extracts. Molecules 18, 1076-1100. DOI: 10.3390/molecules18011076

Maurizi, A., De Michele, A., Ranfa, A., Ricci, A., Roscini, V., Coli, R., Bodesmo, M., Burini, G., 2015: Bioactive compounds and antioxidant characterization of three edible wild plants traditionally consumed in the Umbria Region (Central Italy): *Bunias erucago* L. (corn rocket), *Lactuca perennis* L. (mountain lettuce) and *Papaver rhoeas* L. (poppy). J. Appl. Bot. Food Qual. 88, 109-114. DOI: 10.5073/JABFQ.2015.088.015

Milano, F., Giannetti, V., Gobbi, L., Recchia, L., Tarola, A., 2009: Simultaneous determination of phenolic compounds in selected Italian red wines. J. Comm. Sci. Technol. Qual. 48, 5-15.

Minuti, L., Pellegrino, R.M., Tesei, I., 2006: Simple extraction method and gas chromatography-mass spectrometry in the selective ion monitoring mode for the determination of phenols in wine. J. Chromatogr. A 1114, 263-268. DOI: 10.1016/j.chroma.2006.02.068

Monagas, M., Gómez-Cordovés, C., Bartolomé, B., 2006: Evolution of the phenolic content of red wines from *Vitis vinifera* L. during storage in bottle. Food Chem. 95, 405-412. DOI: 10.1016/j.foodchem.2005.01.004

Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M., Ochi, H., 1996: Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. J. Agric. Food Chem. 44, 37-41. DOI: 10.1021/jf950190a

Simonetti, P., Pietta, P., Testolin, G., 1997: Polyphenol content and total antioxidant potential of selected Italian wines. J. Agric. Food Chem. 45, 1152-1155. DOI: 10.1021/jf960705d

Singleton, V.L., Rossi, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic 16, 144-158.

Tourtoglou, C., Nenadis, N., Paraskevopoulou, A., 2014: Phenolic composition and radical scavenging activity of commercial Greek white wines from *Vitis vinifera* L. cv. Malagousia. J. Food Comp. Anal. 33, 166-174. DOI: 10.1016/j.jfca.2013.12.009

Versari, A., Parpinello, G.P., Mattioli, A.U., 2007: Characterisation of colour components and polymeric pigments of commercial red wines by using selected UV-Vis spectrophotometric methods. S. Afr. J. Enol. Vitic 28, 6-10.

Address of the corresponding author:
University of Perugia, Department of Pharmaceutical Sciences, Section of Food Science and Nutrition, Via San Costanzo, 06126, Perugia, Italy
E-mail: lina.cossignani@unipg.it