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Extraction of phenolic compounds from 'Aglianico' and 'Uva di Troia' grape skins and seeds in model solutions: Influence of ethanol and maceration time

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Summary

The effect of increasing concentration of ethanol (0, 4, 7.5 and 13 %) and contact time (respectively 1, 4, 7 and 10 days) on the extraction of phenolics from berry skins and seeds of the grape, *Vitis vinifera* 'Aglianico' and 'Uva di Troia', were examined. Two assays of post-fermentative maceration in two hydroalcoholic solutions at 11 and 13 % ethanol, were also performed. Chromatic properties and phenolics of medium were analyzed by HPLC and spectrophotometric methods. The extraction of total phenolics, anthocyanins, proanthocyanidins, and vanilline reactive flavans (VRF) from berry skins reached the maximum on the 4th day of maceration. Quercetin and gallic acid were gradually extracted from grape skins. The maximum release of flavan-3-ols from the skins was achieved on the first day of maceration. Total phenolics, tannins and VRF were gradually extracted from seeds. During the post-fermentative maceration, higher the content of ethanol, higher the extraction of total polyphenols and tannins from 'Uva di Troia' skins and the extraction of total polyphenols and tannins from 'Aglianico' seeds. These results clearly indicate that the grape cultivar mainly influences the release of phenolic compounds from the solid parts of berry to the must especially during post-fermentative maceration.

K e y w o r d s : polyphenols, post-fermentative maceration, ethanol, grape skins, grape seeds.

Introduction

The knowledge of the composition of each grape tissue involved in winemaking is fundamental to perform a successful enological practice. A wide number of scientific papers report the content of several phenolics detected in skins and seeds of numerous grape varieties but, to predict phenol content of the corresponding wine it is necessary to consider their specific extraction capacity during fermentation. However the influence on the extraction of skin and seed phenolics during a simulated maceration has been studied for few grape cultivars and phenolics (Oszmianski *et al.* 1986, Canals *et al.* 2005, Del Llaudy *et al.* 2008). In this regard the case of two important grape cultivars of the South of the Italy, 'Aglianico' and 'Uva di Troia' is peculiar. These two grape cultivars give wines rich in polyphenols and, for this reason, were used in the past for blending with other red wines (Lovino 1987, Moio *et al.* 1999). In spite of the similar content of total phenolics and tannins detected in the whole grape berry of both cultivars, a different distribution of phenolic compounds between seeds and skins was detected: 'Aglianico' is higher in anthocyanins from skins and tannins from seeds and 'Uva di Troia' is higher in skin tannins (Suriano and Tarricone 2006). Moreover a different rate in the formation of polymeric pigments during vinification and in the content of total phenolics of final wines was observed (La Notte *et al.* 1992, 1993). Obviously the behaviours detected may be due to differences in the extraction rate and in the nature of proanthocyanidins and copigments released from the solid parts of berries. Unfortunately the kinetics of the extraction of non-anthocyanic phenolics during maceration, as well as the entity of the release of phenolics from seeds and skins, is still unknown.

In this study the berries of the two Italian autochthonous varieties ('Aglianico' and 'Uva di Troia') were submitted to a maceration assay in hydro-alcoholic solution at different ethanol percentages and for 1, 5 and 10 d of contact. Two assays of post-fermentative maceration in a solution at 11 and 13 % ethanol for 10 d were also carried out.

Material and Methods

C h e m i c a l s : All chromatographic solvents were high-performance liquid chromatography (HPLC) ultra gradient grade. Sodium azide and ethanol (95 %) were purchased from J. T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Deionized water (< 18 MΩ cm resistivity) was obtained from a Milli-Q element water purification system (Millipore, Bedford, MA). Sodium metabisulphite and L(+)-tartaric acid (ACS for analysis) were purchased from Carlo Erba (Milan, Italy). *trans*-Resveratrol (purity > 99 %), quercetin dehydrate (purity min. 98 %) and (+)-catechin hydrate (purity min. 98 %) standards were purchased from Sigma (Milan, Italy), (-)-epicatechin (purity 90 %) was purchased from Aldrich (Milan, Italy), gallic acid and

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caffeic acid were purchased from Extrasynthese (Genay, France).

Samples: Berries of 'Uva di Troia' and 'Aglianico' (V. *vinifera* L.) cultivated in the vineyards of Daunia DOC area (Puglia region, Italy) and Taburno DOC area (Campania region, Italy) respectively, were harvested at technological maturity which was fixed according to the established vintage schedule in the area. At the moment of the harvest a representative sample of 100 berries was squeezed for measurement of juice pH, total acidity and total soluble solids according to the Official European Methods (1990). The grapes showed: soluble solids values of 22.4 °Brix for 'Uva di Troia' and 22.6 °Brix for 'Aglianico' grape; pH values were 3.48 and 3.32 respectively; total acidity values were 5.9 $g \cdot l^{-1}$ for 'Uva di Troia' and 6.7 $g \cdot l^{-1}$ for 'Aglianico' grape. The average berry weights were 3.2 g for 'Uva di Troia' and 2.6 g for 'Aglianico' grape. Each fruit sample used for the replicate experiment of extraction consisted of 15 clusters collected from 15 vine plants from the same vineyard. The berries were carefully removed from the rachis to avoid losing juice and were mixed. Samples of 150 berries each were randomly selected from this homogenous mix.

Simulated maceration assays: In order to simulate the maceration phase during alcoholic fermentation, extraction assays were performed in model wine solutions containing 5 $g \cdot l^{-1}$ tartaric acid, 2 $g \cdot l^{-1}$ sodium azide and 60 $mg \cdot l^{-1}$ metabisulfite sodium in hydroalcoholic solutions adjusted to pH 3.2 with NaOH 1N. The content of ethanol in the solution was raised from 0 % to 13 % until the tenth day of maceration. Grape samples were manually separated into skins and seeds. Berries were cut in two with a razor blade, and seeds and skins were carefully removed from each berry-half. The pulp on the inner face of berry skin was removed using a end-flattened spatula trying to preserve the skin integrity. Separate maceration assays were carried out with grape skins and seeds obtained from 150 berries in 300 ml of solution in the ratio reported in a previous paper (Fournard *et al*. 2006). The samples were taken for analysis after 1, 4, 7 and 10 days of maceration corresponding to 0, 4, 7.5, and 13 % ethanol. The duration of simulated maceration was prolonged to 1, 5 and 10 d for the solutions at 11 and 13 % ethanol in order to simulate post-fermentative maceration. All assays were performed in closed glass bottles at 22 °C, skins and seeds were maintained in suspension with gentle magnetic stirring. After decanting, the residual solids and the solution were separated. The skins were gently pressed by hand and the residual liquid was added to the rest of the solution. The solutions were then centrifuged for 10 min at 10,000 rpm to remove the lees. The resulting clear solutions were transferred into a glass bottle and kept at -20 °C until analysis. All experiments were performed in duplicate.

Spectrophotometric measurements: Total phenolics (Folin-Ciocalteau Index) and Absorbances (Abs) were measured according to European Official Methods (1990). Color intensity (CI) and ionization index (I.I.) were evaluated according to Glories (1984). Total anthocyanins were determined by the spectrophotometric method based on SO_2 bleaching (Ribèreau-Gayon and Stonestreet 1965). Tannins were determined according to Ribèreau-Gayon and Stonestreet (1966). The vanillin reactive flavanols (VRF) were analyzed according to the optimized vanillin-HCl method described by Di Stefano *et al.* (1989). Analyses were performed in duplicate.

High-performance liquid chromatography analysis of phenolic compounds in the model solutions: The HPLC used was a Shimadzu apparatus (Shimadzu Italy, Milan) LC10 ADVP, consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 50 µl loop. The column used for this separation was a Nova-Pak C_{18} column (3.9 x 150 mm, 4 µm particles diameter) equipped with a Nova-Pak Sentry C_{18} guard column (3.9 x 20 mm, 4 µm) (Waters Corporation). Samples of 50 µl of sample or calibration standards were directly injected onto the column. The mobile phase consisted of 2 % (v/v) acetic acid in water (eluent A) and 0.5 % acetic acid in water and acetonitrile (50:50, v/v; eluent B). The elution program for the analysis of phenolic acids was as follows: 0 min, 95 % A, 5 % B at a flow rate of 1 $ml \cdot min^{-1}$; 5 min, 90 % A, 10 % B at a flow rate of 1 $ml \cdot min^{-1}$; 6.5 min, 85 % A, 15 % B at 1 $ml \cdot min^{-1}$; 12 min, 75 % A, 25 % B at a flow rate of 1 $ml \cdot min^{-1}$; 25 min, 45 % A, 55 % B at a flow rate of 1 $ml \cdot min^{-1}$; 25.5 min, 100 % B; 28.1 min, 95 % A, 5 % B until the end of analysis at 30 min. Detection was performed by monitoring the absorbance signals at 280 nm (gallic acid) and 306 nm (caffeic acid). The retention times (RT) of the two phenolic acid compounds, identified by comparison with the UV-Visible spectra of pure reference standards and by the injection of co-eluted samples (sample + standard), were as follows: gallic acid (RT = 2.1 min) and caffeic acid (RT = 9.9 min). For the analysis of flavanols, quercetin and *trans*-resveratrol, the gradient program was as follows: 0 min, 90 % A, 10 % B at a flow rate of 1 $ml \cdot min^{-1}$; 10 min, 76 % A, 24 % B at a flow rate of 1 $ml \cdot min^{-1}$; 20 min, 70 % A, 30 % B at 1 $ml \cdot min^{-1}$; 30 min, 45 % A, 55 % B at a flow rate of 1 $ml \cdot min^{-1}$; 37.5 min, 100 % B; 42.5 min, 90 % A, 10 % B until the end of analysis at 45 min. Detection was performed by monitoring the absorbance signals at 280 nm (+)-catechin, (-)-epicatechin), 306 nm (*trans*-resveratrol), and 369 nm (quercetin). The retention times of the four phenolic compounds, identified by comparison with the UV-Visible spectra of pure reference standards and by the injection of co-eluted samples (sample + standard), were as follows: (+)-catechin (RT = 6.4 min), (-)-epicatechin (RT = 10.5 min), *trans*-resveratrol (RT = 26.7 min) and quercetin (RT = 31.5 min). The calibration curves obtained by injecting mixed standard solutions containing (+)-catechin, (-)-epicatechin, *trans*-resveratrol, quercetin, caffeic acid and gallic acid were characterized by a correlation coefficient (r^2) > 0.990 in the range of the concentrations considered (Tab. 1). The model solution samples were injected directly into the chromatographic system after filtration through 0.45 µm (Teknokroma PTFE) and 0.2 µm (Advantec MFS PTFE) membrane-filtered. Analyses were carried out in duplicate.

Table 1

Linearity of assay for seven phenolic constituents of the solution model assessed by regression analysis

	Equation of calibration lines	r^2	linearity range ($mg \cdot l^{-1}$)[a]
Phenolic Acid			
gallic acid	y = -0.652 + 1.43E-05x	0.994	0.4 - 80
caffeic acid	y = -1.79E-02 + 7.77E-06x	0.999	0.0425 - 8.5
Flavonoids			
(-)-epicatechin	y = 0.440 + 6.50E-05x	0.990	0.5 - 100
(+)-catechin	y = 2.046 + 5.22E-05x	0.998	1 - 200
quercetin	y = 0.457 + 5.59E-06x	0.991	0.25 - 50
Stilbens			
trans-resveratrol	y = -3.39E-02 + 4.90E-06x	0.999	0.0375 - 7.5

[a] n = 5

Statistical analysis: All the data are expressed as the arithmetic average ± standard deviation of four replicates. The equation of calibration lines was calculated by linear regression, analysis of variance and *t*-Student and Tukey's test were used to interpret differences in means, if any, at the 95 % confidence level. Elaborations were carried out by means of XLSTAT-Pro 7.5.3 (Addinsoft).

Results

For both 'Aglianico' and 'Uva di Troia' grape skins the concentration of total anthocyanins in the extracts reached the maximum after the first 4 d of maceration and then stabilized (Fig. 1). This trend is similar to those reported by Canals *et al.* (2005) on Tempranillo grape skins where the maximum extraction of anthocyanins was achieved on the third day of maceration. The lower content of anthocyanins extracted at the beginning of maceration (the first day) from 'Uva di Troia' with respect to 'Aglianico' is in agreement with earlier data on these two cultivars (Lovino *et al.* 2006). However this gap disappears when maceration time is prolonged. This evidence is the basis for important analytical implications, already discussed by other authors (Jensen *et al.* 2008, Mattivi *et al.* 2002), on the difficulty of finding an extraction method for the analysis of grape polyphenols, able to predict the future characteristics of the wine. Also the maximum value of colorant intensity and Abs_{520} was detected after four days of maceration (Tab. 2). After this time significant differences between cultivars were detected. This phenomenon is explained by the differences detected in the ionization index (I.I.) (a measure of the percentage of pigments present in solution which contribute to the Abs_{520}) (Tab. 2) due to complex phenomena of copigmentation in which anthocyanins are involved

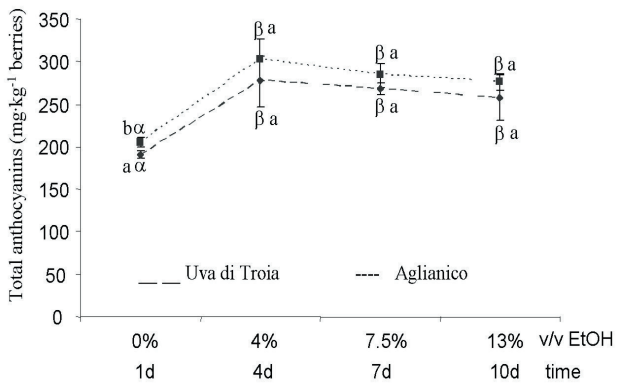

Fig. 1: Trends in release of total anthocyanins from 'Uva di Troia' and 'Aglianico' grape skins, as affected by some fermentative maceration conditions. Varieties (a, b) and maceration time (α, β, χ, δ) sharing the same letters are not significantly different ($p < 0.05$).

Table 2

Trends in some colour indexes of extracts obtained from 'Uva di Troia' and 'Aglianico' grape skins, as affected by some fermentative maceration conditions[a]

	EtOH %	time d	Aglianico				Uva Di Troia			
			Mean	S.D.			Mean	S. D.		
Abs_{420}	0	1	2.0	0.05	b	α	1.5	0.1	a	α
	4	4	3.3	0.1	b	χ	2.8	0.2	a	β
	7.5	7	3.1	0.04	a	β	3.0	0.2	a	βχ
	13	10	3.2	0.1	a	βχ	3.1	0.1	a	χ
Abs_{520}	0	1	4.8	0.1	b	α	3.5	0.3	a	α
	4	4	7.7	0.2	b	χ	6.5	0.5	a	β
	7.5	7	7.0	0.05	a	β	6.8	0.2	a	β
	13	10	7.0	0.1	a	β	6.8	0.4	a	β
Abs_{620}	0	1	0.20	0.05	a	α	0.10	0.08	a	α
	4	4	0.57	0.08	a	β	0.42	0.25	a	αβ
	7.5	7	0.56	0.05	a	β	0.60	0.18	a	β
	13	10	0.65	0.06	a	β	0.69	0.03	a	β
IC	0	1	7.0	0.1	b	α	5.0	0.5	a	α
	4	4	12	0.04	b	χ	9.8	0.6	a	β
	7.5	7	11	0.1	a	β	10	1	a	β
	13	10	11	0.2	a	β	11	0.5	a	β
I.I.	0	1	24	2	b	α	16	1	a	α
	4	4	27	2	b	α	20	1	a	β
	7.5	7	25	1	b	α	23	1	a	χ
	13	10	24	2	a	α	25	2	a	χ

[a] Varieties (a, b) and maceration time (α, β, χ, δ) sharing the same letters are not significantly different ($p < 0.05$).

and that are, probably, cultivar dependent. Previous studies showing that grape cultivar significantly affects the copigmentation process and anthocyanins extraction (Darias-Martin *et al.* 2001, Romero-Cascales *et al.* 2005) support this hypothesis.

In agreement with literature (Meyer and Hernandez 1970, Peyrot Des Gachons and Kennedy 2003), the extraction of tannins from skins make up the early portion of extraction during the first phases of fermentation (Fig. 2). Concerning the chemical structure of tannins we found that, in the first 4 d of maceration, for both cultivars, the percentage of VRF extracted from skins on total tannins rose from 41 to 47 %, then stabilized (Fig. 2 a). On the basis of the correlation shown between the two assays performed on grape phenolics (acid-catalysed cleavage, and VRF) and the mDP (mean degree of polymerization) of condensed tannins reported by Vrhovsek *et al*. (2001) it is clear that, at the beginning of maceration, there was a slight increase in the extraction of monomeric flavanols and proanthocyanidins formed by 2-4 units. Prolonging maceration time the percentage of low molecular weight tannins did not change and was almost fifty percent of total tannins extracted. The data on vanillin reactive flavans VRF extracted from seeds indicate that, for both the cultivars, at the beginning of maceration the extraction of low molecular weight phenols was quicker than total tannins (Fig. 2 b). This is in accordance with results reported by Peyrot Des Gachons and Kennedy (2003) where a slight increase in mDP of procyanidins extracted from seeds after 48 h of maceration (from 11.63 ± 2.04 to 14.09 ± 0.55) was detected.

Maximum release of flavan-3-ols (+)-catechin and (-)-epicatechin from the skins was reached on the first day of maceration for both cultivars (Tab. 3). Gonzales-Manzano *et al.* (2004) detected a modification of flavanol profiles during prolonged maceration. In this study a modification in the ratio (+)-catechin/(-)epicatechin only simulating maceration of 'Uva di Troia' skins, was observed. This is consistent with previous studies showing that considerable modification of flavanol composition takes place during maceration in the process of wine production (Czyzowska and Pogorzelski 2004) and that this modification is cultivar-dependent (Gambuti *et al.* 2004).

Caffeic acid reached its maximum concentration the fourth day of maceration while the gallic acid increased during maceration time. A similar trend was observed by Meyer *et al.* (1997) during the extraction of phenolics from 'Cabernet Sauvignon' and 'Syrah' late grape cultivars. The

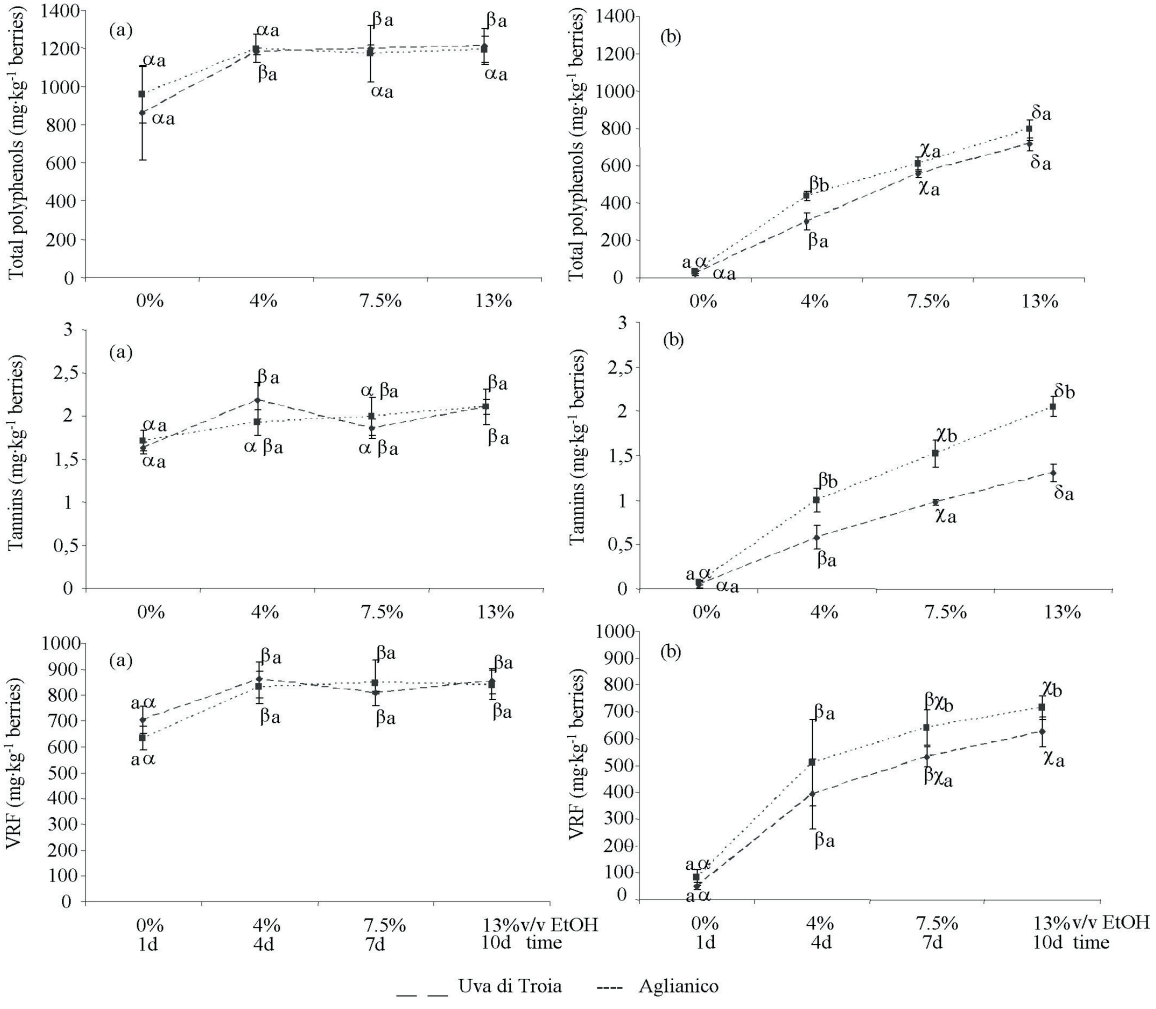

Fig. 2: Trends in release of total polyphenols, tannins and vanilline reactive flavanols VRF from 'Uva di Troia' and 'Aglianico' grape skins (**a**) and seeds (**b**), as affected by some fermentative maceration conditions. Varieties (a, b) and maceration time (α, β, χ, δ) sharing the same letters are not significantly different ($p < 0.05$).

Table 3

Trends in the concentration (mgL^{-1}) of (+)-catechin, (-)-epicatechin, quercetin, caffeic acid, gallic acid and *trans*-resveratrol in the extracts obtained from 'Uva di Troia' and 'Aglianico' grape skins, as affected by some fermentative maceration conditions

	Aglianico															Uva di Troia													
Ethanol	0 %				4 %				7.5 %				13 %			0 %				4 %				7.5 %				13 %		
Time	1 d				4 d				7 d				10 d			1 d				4 d				7 d				10 d		
	Mean	S.D.			Mean	S.D.			Mean	S.D.			Mean	S.D.		Mean	S.D.			Mean	S.D.			Mean	S.D.			Mean	S.D.		
Flavonoids																															
(+)-Catechin	20	5	a	α	25	6	a	α	30	9	a	α	22	5	a	α	21	3	a	α	18	7	a	α	17	6	a	α	24	7	a	α
(-)-Epicatechin	15	5	a	α	11	2	a	α	14	3	a	α	16	2	a	α	25	9	a	α	41	7	b	α	24	8	a	α	12	6	a	α
Quercetin	0.48	0.02		α	0.77	0.32	a	αβ	1.4	0.5	a	β	3.1	0.3	a	χ	n.d.				1.1	0.2	a	β	1.7	0.4	b	β	3.8	1.2	a	χ
Phenolic acids																															
Caffeic acid	0.89	0.11	a	α	4.0	1.0	a	β	2.3	0.6	b	β	0.90	0.22	a	α	1.2	0.1	b	α	6.9	1.7	a	β	1.3	0.2	a	α	0.6	0.1	a	α
Gallic acid	n.d				3.2	0.9	a	β	6.1	1.1	b	χ	7.5	2.2	a	χ	n.d.				1.7	0.9	a	β	2.9	0.7	a	β	5.1	1.2	a	χ
Stilbenes																															
trans-resveratrol	0.36	0.09	a	α	1.2	0.2	a	βχ	1.6	0.3	b	χ	0.89	0.13	a	β	0.64	0.13	b	α	2.3	0.6	b	β	0.87	0.21	a	α	1.35	0.78	a	αβ

[a] Varieties (a, b) and maceration time (α, β, χ, δ) sharing the same letters are not significantly different ($p < 0.05$). nd = not dosable.

involvement of both acids in nonenzymic autoxidation of vicinal dihydroxyphenols (Singleton, 1987) and in copigmentation phenomena (Schwarz *et al*. 2003; Miniati *et al.* 1992) may cause a reduction of the concentration of these compounds during maceration as observed for the caffeic acid. In the case of the gallic acid, the continuous increase is consistent with the fact that: a) it is a poorer copigment (Eiro and Heinonen 2002) and, b) a further release in the medium of significant concentration of this molecule may occur from the hydrolysis of epicatechin gallate (Singleton and Trousdale 1983).

Concerning the extraction kinetics of *trans*-resveratrol, our results, consistent with earlier studies (Gambuti *et al.* 2004), show that both, the amount released and the moment in which the maximum extraction occurs, are linked to differences in grapevine genetics controlling stilbene biosynthesis and skin cell permeability. Gradual extraction of quercetin from skins took place during fermentation-maceration (Tab. 3). This behaviour is comparable with that detected during fermentation of 'Grenache' by Morel-Salmi *et al.* (2006).

Simulation of post-fermentative maceration: 'Aglianico' skins released more anthocyanins in the post-fermentative model solution than 'Uva di Troia' apart from the ethanol content in the medium (Fig. 3). In agreement with previous studies (Mazza *et al.* 1999), a general decrease of these pigments with prolonged skin maceration is observed. Behaviour followed by the colorant intensity of 'Uva di Troia' skins extract confirm this trend in both hydro-alcoholic solutions (Tab. 4). Conversely in the solutions obtained from 'Aglianico' skins at 11 % ethanol an increase of colorant intensity is observed after 5 d of maceration. This may be correlated to a further extraction of phenolics acting as copigments from 'Aglianico' skins as confirmed by the increase of Ionization Index detected after 5 d of post-fermentative maceration.

Ethanol and time did not influence the extraction of total phenolics from 'Aglianico' skins (Fig. 4). A significant enhancement in the extraction of skin tannins is observed only for 'Uva di Troia' grape with 5 d of contact time at 13 % ethanol. The differences observed between cultivars may depend on i) tannin localization in skins (Geny *et al*. 2003), ii) structural characteristics of tannins such as mDP and galloylation degree (Fournand *et al*. 2006), on iii) cell wall polymerization (Gagné *et al*. 2006) and on iv) the pectic polysaccharides of cell walls (Ortega-Regules *et al.* 2006). The 'Uva di Troia' grape seeds release less total phenolics and tannins than 'Aglianico' (Fig. 4). A strong

Fig. 3: Total anthocyanins extracted from 'Uva di Troia' and 'Aglianico' grape skins during simulation of a post-fermentative maceration.

Table 4

Trends in some colour indexes of extracts obtained from'Uva di Troia' and 'Aglianico' grape skins, as affected by some post-fermentative maceration conditions[a]

		11 % EtOH											13 % EtOH									
		Aglianico					Uva di troia						Aglianico					Uva di troia				
	days	Mean	S.D.				Mean	S.D.					Mean	S.D.				Mean	S.D.			
Abs_{420}	1	2.8	0.1	a	α	*	3.0	0.6	a	β	*		4.0	0.1	b	β	**	3.6	0.4	a	β	*
	5	4.1	0.1	b	β	**	2.5	0.3	a	αβ	*		3.4	0.2	b	α	*	2.6	0.1	a	α	*
	10	4.1	0.3	b	β	**	2.4	0.2	a	α	*		3.5	0.3	b	α	*	2.4	0.2	a	α	*
Abs_{520}	1	6.5	0.3	b	β	*	5.5	0.5	a	β	*		9.9	0.2	b	β	**	7.3	0.8	a	β	**
	5	4.9	0.2	a	α	*	4.9	0.4	a	αβ	*		7.0	0.3	b	α	**	5.7	0.04	a	α	**
	10	7.7	0.5	b	χ	**	4.7	0.5	a	α	*		6.9	0.4	b	α	*	4.7	0.5	a	α	**
Abs_{620}	1	0.7	0.1	a	α	*	2.1	0.7	b	β	*		1.1	0.1	a	α	**	2.4	0.6	b	β	*
	5	0.8	0.2	a	α	*	0.9	0.3	a	α	*		1.2	0.2	b	α	**	0.7	0.01	a	α	*
	10	1.7	0.3	b	β	*	0.8	0.1	a	α	*		1.6	0.3	b	β	*	0.8	0.1	a	α	*
IC	1	10	0.3	a	α	*	11	1	a	β	*		15	0.2	b	β	**	13	1	a	β	**
	5	9.7	0.3	b	α	*	8.4	0.6	a	α	*		12	0.4	b	α	**	9.0	0.1	a	α	*
	10	13	0.7	b	β	**	7.9	0.6	a	α	*		12	1	b	α	*	7.9	0.6	a	α	*
I.I. %	1	3.2	0.4	a	α	*	12	1	b	β	*		15	2	b	α	**	11	0.5	a	α	*
	5	23	1	b	β	*	10	1	a	α	*		26	2	b	β	**	14	1	a	β	**
	10	25	3	b	β	*	11	1	a	αβ	*		24	2	b	β	*	12	0.3	a	α	**

[a]Varieties (a, b), maceration time (α, β, χ, δ) and ethanol content of extract (*, **) sharing the same letters are not significantly different ($p < 0.05$).

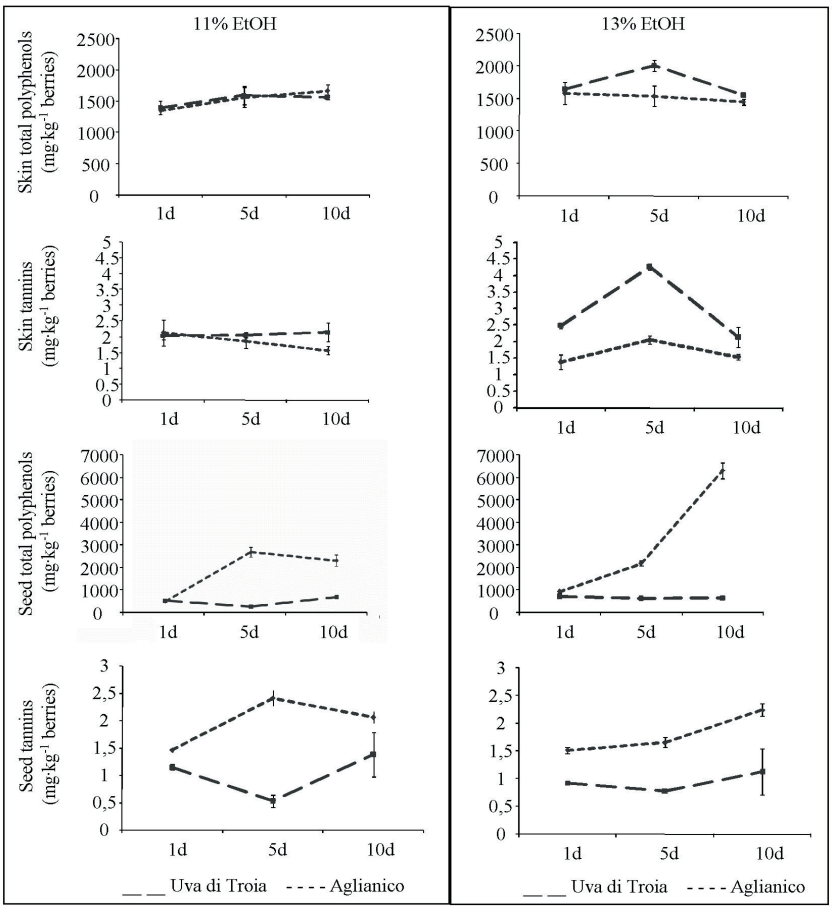

Fig. 4: Total polyphenols, tannins and vanilline reactive flavanols VRF extracted from 'Uva di Troia' and 'Aglianico' grape skins and seeds during simulation of a post-fermentative maceration.

influence of both, ethanol and time, on the extraction of these molecules from 'Aglianico' seeds was also detected.

Conclusion

Ethanol content of the solution and time of contact influence the extraction of phenolics from both skins and seeds. Maximum extraction from skins was reached on the fourth day of maceration, followed by stabilization for anthocyanins, tannins and vanillin reactive flavans VRF. This suggests that an analytical trial to detect the amounts of these important sensory active polyphenols extracted from skins may take almost four days of skin maceration to give a prediction of what happens during winemaking. Concerning the extraction of pigments from skins, great differences between the two varieties with contact time and ethanol content of medium, were observed. Although the lower content of anthocyanins extracted from 'Uva di Troia' grape skins during the first day of maceration, the same value of anthocyanins and of colorant intensity was detected by increasing maceration time and ethanol. The presence of higher amount of reactive copigments (such as caffeic acid) in the skins may be responsible for copigmentation and further extraction of anthocyanins from this grape cultivar. More specific and focused experiments may better elucidate the role of different skins and seeds copigments in the occurrence of copigmentation phenomena.

The extraction of tannins from seeds of 'Aglianico' was more influenced by contact time and ethanol than 'Uva di Troia', indicating that their extraction kinetics is strongly influenced by the solubility of seed cuticle. Further studies focused on the characterization of seed cuticle are necessary to clarify the mechanism whereby seed polyphenols are extracted during maceration.

Given the results obtained in this study and, in the light of the fact that most of the red wines on the world market are made from 'Merlot' and 'Cabernet Sauvignon' grape varieties, future extraction studies including these two commonly used grape cultivars may give interesting informations to better understand the factors influencing the transfer rates of phenolics from solid parts of grape berry into wine under real winemaking conditions.

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