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Constituents of grape pomace from the Sicilian cultivar 'Nerello Mascalese'

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Abstract

A chemical study of grape (*Vitis vinifera*) pomace, from the red Sicilian cultivar 'Nerello Mascalese', has been carried out. The methanolic extract was further fractionated and analysed by HPLC–UV–DAD and HPLC–MS–ESI, allowing identification of 16 flavonols or flavonol glucosides (**1**–**16**), the flavan-3-ols catechin (**17**) and epicatechin (**18**), as well as their gallate esters **19** and **20**, 10 anthocyanins (**21**–**30**), 5 pyranoanthocyanins (**31**–**35**), and 3 low-molecular weight proanthocyanins (**36**–**38**). The pyranoanthocyanins cyanidin 3-*O*-glucoside acetaldehyde (**31**), peonidin 3-*O*-glucoside acetaldehyde (**32**), petunidin 3-*O*-glucoside acetaldehyde (**33**) and malvidin 3-*O*-glucoside acetaldehyde (**34**) have been identified for the first time in grape pomace. From quantitative analysis of the main constituents, quercetin 3-*O*-glucoside (**5**) and quercetin 3-*O*-glucuronide (**9**) proved to be the most abundant flavonol glycosides, and malvidin 3-*O*-glucoside (**27**) the main anthocyanin. Flash-chromatography of the EtOAc extract allowed isolation and spectral identification of lupeol (**39**), oleanoic acid (**40**), quercetin (**3**) and β-sitosterol glucoside (**41**).

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

1. Introduction

Grape, one of the world's largest fruit crops, with more than 60 million metric tons is cultivated mainly as *Vitis vinifera* for wine production (FAO STAT Database). Wine-making affords grape pomace as a by-product in an estimated amount of 13% by weight of the grapes (Torres et al., 2002). This waste, consisting of skins, seed and stems, should be treated as a special solid residue, due to its high levels of residual phenolic compounds which may have an adverse enviromental impact, mainly because of the inhibition of germination properties of polyphenols (Morthup, Dahlgren, & McColl, 1998). Thus, wine-making industries will have increasing costs for waste treatment. On the other hand, it is known that grape pomace is a rich source of polyphenols possessing beneficial effects on human health (Lu & Foo, 1999; Torres et al., 2002). The available studies on grape pomace are relatively scarce in comparison with those carried out on wine components. Indeed, a variety of epidemiological, clinical and in vitro studies have been carried out in support of the positive role of wine polyphenols in preventing CHD (*Cardiovascular Health Diseases*), starting from the so-called 'French paradox', namely the negative correlation observed between moderate red wine consumption and CHD incidence (Burns et al., 2000; Frankel, Kanner, German, & Kanner, 1993; Renaud & de Lorgeril, 1992). Further studies have shown the chemopreventive properties of phenolic components of wine, and in particular of flavonoids and stilbenoids, in countering cancer and other degenerative diseases associated with oxidative or inflammatory processes (Hertog et al., 1995; Jang et al., 1997; Soleas, Grass, Josephy, Diamandis, & Goldberg, 2002; Waffo-Teguo et al., 2001). The investigations of chemical constituents of grape pomace have shown the presence of polyphenols, identified also in red wine,

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mainly anthocyanins, flavonols, flavonol glycosides, and phenolic acids (Renaud & de Lorgeril, 1992; Torres et al., 2002). Also, less common compounds have been identified, which may have originated from seeds and stems or formed in biotransformations during wine-making (Foo, Lu, & Wong, 1998; Souquet, Labarbe, Le Guernevéc, Cheynier, & Moutounet, 2000; Torres et al., 2002). These data suggest that grape pomace, if conveniently processed, could furnish useful products that may balance out waste treatment costs. However, a significant variability in quantitative and qualitative distribution of chemical constituents in grape pomace has to be expected depending on an array of important factors, such as the varietal differences of *V. vinifera*, the location of cultures and the wine-making procedures (destemming, crushing, maceration and pressing). For instance, it is well-known that anthocyanin distributions show large variations among different grape cultivars (Mazza, 1995). More generally, quantitative and qualitative varietal differences have been reported among polyphenol profiles of table grape cultivars (Cantos, Espin, & Tomas-Barberan, 2002).

On this basis, we have carried out a chemical study of the constituents of grape pomace from a *V. vinifera* cultivar largely employed in Sicily for red wine production, namely 'Nerello Mascalese' (NM), with the aim of exploiting the potential added-value of this by-product. Italy is one of the main wine producing countries in the world, and wine-making in the Sicily region shows an increasing trend. Thus, considerable amounts of grape pomace are obtained as a by-product in this area. Nevertheless, no chemical study on Sicilian grape pomace has been carried out to date.

As a first step, we have focussed our study on the main anthocyanins, flavonols and flavonol glycosides, identified and, when possible, quantified through LC–UV–DAD and LC–MS–ESI analysis of a MeOH/HCl extract, as detailed below. Common flavan-3-ols and some low-molecular weight proanthocyanidins were also detected. For the sake of completeness, we also examined the main lipophilic constituents, isolated from an EtOAc extract and identified through spectral analysis.

2. Materials and methods

2.1. General methods

Column chromatography was performed by flash chromatography on Lichroprep Si 60 or silica gel DIOL 40–63 μm (Merck). Thin-layer chromatography (TLC) was carried out on Merck 60 F_{254} plates, using cerium sulphate and phosphomolybdic acid as chromogenic reagents. The adsorption of MeOH extracts was performed on sea sand, 40–200 mesh (Fluka). Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded using a Varian Unity Inova spectrometer (respectively at 500 and 125 MHz) and performed at constant temperature (27 °C) in deuterated chloroform ($CDCl_3$). Electron Impact (EI–MS) and Fast Atom Bombardment (FAB–MS) mass spectra were recorded on a Kratos MS 50 instrument, using 3-nitrobenzylalcohol (NBA) as matrix. Liquid chromatography–mass spectrometry (LC–MS) was carried out on Waters Micromass ZQ2000 equipped with 1525 pump module and 996 PDA detector.

2.2. Material and extraction

Nerello mascalese grape pomace was obtained from 'Torrevecchia' Winery, Ragusa, Italy. After pressing of the grapes and 48 h maceration, an appropriate amount of the residual pomace was freeze-dried, finely ground and stored in a freezer until used. Two separate samples were processed as follows. One sample (powder, 100 g) was extracted under continuous stirring with acidified methanol (MeOH/HCl 1 N 99:1, 3×1500 ml, 12 h). After evaporation under vacuum a dark red wine residue was obtained (15.57 g, 15.57% of grape pomace dry weight). A second sample (400 g) was defatted with *n*-hexane (3×1500 ml, 12 h; 9.11 g, 2.27% of grape pomace dry weight), and subsequently extracted with ethyl acetate (3×1500 ml, 12 h). The extract was concentrated under vacuum to afford 8.88 g (2.22% of grape pomace dry weight) of a dark green viscous residue.

2.3. Fractionation of the MeOH extract

The crude MeOH extract was adsorbed on sea sand (ratio 50:1 w/w) and defatted with *n*-hexane (750 ml). Half of the residue was directly eluted with methanol (400 ml). The eluate, after evaporation of the solvent, was used for quantitative HPLC analysis of the main flavonols and anthocyanins (MeOH extract). The remaining portion was percolated in sequence with tetrahydrofuran (500 ml) and methanol (300 ml) to obtain respectively, after evaporation of the solvents, the fractions THF and MeOH, used for qualitative HPLC analysis.

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

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) – $t_{0\ min}$ B (5%), $t_{20\ min}$ B (15%), $t_{40\ min}$ B (30%), $t_{55\ min}$ B (100%), $t_{65\ min}$ B (100%). The flow rate was set at 1 ml min^{-1}. ESI-MS was used for detection and characterization of metabolites, using negative mode with a capillary voltage of 3.5 kV, a cone voltage

of 40 V, a vapourizer 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 range. DAD analysis was carried out in the range between 200 and 700 nm, setting the detector at 280 nm for identification of flavan-3-ols and proanthocyanidins, at 350 nm for flavonols and flavonols glycosides, at 480 nm for pyranoanthocyanins, and at 530 nm for anthocyanins. UV–DAD analysis was also performed for quantification of the main components using a calibration curve; quercetin 3-*O*-glucoside and malvidin 3-*O*-glucoside were used as standards for flavonols and anthocyanins, respectively.

2.5. Fractionation of the EtOAc extract

The crude EtOAc extract was applied to a DIOL Silica column (60×4 cmID) and subjected to 'flash chromatography' under light nitrogen pressure, eluting first with an increasing gradient of ethyl acetate in petroleum ether (10–100%), and subsequently with an increasing gradient of methanol in ethyl acetate (2–100%). The eluates, analysed by TLC, were pooled in five fractions (A–E). Fraction A (3170 mg), containing fatty acids and minor amounts of low polar metabolites, was not analysed. Fraction B (1069 mg) was submitted to further flash-chromatography on a DIOL Silica column (50×2 cmID), eluted with dichloromethane:petroleum ether (9:1) in isocratic mode, to yield three subfractions: B_1, B_2 and B_3. Fraction B_1 was identified as lupeol (**39**, 34 mg, 0.0085% of pomace dry weight). The core fraction B_2 was identified as oleanolic acid (**40**, 630 mg, 0.1575% of pomace dry weight). Fraction C (129.9 mg) was applied to a DIOL Silica column (40×2 cmID) and flash-chromatographed with a gradient of increasing percentage of methanol in dichloromethane (1–50%) to give subfractions C_1–C_3. Fraction C_2 was identified as quercetin (**3**, 19.8 mg, 0.0049% of dry weight). The more complex fraction D (408.5 mg) was applied to a DIOL Silica column (40×2 cmID) and flash-chromatographed with a gradient of increasing percentage of acetone in petroleum ether (20–60%) to give subfractions D_1–D_4. The D_1 fraction was identified as β-sitosterol 3-*O*-glycoside (**42**, 99.6 mg, 0.0249% of dry weight). Fractions D_3, D_4, and E (3447 mg), containing mainly polyphenols, were not further analysed.

3. Results and discussion

3.1. General

Two separate samples of grape pomace, obtained from wine-making of the red Sicilian cultivar NM, after freeze-drying and grinding, were submitted to different extraction/fractionation protocols, respectively aimed at identification of polyphenols (extracted with acidified methanol) and of lipophilic constituents (extracted with ethyl acetate). The crude MeOH/H^+ extract was adsorbed on sand and this latter was percolated with *n*-hexane to remove fats. One half of the residue was directly eluted with methanol, affording the MeOH extract, used for quantitative analysis of the main flavonols and anthocyanins, based on UV–DAD determination. Preliminary HPLC suggested that a more effective qualitative analysis, also allowing identification of minor constituents, would be more conveniently carried out on separate fractions, selectively enriched in flavonols and flavonol glycosides or anthocyanins. Thus, the remaining portion was percolated in sequence with: (i) tetrahydrofuran, to elute mainly flavanols, flavonols and flavonol glycosides (THF fraction) and (ii) methanol, to elute anthocyanins and low-molecular weight proanthocyanidins (MeOH fraction). Subsequently, these fractions were submitted to HPLC–UV–MS analysis. Each compound was identified on the basis of HPLC retention time (RT), UV spectrum (obtained from DAD), and MS spectrum (obtained from ESI detector). Stereochemistry of the reported structures (**1**–**41**) 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 (Scheme 1).

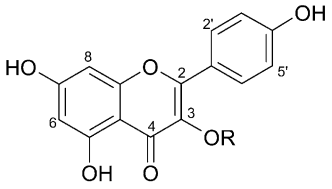
The crude EtOAc extract was submitted to preparative flash-chromatography on DIOL silica gel, and afforded fractions A–E, which were submitted to further DIOL flash-chromatography, as detailed below.

3.2. Constituents of the THF fraction

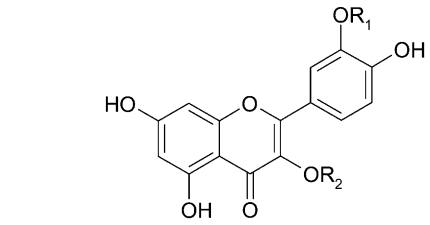
The THF fraction was submitted to HPLC analysis, splitting the eluate between the UV–DAD and MS–ESI detectors (see Section 2). The combined RT/UV/MS data allowed identification of 16 flavonols and flavonol glycosides, namely: kaempferol (**1**), kaempferol 3-*O*-glucoside (**2**), quercetin (**3**), isorhamnetin (**4**), quercetin 3-*O*-glucoside (**5**), quercetin 3-*O*-(6″-*O*-acetyl)-glucoside (**6**), quercetin 3-*O*-rhamnoside (**7**), quercetin 3-*O*-rutinoside (**8**), quercetin 3-*O*-glucuronide (**9**), isorhamnetin 3-*O*-glucoside (**10**), laricitrin (**11**), syringetin (**12**), laricitrin 3-*O*-glucoside (**13**), myricetin 3-*O*-glucoside (**14**), myricetin 3-*O*-glucuronide (**15**) and astilbin (**16**). In addition, the flavan-3-ols catechin (**17**) and epicatechin (**18**), as well as their 3-gallate esters (**19** and **20**) were identified as constituents of this fraction. All RT/UV/MS data were in perfect agreement with those previously reported for the above cited flavonoids.

3.3. Constituents of the MeOH fraction

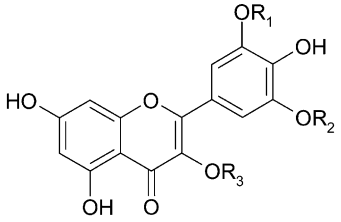
The MeOH fraction, as expected, was deep red-wine coloured due to the presence of anthocyanins. These



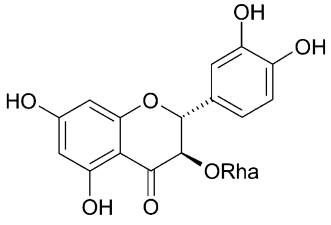
1 R = H, kaempferol
2 R = Glc, kaempferol 3-*O*-glucoside



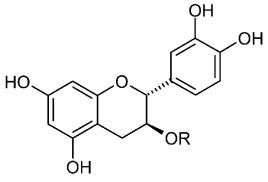
3 R_1 = H, R_2 = H, quercetin
4 R_1 = Me, R_2 = H, isorhamnetin
5 R_1 = H, R_2 = Glc, quercetin 3-*O*-glucoside
6 R_1 = H, R_2 = GluAc, quercetin 3-*O*-(6"-*O*-acetyl)-glucoside
7 R_1 = H, R_2 = Rha, quercetin 3-*O*-rhamnoside
8 R_1 = H, R_2 = Rut, quercetin 3-*O*-rutinoside
9 R_1 = H, R_2 = GlcA, quercetin 3-*O*-glucuronide
10 R_1 = Me, R_2 = Glc, isorhamnetin 3-*O*-glucoside



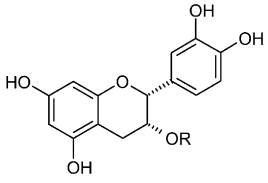
11 R_1 = Me, R_2 = H, R_3 = H, laricitrin
12 R_1 = Me, R_2 = Me, R_3 = H, syringetin
13 R_1 = Me, R_2 = H, R_3 = Glc, laricitrin 3-*O*-glucoside
14 R_1 = H, R_2 = H, R_3 = Glc, myricetin 3-*O*-glucoside
15 R_1 = H, R_2 = H, R_3 = GlcA, myricetin 3-*O*-glucuronide



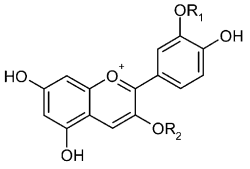
16 astilbin



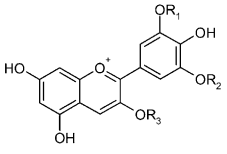
17 R = H, catechin
19 R = Gal, catechin 3-*O*-gallate



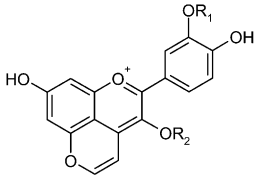
18 R = H, epicatechin
20 R = Gal, epicatechin 3-*O*-gallate



21 R_1 = H, R_2 = Glc, cyanidin 3-*O*-glucoside
22 R_1 = H, R_2 = GlcAc, cyanidin 3-*O*-(6"-*O*-acetyl)-glucoside
23 R_1 = Me, R_2 = Glc, peonidin 3-*O*-glucoside
24 R_1 = Me, R_2 = GlcCoum, peonidin 3-*O*-(6"-*p*-*O*-coumaroyl)-glucoside



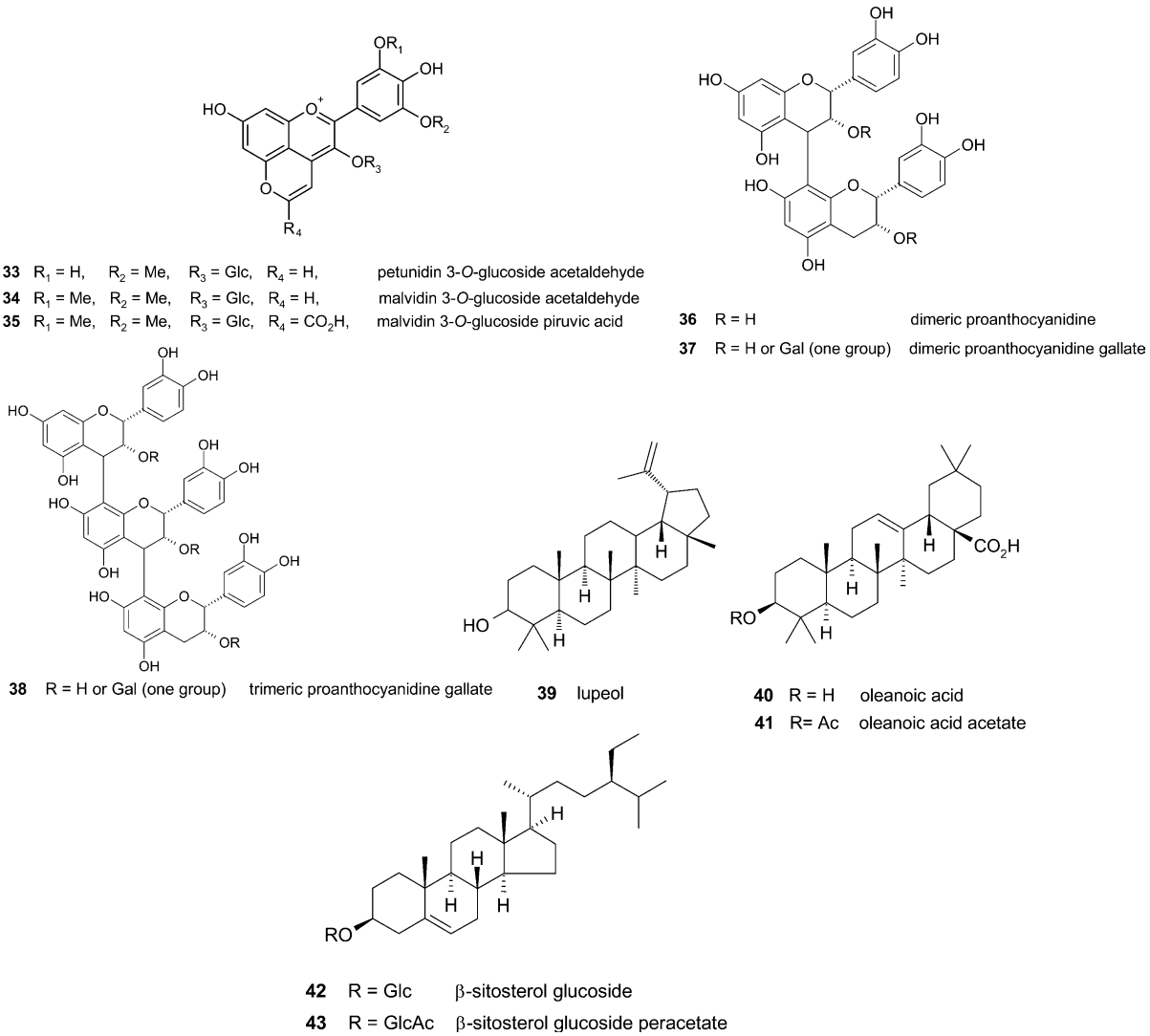
25 R_1 = H, R_2 = H, R_3 = Glc, delphinidin 3-*O*-glucoside
26 R_1 = H, R_2 = Me, R_3 = Glc, petunidin 3-O-glucoside
27 R_1 = Me, R_2 = Me, R_3 = Glc, malvidin 3-*O*-glucoside
28 R_1 = Me, R_2 = Me, R_3 = GlcAc, malvidin 3-*O*-(6"-*O*-acetyl)-glucoside
29 R_1 = Me, R_2 =Me, R_3 = GlcCoum, malvidin 3-*O*-(6"-*p*-*O*-coumaroyl)-glucoside
30 R_1 = Me, R_2 = Me, R_3 = GlcCaff, malvidin 3-*O*-(6"-*p*-*O*-caffeoyl)-glucoside



31 R_1 = H, R_2 = Glc, cyanidin 3-*O*-glucoside acetaldehyde
32 R_1 = Me, R_2 = Glc, peonidin 3-*O*-glucoside acetaldehyde

Scheme 1.

could be identified through HPLC–UV–MS data as ten common anthocyanins of *V. vinifera* (Mazza, 1995), namely cyanidin 3-*O*-glucoside (**21**), cyanidin 3-*O*-(6″-*O*-acetyl)-glucoside (**22**), peonidin 3-*O*-glucoside (**23**), peonidin 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside (**24**), delphinidin 3-*O*-glucoside (**25**), petunidin 3-*O*-glucoside (**26**), malvidin 3-*O*-glucoside (**27**), malvidin 3-*O*-(6″-*O*-acetyl)-glucoside (**28**), malvidin 3-*O*-(6″-*O*-*p*-coumaroyl)-glucoside (**29**) and malvidin 3-*O*-(6″-*O*-caffeoyl)-glucoside (**30**). Also for these anthocyanins, RT/UV/MS data were in agreement with previous reports. In the frame of this work we were also able to identify five pyranoanthocyanins, namely cyanidin 3-*O*-glucoside acetaldehyde (**31**), peonidin 3-*O*-glucoside acetaldehyde (**32**), petunidin 3-*O*-glucoside acetaldehyde (**33**), malvidin 3-*O*-glucoside acetaldehyde (**34**) and malvidin 3-*O*-



Scheme 1. (*continued*)

glucoside pyruvic acid (**35**). These modified anthocyanins have been previously identified mainly in aged wines (Lu & Foo, 2001) and, according to the work of Mateus and De Freitas (2001) and Mateus, Silva, Santoz-Buelga, Rivas-Gonzalo, and De Freitas (2002), are the products of condensation between anthocyanins and acetic aldehyde or pyruvic acid, generating a further oxygenated cycle joining C-4 with the OH group in C-5. Also, identification of these compounds has been based on UV and MS data. The UV spectrum of these compounds shows a typical hypsochromic shift of ca. 15 nm of the visible absorbance maximum with respect to the corresponding anthocyanin. The MS–ESI spectrum allowed identification of all $[M–H]^-$ ions as well as of the characteristic aglyconic ion due to the loss of a glucoside moiety.

Condensed tannins (proanthocyanidins) were not examined in detail in this step of the work, mainly due to the complexity of the mixture and the difficulty of analysing it without chemical breakdown. Nevertheless, we obtained MS evidence at least for the presence, in the MeOH fraction, of low-molecular weight components (Gabetta et al., 2000), namely dimeric proanthocyanidin (**36**, $[M–H]^- = 577$ *m/z*) and dimeric proanthocyanidin gallate (**37**, $[M–H]^- = 729$ *m/z*), trimeric proanthocyanidin gallate (**38**, $[M–H]^- = 1017$ *m/z*), thus confirming that ESI-MS is a valuable tool for analyzing this class of substances. Nevertheless, stereochemical details, as well as the exact location of gallate group in **37** and **38**, could not be determined.

3.4. Quantitative determination of the main flavonoids (MeOH extract)

The quantitative determination of the main flavonoids (flavonols and anthocyanins) was carried out on

defatted MeOH extract. Fig. 1 depicts an HPLC–UV chromatographic profile of this extract, detected at 350 nm and showing the complex mixture of flavonols and flavonol glycosides. In Table 1, the quantitative data for compounds **1**, **2**, **3, 5**, **9**, **10**, **14** are reported as mg/kg of dried pomace and referred to quercetin 3-*O*-glucoside. Quercetin 3-*O*-glucoside (179 mg/kg) and quercetin 3-*O*-glucuronide (130 mg/kg) are the main flavonoids. Fig. 2 depicts an HPLC–UV chromatogram of MeOH extract, detected at 530 nm and showing the anthocyanin profile. All the identified anthocyanins **21**–**30**, could be quantified, with reference to malvidin 3-*O*-glucoside, as listed in Table 2 (mg/kg dried pomace). Malvidin 3-*O*-glucoside (64.6 mg/kg) is clearly the main anthocyanin. Peonidin 3-*O*-glucoside (18.7 mg/kg) and petunidin 3-*O*-glucoside (10.7 mg/kg) are significantly more abundant than the other anthocyanins, and this may be a peculiarity of NM grape pomace.

Table 1
Quantitative data on the main flavonols and flavonol glycosides

Compound	mg/kg[a]
Kaempferol (**1**)	2.37
Kaempferol 3-*O*-glucoside (**2**)	13.50
Quercetin (**3**)	15.30
Quercetin 3-*O*-glucoside (**5**)	179.00
Quercetin 3-*O*-glucuronide (**9**)	130.00
Isorhamnetin 3-*O*-glucoside (**10**)	63.80
Laricitrin 3-*O*-glucoside (**13**)	3.84
Myricetin 3-*O*-glucoside (**14**)	21.30

[a] Based on spectrophotometrical determination (UV–DAD) and referred to quercetin 3-*O*-glucoside. Values are in mg/kg of dried pomace.

3.5. Constituents of the EtOAc extract

The EtOAc crude extract was fractionated on DIOL silica gel, affording fractions A–E. Fraction A gave, as main constituent, compound **39**, submitted to spectral analysis (EIMS, NMR) and identified as the triterpene lupeol. Fraction B afforded compound **40** which, upon preliminary NMR analysis, suggested a triterpenoid structure. The molecular weight of 456 was determined by EIMS. The compound was poorly soluble in $CDCl_3$ and by treatment with acetic anhydride, gave a monoacetate **41** (EIMS: *m/z* 498), largely soluble in $CDCl_3$. Thus, a complete ^1H and ^{13}C NMR study was carried out on **41** in $CDCl_3$. Careful analysis of these data, aided by a bibliography search, allowed us to establish **41** as the oleanoic acid acetate and consequently **40** was identified as oleanoic acid. Fraction C afforded compound **3**, whose preliminary ^1H NMR clearly suggested a flavonoid constituent. Following MS–FAB, high-field ^1H NMR analysis and literature search, **3** was easily established as the widespread flavonoid quercetin. From fraction D three subfractions (D_1–D_3) were obtained. Subfraction D_2 afforded compound **42**, whose MS–FAB spectrum showed an $[M–H]^-$ at *m/z* 557. Acetylation of

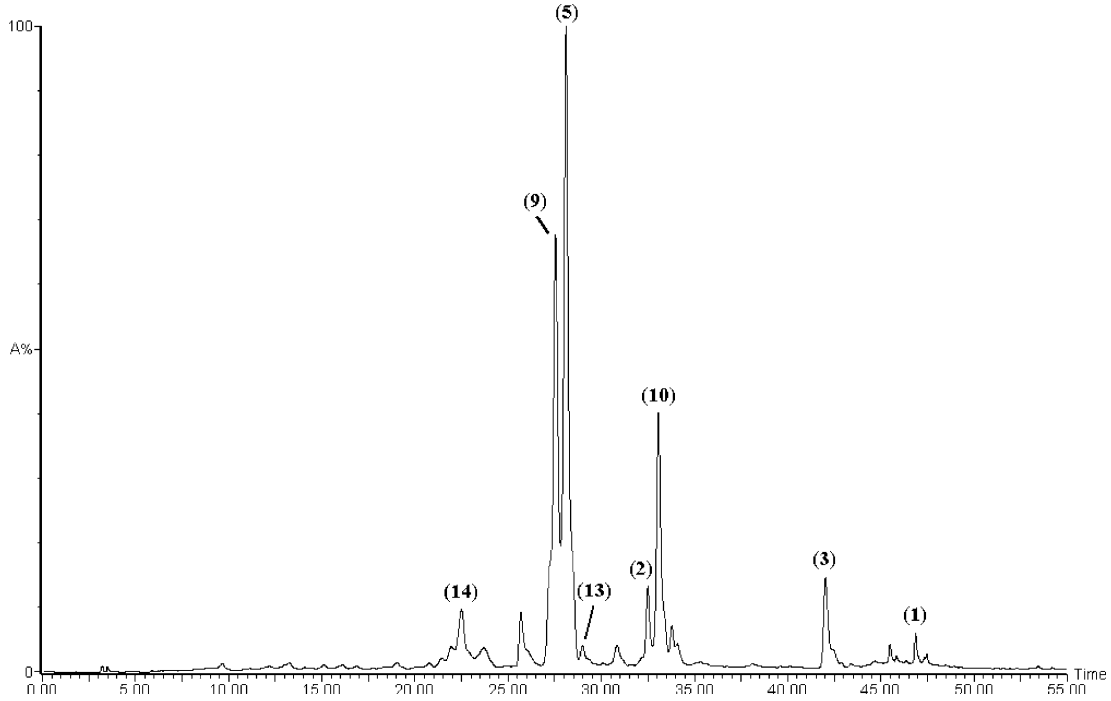


Fig. 1. HPLC–UV chromatogram of MeOH extract, detected at 350 nm.

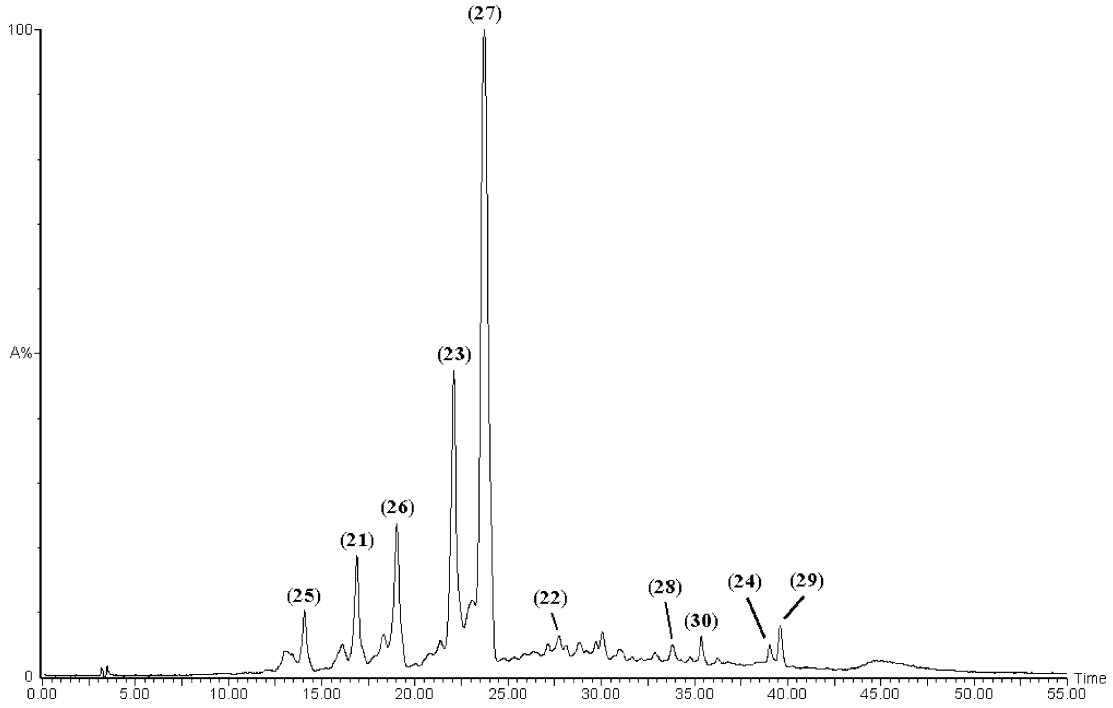


Fig. 2. HPLC–UV chromatogram of MeOH extract, detected at 530 nm.

Table 2
Quantitative data on the anthocyanins

Compound	mg/kg[a]
Cyanidin 3-*O*-glucoside (**21**)	6.99
Cyanidin 3-*O*-(6′-*O*-acetyl)-glucoside (**22**)	1.77
Peonidin 3-*O*-glucoside (**23**)	18.70
Peonidin 3-*O*-(6′-*O*-*p*-coumaroyl)-glucoside (**24**)	1.18
Delphinidin 3-*O*-glucoside (**25**)	3.73
Petunidin 3-*O*-glucoside (**26**)	10.70
Malvidin 3-*O*-glucoside (**27**)	64.6
Malvidin 3-*O*-(6′-*O*-acetyl)-glucoside (**28**)	0.96
Malvidin 3-*O*-(6′-*O*-*p*-coumaroyl)-glucoside (**29**)	2.52
Malvidin 3-*O*-(6′-*O*-caffeoyl)-glucoside (**30**)	1.80

[a] Based on spectrophotometrical determination (UV–DAD) and referred to malvidin 3-*O*-glucoside. Values are in mg/kg of dried pomace.

42 gave a peracetate **43**, whose MS–FAB suggested a m.w. of 744, accounting for four introduced acetyl groups. ^{1}H and ^{13}C NMR spectral analysis of compound **42** was carried out mainly on the more soluble acetate, **43**. Both spectra showed signals attributable to a terpenoid moiety and a glucopyranosyl moiety. Also, in this case, careful literature search allowed identification of **43** as β-sitosterol glucoside tetraacetate, and consequently **42** as β-sitosterol glucoside. A TLC analysis of fraction E showed a complex profile, which appeared very similar to that of the MeOH/H^+ extract, in particular when polyphenol-specific chromogenic reagents were used. Thus, this fraction was not further submitted to preparative chromatography.

4. Conclusions

This work is the first chemical study on grape pomace from a Sicilian cultivar (NM). We identified 10 anthocyanins, 5 pyranoanthocyanins, 16 flavonols or flavonol glycosides, the widespread flavan-3-ols catechin and epicatechin, as well as their gallate esters, and 3 low-molecular weight proanthocyanins. Quantitative determination was successfully carried out for a number of flavonoids and all the identified anthocyanins as listed in Tables 1 and 2, respectively. Quercetin 3-*O*-glucoside (**5**) and quercetin 3-*O*-glucuronide (**9**) were the main flavonol glycosides, in agreement with previous data on grape pomace constituents (Bonilla, Mayen, Merida, & Medina, 1999). Known data on distribution of anthocyanins in grapes from different cultivars of *Vitis vinifera* show that malvidin 3-*O*-glucoside (**27**) is generally the main anthocyanin, whereas the other anthocyanins show large variations (Mazza, 1995). The anthocyanins in grape pomace have scarcely been investigated, so that we cannot directly compare our data with previous quantitative results. As expected, malvidin 3-*O*-glucoside is the main anthocyanin in the NM sample, reaching almost 57.0% of the total amount of anthocyanins extracted. Less obvious is the relatively abundant percentage observed for peonidin 3-*O*-glucoside (16.5%). The presence of pyranoanthocyanins is worthy of particular mention. These modified anthocyanins have been identified in aged red wines and are generally reputed to be the products of condensation of anthocyanins with acetaldehyde or pyruvic acid during fermentation.

Nevertheless, at least from one sample of grape pomace the presence of malvidin 3-*O*-glucoside pyruvic acid (**35**) has been previously reported (Fulcrand, Benabdeljalil, Riguaud, Cheynier, & Moutounet, 1998). This was also the main pyranoanthocyanin in our sample, but we were able to identify four further pyranoanthocyanins, namely cyanidin 3-*O*-glucoside acetaldehyde (**31**), peonidin 3-*O*-glucoside acetaldehyde (**32**), petunidin 3-*O*-glucoside acetaldehyde (**33**) and malvidin 3-*O*-glucoside acetaldehyde (**34**), which have been found for the first time in grape pomace.

Most of the polyphenols herein reported are well known for promising biological properties, especially for their antioxidant and radical scavenging activity (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Kaehkoenen & Heinonen, 2003). In particular, it has been recently reported that the anthocyanin fraction from an Italian red wine was the most effective phenolic fraction in scavenging reactive oxygen species (ROS) and in inhibiting lipoprotein oxidation and platelet aggregation (Burda & Oleszek, 2001).

The main lipophilic constituents of grape pomace have not been considered in previous chemical studies on grape pomace. Nevertheless, lupeol, oleanoic acid and β-sitosterol glucoside have previously been identified in grapes and their presence in the EtOAc fraction is not surprising. Also these compounds are known for important biological activities. In particular, in recent studies lupeol is reported as anti-inflammatory (Fernandez, De Las Heras, Garcia, Saenz, & Villar, 2001), an inducer of apoptosis (Hata, Hori, & Takahashi, 2002) and inhibitor of tumor promotion (Saleem et al., 2001). Oleanoic acid, a well-known component of grape cuticular wax, has recently been reported as an anti-HIV (Mengoni et al., 2002) and gastroprotective agent (Astudillo, Rodriguez, & Schmeda-Hirschmann, 2002). β-Sitosterol glucoside is known for a variety of beneficial properties (Villasenor, Angelada, Canlas, & Echegoyen, 2002) and has recently been reported as an antimutagenic agent (Park, Jung, Rhee, & Choi, 2003). Nevertheless, it is worth noting here that this sterol has been identified as a potential neurotoxic component of cycad (*Cycas circinalis*) seeds (Wilson et al., 2002).

In conclusion, grape pomace from the Sicilian cultivar 'Nerello Mascalese' appears a promising source of useful compounds. In particular, the MeOH extract is rich in flavanols and anthocyanins, known to be chemopreventive nutraceuticals.

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