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Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars

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Dedicated to Prof. Mario Piattelli on the occasion of his 80th birthday

Abstract

The methanolic extracts (MeOH) obtained from de-stemmed grape pomace samples of five Sicilian red grape cultivars (Nero d′Avola-NA, Nerello Mascalese-NM, Nerello Cappuccio-NC, Frappato-FR and Cabernet Sauvignon-CS) were evaluated for their DPPH$^{\cdot}$ and ABTS$^{\cdot-}$ radical scavenging capacity, and submitted to HPLC–UV–DAD and HPLC–MS–ESI analysis to determine the main polyphenolic constituents, namely anthocyanins and flavonols. All the MeOH extracts showed significant antioxidant activity, with some differences between the two methods employed. The NM sample was the most active in both tests. A large variability in the total anthocyanin (TA) and flavonol (TF) contents of the MeOH extracts, as well as in the quantitative distribution of the single anthocyanins and flavonols was observed. Statistically insignificant correlations between the TA + TF content and antioxidant activity, as measured by DPPH$^{\cdot}$ and ABTS$^{\cdot-}$ model systems ($r^2 = 0.0607$, $P > 0.05$; $r^2 = 0.3471$, $P > 0.05$), were established, but the most active sample, NM, showed the highest content of anthocyanins including a free catechol moiety in their structure.

Keywords: Grape pomace; *Vitis vinifera*; Anthocyanins; Flavonols; Antioxidants; HPLC–UV–MS; DPPH; TEAC

1. Introduction

The increasing demand for production of safer foods nowadays is accompanied by the request for cleaner production processes. The vegetable/beverage industry will have to sustain increasing costs for treating solid and liquid wastes: their use for animal feed or fertilizer without pre-treatments is not simple, because of the intolerance of some animals to some waste components (Laufenberg, Kunz, & Nystroem, 2003), and the known germination inhibition properties of many polyphenols (Northup, Dahlgren, & McColl, 1998). This is particularly true for wine production, which affords a substantial volume of solid organic wastes: grape pomace as by-product is approximately 20% of the harvested grapes (Laufenberg et al., 2003). Grape (except for orange) is the world's largest fruit crop, with more than 61 million metric tons, cultivated mainly as *Vitis vinifera* for wine production (FAO STAT Database in www.fao.org; Schieber, Stintzing, & Carle, 2002). The main by-products are collected during de-stemming (stems), grape crushing and pressing (skins, seeds and lees). At present, only minimum amounts of these wastes are up-graded or recycled and this is particularly true in Europe, where vegetable wastes are generally dumped or used for animal feed or compost, without any pre-treatment. Part of the grape pomace is destined for distillation but this allows recovery of a minimum amount of the material as volatile constituents of alcoholic beverages (grappa). With respect

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to the chemical studies carried out on wine constituents, grape pomace has scarcely been investigated, but it is undoubtedly rich in polyphenols (Torres et al., 2002; Amico et al., 2004; Kammerer, Claus, Carle, & Schieber, 2004). The high level of polyphenols in this waste, a disadvantage for their possible use as animal feed, composting or discharge, may be turned to an advantage through the extraction of polyphenols before further utilisation or treatment. In fact, many recent studies have highlighted the beneficial effects of grape or wine polyphenols for human health (Simic & Jovanovic, 1994; Rice-Evans, Miller, & Paganga, 1997; Lurton, 2003; Frankel, 1999). More specifically, the antioxidative properties of many natural polyphenols may exert a chemopreventive role toward cardiovascular and degenerative diseases, (Halpern et al., 1998; Renaud & De Lorgeril, 1992), including neurodegenerative pathologies (Esposito et al., 2002) and cancer (Bozidar, 1995). In particular, skins are rich in anthocyanins, a group of polyphenols well-know for their beneficial properties (Katsube, Iwashita, Tsushida, Yamaki, & Kobori, 2003; Wang, Cao, & Prior, 1997; Ghiselli, Nardini, Baldi, & Scaccini, 1998; Kong, Chia, Goh, Chia, & Brouillard, 2003; Kähkönen & Heinonen, 2003). Quantitative and qualitative distribution of polyphenols in grape pomace may show significant differences, depending on several factors, such as the varietal differences of *Vitis vinifera*, the location of cultures and the wine-making procedures.

Notwithstanding that Italy is the first grape-producing country in the world, investigations on grape pomace coming from Italian wineries were absent until recent times. We have recently carried out a study of grape pomace constituents from the Sicilian cultivar 'Nerello mascalese', (Amico et al., 2004) in view of the increasing trend for grape cultivation and wine-making in Sicily and consequently of the considerable amounts of wine by-products which will accumulate in the near future in this region. Pursuing this effort we wish to report here the study of the main polyphenol constituents and the antioxidant effectiveness (evaluated as radical-scavenging activity) of the extracts from de-stemmed samples of grape pomace of five important Sicilian cultivars of red wine grape, namely Nero d′Avola, Nerello Mascalese, Nerello Cappuccio, Frappato and Cabernet Sauvignon, globally accounting for 80% of the total Sicilian area devoted to red wine grape cultivation. In view of the possible exploitation of these agro-industrial wastes, we have focussed our attention on anthocyanins and flavanols, well-known for their antioxidative and beneficial properties and, at the same time, rapid detectability by HPLC–UV–MS, for a possible future standardisation of the crude extracts.

2. Materials and methods

2.1. General methods

Liquid chromatography–mass spectrometry (LC–MS–ESI) was carried out on an HPLC Waters mod. 1525 equipped with mass spectrometer Waters mod. Micromass ZQ2000. HPLC-UV-DAD analyses were run on the same HPLC connected with a Waters mod. 996 PDA detector. Ultraviolet–visible (UV–Vis) spectra were recorded using a Perkin-Elmer model Lambda 25 spectrophotometer. Thin-layer chromatography (TLC) was carried out on Merck 60 F_{254} plates using cerium sulphate, phosphomolybdic acid and ferric chloride solutions as chromogenic reagents. UV–DAD analysis was also performed for quantification of the main components using a calibration curve. Malvidin 3-*O*-glucoside (**8**) and quercetin 3-*O*-glucoside (**21**) were used as standards for anthocyanins and flavonols, respectively.

Pure standards were purchased from Aldrich Chemical Co. and Extrasynthese, France.

2.2. Plant material

Grape pomaces of Nero d′Avola (NA) and Frappato (FR) were a gift of "Valle dell′Acate" winery, Acate RG, Italy. Nerello Mascalese (NM) was from "Valle Galfina" winery, Linguaglossa CT, Italy. Nerello Cappuccio (NC) was a gift of "Benanti" winery, Viagrande CT, Italy. Cabernet Sauvignon (CS) was obtained from "Emanuele Scammacca Barone del Murgo" winery, Santa Venerina CT, Italy. Each sample was collected after the maceration (5–8 days) of destemmed grape. All materials were collected during the 2002 vintage.

2.3. Preparation of extracts

Fresh grape pomaces were freeze-dried and successively finely ground and stored at −20 °C until used. This material (50 g) was extracted with methanol with 1% 1N HCl at 25 °C for 4 h (300 ml × 3) with continuous stirring. After concentration of the whole solution to 300 ml, this was extracted three times with hexane (300 ml). Both organic phases were taken to dryness and the methanol residue subjected to the subsequent analyses. The percentage yields (g extract / 100 g dry pomace) for the *n*-hexane and methanol residues, respectively, were the following: NA: 1.03 and 4.76; NM: 0.98 and 5.76; NC: 1.62 and 5.75; FR: 1.02 and 3.44. CS: 0.96 and 3.54.

2.4. HPLC–UV–DAD and HPLC–MS–ESI analyses

The HPLC analyses were performed on a Phenomenex® Luna®, C18 250 × 4.6 mm (5 μm) column held at 20 °C using the following eluent system: eluent A: water-formic acid, 9:1 (v/v) – eluent B acetonitrile – formic acid, 9:1 (v/v) – $t_{0\text{min}}$ B (5%), $t_{20\text{min}}$ B (15%), $t_{40\text{min}}$ B (30%), $t_{55\text{min}}$ B (100%), $t_{65\text{min}}$ B (100%). The flow rate was set at 1 ml/min. ESI–MS was used for detection and characterization of metabolites, using positive mode for anthocyanins and negative mode for flavonols, with a capillary voltage of 3.5 kV, a cone voltage of 40 V, a vaporizer temperature of 250 °C, and a carrier gas flow (nitrogen) of 500 l/h.

The mass acquisition was carried out between 100 and 1500 Da. DAD analysis was carried out in the range between 200 and 700 nm, setting the detector at 350 nm for flavonols and flavonol glycosides, at 480 nm for pyranoanthocyanins, and at 530 nm for anthocyanins.

2.5. Measurement of the DPPH$^{\bullet}$ scavenging activity

The radical-scavenging activity of grape extracts was evaluated according to a modified version of the method of Brand-Williams, Cuvelier, and Berset (1995) The initial concentration of 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) radical in methanol, 184 μM, was controlled for every experiment from a calibration curve made by measuring the absorbance at 515 nm of standard samples of DPPH$^{\bullet}$ at different concentrations, in a quartz cuvette (1 cm of light path). The equation of the curve was $ABS_{515nm} = 10865 \times [DPPH]$, as determined by linear regression. In a typical procedure, to 2 ml of the freshly prepared solution of DPPH$^{\bullet}$, 10, 20 and 30 μl of freshly prepared (2 mg/ml) solution in methanol of extracts were added. The reaction mixtures were shaken and incubated for 5 h in the dark at 25 °C, in order to reach the steady state; each measurement was acquired in triplicate. The results were plotted as the percentage of reacted DPPH$^{\bullet}$ $\left(\frac{ABS_0 - ABS}{ABS_0} \times 100\right)$ against the concentration (μg/ml) of the added samples. SC_{50} (*scavenging concentration*) is the concentration (μg/ml) of extract which was required to quench 50% of the initial DPPH$^{\bullet}$ radicals under the experimental conditions given.

2.6. Measurement of the ABTS$^{\bullet-}$ scavenging activity

Evaluation of free radical-scavenging activity was performed with trolox equivalent antioxidant capacity (TEAC) assay. The TEAC value is based on the ability of the antioxidant to scavenge the radical anion 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS$^{\bullet-}$) (Thomas et al., 2004) with spectrophotometric analysis according to Re et al. (1999). Samples were diluted with methanol to produce solutions of 0.3, 0.5, 1.0, 1.5 and 2.0 mg/l. The reaction was initiated by the addition of 1 ml of diluted ABTS$^{\bullet-}$ to 10 μl of each solution of samples or trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, standard) or 10 μl of MeOH (control). The percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to a blank absorbance (methanol). All analyses were done in triplicate.

2.7. Statistical analyses

The determinations of anthocyanin and flavonol contents, and the evaluation of the radical-scavenging activity were carried out in triplicate, and results are given as means ± standard deviation (SD). Correlation between anthocyanin and flavonol contents and radical-scavenging efficiency was quantified by the correlation factor "*r*". The *P*-value $\leqslant 0.05$ was considered statistically significant.

3. Results and discussion

3.1. General

The cultivars of *Vitis vinifera* selected for this study are to date widely cultivated in Sicily. 'Nero d′Avola' (NA) is the main Sicilian variety (50% of total Sicilian red grape wine cultivation), followed by 'Nerello Mascalese' (NM, 15%) and 'Nerello Cappuccio' (NC, 8%). 'Frappato' (FR, 3%), has a limited cultivation area in the South-East of Sicily. 'Cabernet Sauvignon' (CS, 6%) is a French variety, the only cultivar used in this study not originally from Sicily.

Grape pomaces were collected immediately after maceration of destemmed grapes in the wineries, freeze-dried and ground. The analytical procedure was slightly simplified with respect to our previous work on 'Nerello Mascalese' (Amico et al., 2004). The acidified MeOH extracts were defatted with *n*-hexane. The percentage yields in defatted MeOH fractions were in the range 3.44% (FR) – 5.77% (NM) of dried pomace, whereas the extractable fats with *n*-hexane were around 1%. (See Materials and methods for details). The defatted MeOH fractions were submitted to HPLC–UV–MS analysis. Each compound was identified on the basis of HPLC retention time, UV–Vis spectrum (obtained from DAD) and MS spectrum (obtained from ESI detector). Stereochemistry of the reported structures (**1**–**28**) has been tentatively assigned on the basis of previous citations in the literature or assuming that the more common stereoisomer would be present in the extract. Quantitative analysis was focussed on the main anthocyanin and flavonol/flavonol glycosides. The anthocyanins identified and, when possible, quantified in extracts from grape pomace are listed in Table 1 (see Fig. 1 for structural formulas). Flavonols and their glucosides or glucuronides are listed in Table 2 (see Fig. 2 for structural formulas). For a better comparison, the total amount of quantified anthocyanins (TA) and flavonols (TF), resulting simply from the addition of the quantified anthocyanins or flavanols, as well as the total polyphenols (TP = TA + TF) and the relative percentages of anthocyanins (%TA) and flavanols (%TF) are reported in Table 3 for each sample.

Concerning the study of the antioxidant effectiveness it has recently been recommended to employ at least two different in vitro models, due to the differences between the various free radical-scavenging assay systems (Schlesier, Harwat, Bohm, & Bitsch, 2002; Aruoma, 2003). Thus, the defatted MeOH extracts were submitted to two different free radical-scavenging bioassays, employing, respectively, the DPPH$^{\bullet}$ and the ABTS$^{\bullet-}$ radicals. The DPPH$^{\bullet}$ assay has been widely used for evaluation of radical-scavenging activity of natural products and crude extracts, (Mavi, Terzi, Ozgen, Yildirim, & Coskun, 2004; Fernandez-Pachon, Villano, Garcia-Parrilla, & Troncoso, 2004) and antioxidant activity is herewith reported as SC_{50} (concentration scavenging 50% of free radicals). We also used

Table 1
Concentrations of anthocyanins in grape pomace extracts of Sicilian cultivars[a]

Compound[b]	NA	NM	NC	FR	CS
	mg/g[c]				
Delphinidin 3-*O*-glucoside (**4**)	0.21 (0.02)	1.17 (0.15)	0.81 (0.08)	0.21 (0.02)	0.16 (0.01)
Cyanidin 3-*O*-glucoside (**1**)	t	0.64 (0.02)	t	0.09 (0.01)	nd
Petunidin 3-*O*-glucoside (**6**)	0.43 (0.05)	1.43 (0.06)	1.30 (0.16)	0.38 (0.04)	0.32 (0.02)
Peonidin 3-*O*-glucoside (**2**)	0.20 (0.02)	1.48 (0.05)	0.42 (0.06)	0.39 (0.05)	0.15 (0.03)
Malvidin 3-*O*-glucoside (**8**)	5.61 (0.36)	4.09 (0.61)	10.38 (0.51)	2.16 (0.09)	5.70 (0.08)
Delphinidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**5**)	0.81 (0.11)	nd	2.25 (0.18)	nd	nd
Petunidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**7**)	1.22 (0.17)	nd	nd	nd	nd
Peonidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**3**)	0.22 (0.01)	nd	2.97 (0.30)	t	t
Malvidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**11**)	19.32 (0.02)	0.29 (0.02)	27.14 (2.18)	0.43 (0.01)	0.99 (0.02)
Malvidin 3-*O*-(6″-*O*-acetyl)-glucoside (**9**)	0.68 (0.05)	nd	nd	nd	2.02 (0.16)
Malvidin 3-*O*-(6″-*O*-caffeyl)-glucoside (**10**)	t	nd	nd	0.10 (0.01)	0.27 (0.01)

[a] NA = Nero d'Avola; NM = Nerello Mascalese; NC = Nerello Cappuccio; FR = Frappato ; CS = Cabernet Sauvignon.
[b] Compounds are listed according to the elution order in the HPLC analysis. Standard deviation ($n = 3$) in parentheses; nd = not detected; t = trace (<0.05 mg/g).
[c] Referred to 1 g of MeOH extract.

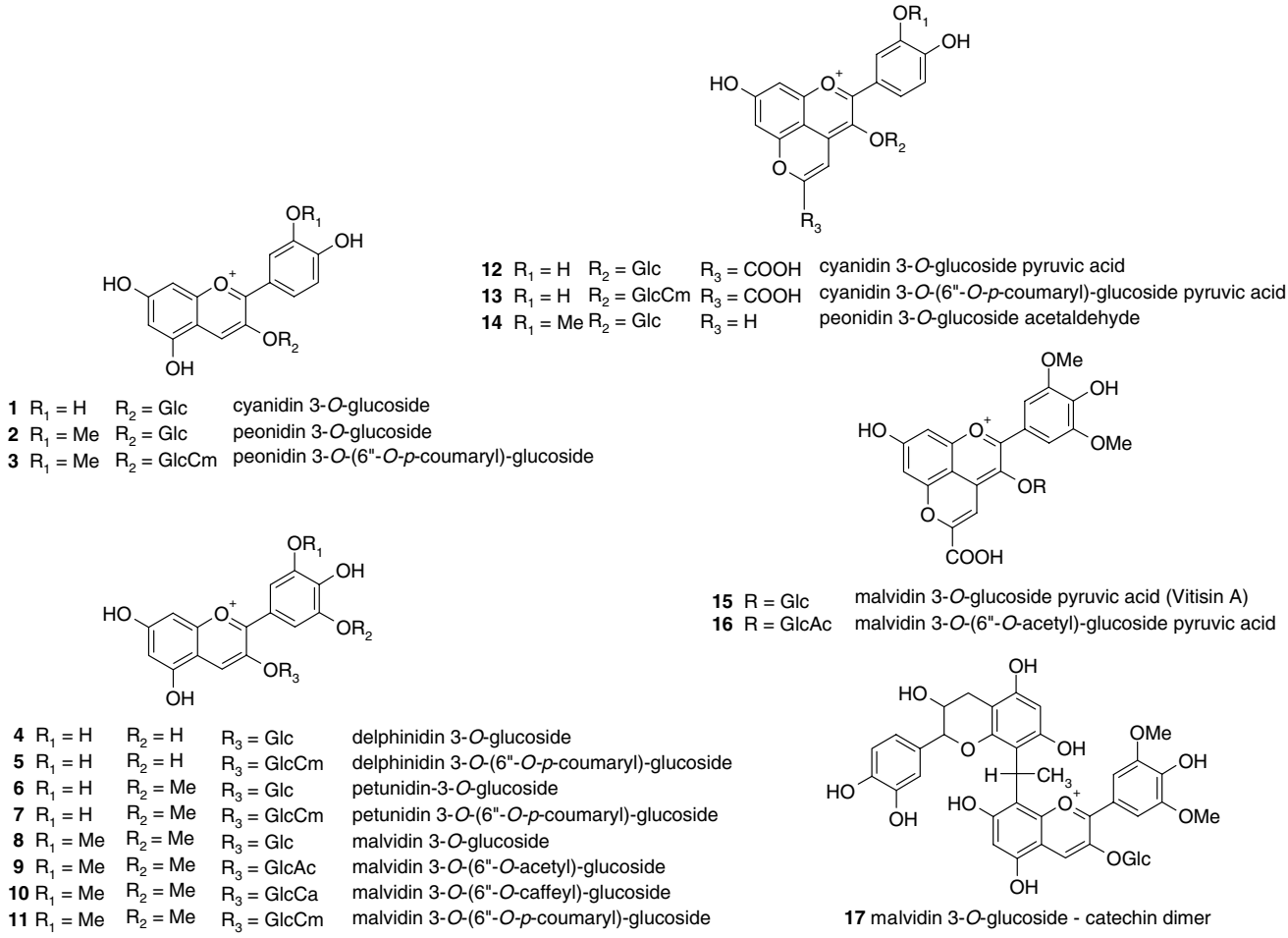

Fig. 1. Structures of anthocyanins identified in grape pomace extracts (Glc = glucoside; GlcCm = coumaryl glucoside; GlcAc = acetyl glucoside; GlcCa = caffeyl glucoside).

the assay based on $ABTS^{\cdot-}$ anion radical, whose results are normally reported as trolox equivalent antioxidant concentration (TEAC) and referred to the well-known free radical scavenger trolox (Van den Berg, Haenen, Van den Berg, & Bast, 1999). This assay has been employed for establishing the antioxidant activity of a variety of sub-

Table 2
Concentrations of flavonols in grape pomace extracts of Sicilian cultivars[a]

Compound[b]	NA	NM	NC	FR	CS
	mg/g[c]				
Quercetin 3-*O*-glucuronide (**22**)	1.10 (0.08)	0.71 (0.06)	nd	1.34 (0.19)	nd
Quercetin 3-*O*-glucoside (**21**)	0.50 (0.03)	0.86 (0.07)	t	0.72 (0.03)	t
Quercetin 3-*O*-rutinoside (**23**)	0.49 (0.07)	nd	nd	nd	nd
Kaempferol 3-*O*-glucoside (**19**)	nd	nd	0.17 (0.01)	nd	nd
Isorhamnetin 3-*O*-glucoside (**25**)	0.12 (0.01)	nd	nd	nd	nd
Isorhamnetin 3-*O*-glucuronide (**26**)	0.88 (0.03)	0.37 (0.05)	nd	nd	nd
Myricetin (**27**)	nd	nd	nd	nd	t
Quercetin (**20**)	1.19 (0.09)	0.88 (0.09)	2.77 (0.40)	0.68 (0.08)	0.54 (0.02)
Laricitrin (**28**)	nd	nd	nd	nd	0.25 (0.03)
Kaempferol (**18**)	0.36 (0.05)	0.25 (0.03)	1.12 (0.05)	0.29 (0.03)	t
Isorhamnetin (**24**)	nd	0.19 (0.01)	nd	0.13 (0.01)	0.25 (0.01)

[a] NA = Nero d'Avola; NM = Nerello Mascalese; NC = Nerello Cappuccio; FR = Frappato ; CS = Cabernet Sauvignon.
[b] Compound are listed according to the elution order in the HPLC analysis; Standard deviation ($n = 3$) in parentheses; nd = not detected; t = trace (<0.05 mg/g).
[c] Referred to 1 g of MeOH extract.

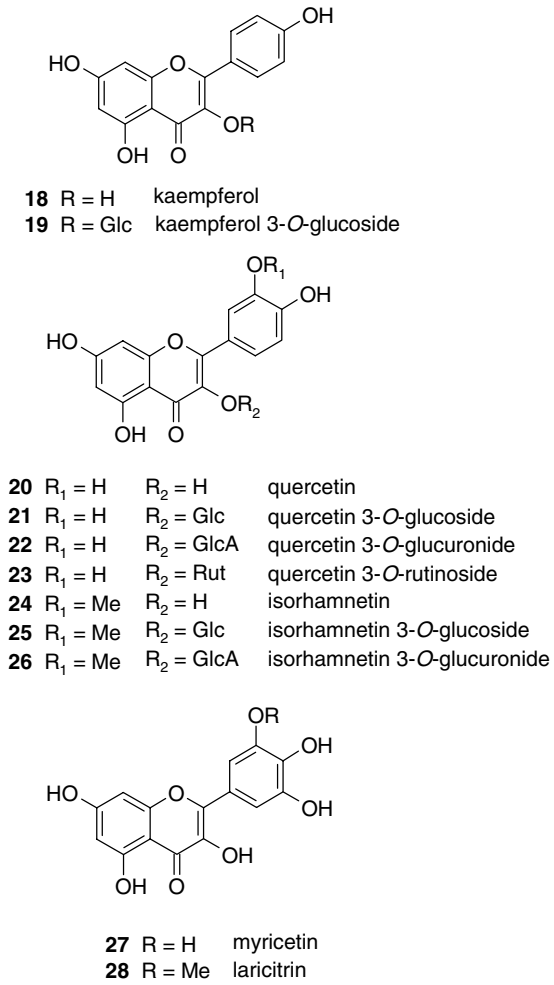

Fig. 2. Structures of flavonols identified in grape pomace extracts (Glc = glucoside; GlcA = glucuronide; Rut = rutinoside).

strates, including water-soluble compounds or vegetable juices or beverages (Ruiz-Larrea et al., 2000; Djuric & Powell, 2001).

3.2. Anthocyanins and derivatives

The main anthocyanins (Table 1) were 3-*O*-glucosides of the five common anthocyanidins: cyanidin, peonidin, delphinidin, petunidin, malvidin (**1**, **2**, **4**, **6**, **8**), and malvidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**11**). The acylated 3-*O*-glucosides **3**, **5**, **7**, **9** and **10** were identified only in some of the samples. We also identifed the pyranoanthocyanins **12**–**16**: these minor constituents, normally encountered in aged wines, (Mateus & De Freitas, 2001) were previously identified by us in a 'Nerello Mascalese' grape pomace sample (Amico et al., 2004). Malvidin 3-*O*-glucoside-catechin dimer (**17**) was also identified in CS sample. Qualitative differences were observed for the different cultivars analysed (see for instance **5**, **7**, and **9**, not detected in at least three samples) but quantitative differences were of particular significance. The TA (see Table 3), ranged from 28.70 mg/g of NA to 3.75 mg/g of FR. The main anthocyanin was malvidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**11**) in both samples with the highest TA, namely NA (19.32 mg/g, 67% TA) and NC (27.14 mg/g, 60% TA) samples, but a concentration approximately two orders of magnitude lower (0.29 mg/g, 3% TA) was determined in NM. Malvidin 3-*O*-glucoside (**8**), reported to be the main anthocyanin in a number of wine grapes (Mazza, 1995),was the main anthocyanin only in FR (57% TA) and CS (59% TA), being less than 20% TA in the NA sample. Cyanidin 3-*O*-glucoside (**1**) is among the minor anthocyanins, with the exception of NM (7% TA).

3.3. Flavonols and flavonol glycosides

Flavonols and their glucosides or glucuronides (**18**–**28**) are listed in Table 2 (Fig. 2). The total amount of flavonols (TF, see Table 3), ranging from 4.64 mg/g (NA) to 1.04 mg/g (CS) was significantly lower than TA within each sample, with the exception of FR, showing comparable amounts of both anthocyanins (3.75 mg/g) and flavonols (3.16 mg/g). Qualitative and quantitative differences were

Table 3
Comparative data on quantified polyphenols in grape pomace extracts of Sicilian cultivars[a]

	NA	NM	NC	FR	CS
TA[b]	28.70	9.10	45.27	3.75	9.61
TF[c]	4.64	3.26	4.06	3.16	1.04
TP[d]	33.33	12.36	49.33	6.91	10.65
%TA[e]	86.1	73.6	91.8	54.3	90.2
%TF[f]	13.9	26.4	8.2	45.7	9.8

[a] NA = Nero d' Avola; NM = Nerello Mascalese; NC = Nerello Cappuccio; FR = Frappato ; CS = Cabernet Sauvignon.
[b] TA = Total anthocyanins reported from Table 1 (mg/g MeOH extract).
[c] TF = Total flavonols reported from Table 2 (mg/g MeOH extract).
[d] TP = Total polyphenols = TA + TF.
[e] %TA = Percentage of TA with respect to TP.
[f] %TF = Percentage of TF with respect to TP.

observed, higher than those above discussed for anthocyanins. For instance, quercetin 3-*O*-glucuronide (**22**) is the main flavonol glycoside in FR (1.34 mg/g, 42% TF), but was not detectable in NC and CS samples, where quercetin 3-*O*-glucoside (**21**) was also present only in trace amounts. Quercetin (**20**) was the main flavonol in all samples except FR, reaching the higher relative percentage in NC (2.77 mg/g, 68% TF). Some flavonols were detected only in one extract, such as quercetin 3-*O*-rutinoside (**23**) and isorhamnetin 3-*O*-glucoside (**25**) in NA, kaempferol 3-*O*-glucoside (**19**) in NC, laricitrin (**28**) in CS.

3.4. Free radical-scavenging activity

The results of both DPPH· and TEAC for the five MeOH crude extracts assays are listed in Table 4. The SC_{50} values obtained for the five samples of grape pomace submitted to the DPPH· assay are approximately in the range 14–39 μg/ml; TEAC values are approximately in the range 1.5–2.2, higher than trolox and comparable to those measured for pure reference flavonoids (Baderschneider & Winterhalter, 2001). All the examined extracts show significant antioxidant activity and in particular NM has the highest activity in both tests. In two cases, namely CS and FR, the DPPH· and TEAC assays show different levels of activity, whereas, for the other samples, there is a good correlation between the two assay methods.

3.5. Polyphenol constituents and antioxidant activity

Tables 1–4 clearly show that there is no simple quantitative correlation of the antioxidant activity with the total amount of quantified polyphenols: for instance, the TP of NM (12.36 mg/g), the most active sample in both tests, is less than one half that of the NA sample (33.33 mg/g), showing the lowest activity in both tests. Although TA are generally much more abundant than TF (reaching 92% ca. in NC), even this parameter shows large variations, and in FR, a sample with a particularly low yield in TP, TA (ca. 54%) are comparable with TF. Even with a more detailed analysis, the above-discussed large variations in the levels of the single anthocyanins and flavanols make it difficult to find a clear correlation with the antioxidant activity. Nevertheless, the most active (NM) and the less active (NA) samples show marked differences in the relative percentages of some minor anthocyanins, namely petunidin 3-*O*-glucoside (**6**) (15.7% and 1.5%, respectively), delphinidin 3-*O*-glucoside (**4**) (13% and 0.7% respectively) and cyanidin 3-*O*-glucoside (**1**) (7% and traces, respectively). All these anthocyanins include a free (not methylated) catechol moiety in their structure and could contribute to the overall antioxidant activity to a much larger extent than the main anthocyanins malvidin 3-*O*-glucoside (**8**) and malvidin 3-*O*-(6″-*O*-*p*-coumaryl)-glucoside (**11**), bearing only one free OH group. For instance, the radical-scavenging ability of flavonoids with hydroxyl groups in the 3′, 4′ and 5′ position in the B ring has been estimated, in different model systems, as higher than that of corresponding flavonoids bearing only one hydroxy group on the same ring (Kähkönen & Heinonen, 2003; Foti, Piattelli, Baratta, & Ruberto, 1996; Goupy, Dufour, Loonis, & Dangles, 2003).

In conclusion, all the data presented and discussed above clearly show that there is a large variability in the quantitative distribution of the main extractable anthocyanins and

Table 4
Antioxidant activity of grape pomace extracts of Sicilian cultivars[a]

	NA	NM	NC	FR	cs
DPPH[b]	38.93 (0.24)	14.45 (0.13)	23.99 (1.80)	15.90 (0.89)	34.20 (1.48)
TEAC[c]	1.58 (0.01)	2.24 (0.05)	1.66 (0.03)	1.59 (0.02)	2.21 (0.04)

[a] NA = Nero d'Avola; NM = Nerello Mascalese; NC = Nerello Cappuccio; FR = Frappato ; CS = Cabernet Sauvignon.
[b] Expressed as SC_{50}, that is the concentration (μg/ml) of extract required to quench 50% of the initial DPPH. Standard deviation ($n = 3$) in parentheses.
[c] Expressed as TEAC trolox equivalent antioxidant (capacity). Standard deviation ($n = 3$) in parentheses.

flavonols from grape pomace, at least in the five Sicilian cultivars examined in this study. Of course, this may be related, not only to the cultivar, but also to the production site, the wine-making methods, and other factors. Thus, a preliminary chemical analysis has to be carried out before planning any possible exploitation of these agro-industrial wastes for isolation of single constituents or enriched mixtures.

This study demonstrates how the defatted MeOH extracts, obtainable from Sicilian grape pomace, are normally highly active as radical scavengers and are worthy of further study for their possible use as food additives or chemopreventive agents. At the same time, however, statistically insignificant correlations have been revealed between the anthocyanin and flavonol (TA + TF) contents and the antioxidant efficiency as measured by DPPH$^{\cdot}$ and ABTS$^{\cdot-}$ models, $r^2 = 0.0607$, $P > 0.05$ and $r^2 = 0.3471$, $P > 0.05$, respectively. This is also confirmed by recent studies which, using the same model systems, show a low and/or statistically insignificant correlation between radical-scavenger activity and anthocyanin/flavonol contents of similar samples, suggesting that the presence of further phenolic components or synergism may be involved in the antioxidative action (Alonso, Guilltén, Barroso, Puertas, & García, 2002; Kallithraka, Mohdaly, Makris, & Kefalas, 2005). Finally, it is worth noting here that, among the five cultivars examined, the 'Nerello Mascalese' (NM), a typical Sicilian vine, appears particularly promising for the possible re-use of residual pomace: in fact, it affords the highest yield in defatted MeOH extract (57.6 g/kg of dried pomace) and has the highest activity in scavenging both DPPH$^{\cdot}$ and ABTS$^{\cdot-}$ radicals. Our previous study on NM pomace, coming from grapes collected in an entirely different area, showed a similar quantitative distribution of the main anthocyanins, in particular the higher relative percentage of the 3-*O*-glucosides of the anthocyanidins bearing a free catechol: cyanidin, petunidin, delphinidin (Amico et al., 2004).

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References

Alonso, A. M., Guilltén, D. A., Barroso, C. G., Puertas, B., & García, A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry, 50*, 5832–5836.

Amico, V., Napoli, E. M., Renda, A., Ruberto, G., Spatafora, C., & Tringali, C. (2004). Constituents of grape pomace from the Sicilian cultivar 'Nerello Mascalese'. *Food Chemistry, 88*(4), 599–607.

Aruoma, O. I. (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutation Research, 523–524*, 9–20.

Baderschneider, B., & Winterhalter, P. (2001). Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 2788–2798.

Bozidar, S. (1995). The role of polyphenols as chemopreventers. *Polyphénols Actualités, 13*, 24–25.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*(1), 25–30.

Djuric, Z., & Powell, L. C. (2001). Antioxidant capacity of lycopene-containing foods. *International Journal of Food Science and Nutrition, 52*(2), 143–149.

Esposito, E., Rotilio, D., Di Matteo, V., Di Giulio, C., Cacchio, M., & Algeri, S. (2002). A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiology of Aging, 23*(5), 719–735.

FAO STAT Database in www.fao.org.

Fernandez-Pachon, M. S., Villano, D., Garcia-Parrilla, M. C., & Troncoso, A. M. (2004). Antioxidant activity of wines and relation with their polyphenolic composition. *Analitica Chimica Acta, 513*(1), 113–118.

Foti, M., Piattelli, M., Baratta, M. T., & Ruberto, G. (1996). Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system. Structure-activity relationship. *Journal of Agricultural and Food Chemistry, 44*, 497–501.

Frankel, E. N (1999). Food antioxidants and phytochemicals. Present and future perspectives. *Fett-Lipid, 101*(12), 450–455.

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separeted from italian red wine. *Journal of Agricultural and Food Chemistry, 46*, 361–367.

Goupy, P., Dufour, C., Loonis, M., & Dangles, O. (2003). Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *Journal of Agricultural and Food Chemistry, 51*, 615–622.

Halpern, M. J., Dahlgren, A.-L., Laakso, I., Seppanen-Laakso, T., Dahlgren, J., & McAnulty, P. A. (1998). Red-wine polyphenols and inhibition of platelet aggregation: Possible mechanisms, and potential use in health promotion and disease prevention. *Journal of International Medical Research, 26*(4), 171–180.

Kähkönen, M. P., & Heinonen, M. (2003). Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry, 51*, 628–633.

Kallithraka, S., Mohdaly, A. A.-A., Makris, D. P., & Kefalas, P. (2005). Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. *Journal of Food Composition and Analysis, 18*, 375–386.

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC–DAD–MS/MS. *Journal of Agricultural and Food Chemistry, 52*, 4360–4367.

Katsube, N., Iwashita, K., Tsushida, T., Yamaki, K., & Kobori, M. (2003). Induction of apoptosis in cancer cells by bilberry *(Vaccinium myrtillus)* and the anthocyanins. *Journal of Agricultural and Food Chemistry, 51*, 68–75.

Kong, J.-M., Chia, L.-S., Goh, N.-K., Chia, T.-F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry, 64*(5), 923–933.

Laufenberg, G., Kunz, B., & Nystroem, M. (2003). Transformation of vegetable waste into value added products: (A) the upgrading concept, (B) practical implementations. *Bioresource Technology, 87*(2), 167–198.

Lurton, L. (2003). Grape polyphenols: a new powerful health ingredients. *Innovations in Food Technology, 18*, 28–30.

Mateus, N., & De Freitas, V. (2001). Evolution and stability of anthocyanin derived during port wine aging. *Journal of Agricultural and Food Chemistry, 49*, 5217–5222.

Mavi, A., Terzi, Z., Ozgen, U., Yildirim, A., & Coskun, M. (2004). Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* (Urticaceae). *Biological and Pharmaceutical Bulletin, 27*(5), 702–705.

Mazza, G. (1995). Anthocyanins in grapes and grape products. *Critical Reviews in Food Science and Nutrition, 35*(4), 341–371.

Northup, R. R., Dahlgren, R. A., & McColl, J. G. (1998). Polyphenols as regulators of plant-litter-soil interactions in northern California's pygmy forest: a positive feedback? *Biogeochemistry, 42*(1-2), 189–220.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9/10), 1231–1237.

Renaud, S., & De Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart. *Lancet, 339*(8808), 1523–1526.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4), 152–159.

Ruiz-Larrea, M. B., Martin, C., Martinez, R., Navarro, R., Lacort, M., & Miller, N. J. (2000). Antioxidant activities of estrogens against aqueous and lipophilic radicals, differences between phenol and catechol estrogens. *Chemistry and Physics of Lipids, 105*(2), 179–188.

Schieber, A., Stintzing, F. C., & Carle, R. (2002). By-products of plant food processing as a source of functional compounds – recent developments. *Trends in Food Science and Technology, 12*(11), 401–413.

Schlesier, K., Harwat, M., Bohm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research, 36*(2), 177–187.

Simic, M. G., & Jovanovic, S. V. (1994). Inactivation of oxygen radicals by dietary phenolic compounds in anticarcinogenesis. In C.-T. Ho, T. Osawa, M.-T. Huang, & R. T. Rosen (Eds.), *Food phytochemicals for cancer prevention II – teas, spices, and herbs. ACS Symposium Series* (vol. 547, pp. 20–32). Washington DC: American Chemical Society.

Thomas, J. H., Drake, J. M., Paddock, J. R., Conklin, S., Johnson, J., Seliskar, C. J., et al. (2004). Characterization of ABTS at a polymer-modified electrode. *Electroanalysis, 16*(7), 547–555.

Torres, J. L., Varela, B., Garcia, M. T., Carilla, J., Matito, C., Centelles, J. J, et al. (2002). Valorization of grape *(Vitis vinifera)* byproducts. Antioxidant and biological properties of polyphenolic fractions differing in procyanidin composition and flavonol content. *Journal of Agricultural and Food Chemistry, 50*, 7548–7555.

Van den Berg, R., Haenen, G. R. M. M., Van den Berg, H., & Bast, A. (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixture. *Food Chemistry, 66*(4), 511–517.

Wang, H., Cao, G., & Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry, 45*, 304–309.