# DETERMINATION OF MAJOR ANTHOCYANIN PIGMENTS AND FLAVONOLS IN RED GRAPE SKIN OF SOME TABLE GRAPE VARIETIES (*VITIS VINIFERA* SP.) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–PHOTODIODE ARRAY DETECTION (HPLC-DAD)

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**Abstract**

**Aim**: The aim of this study was the investigation of the anthocyanin and flavonol content in grape skin extracts. Five prevalent anthocyanin-types and four flavonol-types were determined in the skin of three red table grape varieties widely cultivated in El-Tarf (Algeria).

**Methods and results**: The identification of the compounds was performed by HPLC-DAD based on C-18 reversed phase column separation. Results from HPLC analysis showed that malvidin and petunidin-3-*O*-glucoside were the major anthocyanin glucoside, whereas quercetin-3-*O*-glucoside was the major flavonol among the four identified.

**Conclusion**: The content of anthocyanins and flavonols in the grape skin of three grapevine (*Vitis vinifera*) varieties exhibits notable differences among the cultivars studied, confirming their importance in the varietal characterization. The highest concentrations of total anthocyanins and flavonols corresponded to the Gros noir variety. The results of the present study also indicate that the grape skin extracts of these Algerian cultivars can be used as easily accessible source of natural antioxidants.

**Significance and impact of the study**: To the best of our knowledge, this is the first report on the identification of different anthocyanins and flavonols in berry skin from some red grape varieties largely cultivated in this region of Algeria.

**Key words**: anthocyanins, flavonols, *Vitis vinifera*, HPLC, table grape

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## INTRODUCTION

Grape (*Vitis vinifera*) is one of the world's largest fruit crops, and its composition and properties have been extensively investigated, with several reports of the presence of large amounts of phenolic compounds. Most phenolic compounds found in grape can act as antioxidants. Grape skin is a source of natural pigments (anthocyanins and flavonols), which are known to possess broad pharmacological activities and therapeutic potentials (Bagchi *et al.*, 2004; Leifert and Abeywardena, 2008). Anthocyanins, the major polyphenols found in red grape skins, have been reported to show cardioprotective effects against ischemic reperfusion injury and to possess other diverse biological properties and therefore are considered as secondary metabolites with potential nutritional value (Kallithraka *et al.*, 2005; Das *et al.*, 2007). Anthocyanins, being located in the berry skin, are the main flavonoids responsible for the red color of grape cultivars. It is well known that the anthocyanin concentration can vary widely among different vintages of a given cultivar, due to both environmental (seasonal conditions) and agronomical factors. Therefore, the anthocyanin profile has been used as a chemotaxonomic parameter for the classification of red *Vitis vinifera* varieties (Revilla *et al.*, 2001; Mattivi *et al.*, 2006). Grape anthocyanins are monoglucosides of five anthocyanidins, namely delphinidin, cyanidin, petunidin, peonidin and malvidin. The acylated anthocyanins are esters of the glucose moiety of the free anthocyanins with acetic, *p*-coumaric or caffeic acids. Flavonols are one of the most studied classes of polyphenolic phytochemicals, because of the importance pertaining to their antioxidant potency and other biological activities (O'Byrne *et al.*, 2002). Flavonols constitute a group of flavonoids that vary in color from white to yellow and are closely related in structure to the flavones. Derivatives of the most commonly encountered aglycones, including quercetin, myricetin, kaempferol and isorhamnetin, have been found in grapes (*Vitis vinifera* sp.). The conjugates are exclusively 3-*O*-glycosides, whereas sugar attachment on other positions of the flavonol skeleton has never been reported. For isorhamnetin, only glucose derivatives have been identified, but myricetin, quercetin and kaempferol may also occur as glucuronides (Makris *et al.*, 2006).

The objective of the present study is to identify phenolic compounds in skin of red grape varieties grown in Mediterranean climate. The grapes of three varieties commonly used in Algeria (Cardinal, Gros noir and Muscat noir) were analyzed and compared for their anthocyanin and flavonol content. To the best of our knowledge, no research has examined the anthocyanin and flavonol content in these grape varieties grown in El-Tarf (Algeria).

## MATERIALS AND METHODS

### 1. Plant material

Skins from three grape cultivars, namely Cardinal, Gros noir and Muscat noir, were examined. Grape samples were grown in the region of El-Tarf located in the north-east of Algeria (36° 45' 00» N; 81° 10' 00» E) and collected at maturity. The region has a Mediterranean climate, which is divided into a hot season from June to October and a rainy season from November to April. The rest of the year the region enjoys a mild and pleasant climate.

### 2. Sample preparation

Before extraction, skins were manually separated from the whole berries and dried in oven at 50°C until constant weight. Dried grape skins were crushed in a grinder for 2 min and then used for extractions.

### 3. Extraction of anthocyanins and flavonols

The extraction procedures was done according to Cadot *et al.* (2012) and Brossaud *et al.* (1999). Briefly, dried skin powder (2 g) was extracted successively with 80 mL of methyl alcohol/water/TFA (80:20:0.05) and 50 mL of acetone/water (60:40). The extraction was repeated twice: 25°C, 15 min, 250 rpm and the extract was centrifuged (10°C/10 min/10 000 rpm). The supernatant was then filtered through a glass microfiber filter (GF/A Whatman® 1.6 µm) before drying under vacuum at 30°C using a rotary evaporator (Buchi®) and dissolved in 5 mL of methanol to yield a crude skin flavonoid extract.

### 4. HPLC analysis of anthocyanins and flavonols

Analysis of monomeric phenolics was performed following a method described by Cadot *et al.* (2012) with some modifications. Prior to analysis, skin extracts were filtered using a HVLP 0.45-µm filter (Millipore). Quercetin and malvidin-3-*O*-glucoside were used as external standards for flavonols and anthocyanins, respectively (Extrasynthese, Genay, France).

The analyses were done on extract as described previously by Roggero *et al.* (1992) using a Waters Millennium HPLC-DAD system (Milford, MA). The column consisted of a reversed-phase (Phenomenex Kinetex 18 RP 100 Å 5 mm (250x4.6)), protected by

**Table 1 - Linear gradient used for the separation of anthocyanins and flavonols in grape skins.**

| Time (min) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 20 | 80 |
| 26 | 85 | 15 |
| 30 | 20 | 80 |

security guard cartridges C-18 (Merck, Darmstadt, Germany; 4 mm × 4.6 mm i.d.). Oven temperature was set at 10°C. The mobile phase was a linear gradient of formic acid/water (10:90 v/v; solvent B) in formic acid/water/acetonitrile (10:60:30 v/v; solvent A), at a flow rate of 0.8 mL/min. Proportions of solvent B were as follows: 0–26 min, 80–15%; 26–30 min, 15–80% (Table 1). The elution was monitored on a Waters 996 photodiode array (265-650 nm) and Empower software. All analyses were performed in duplicate. Compounds were identified

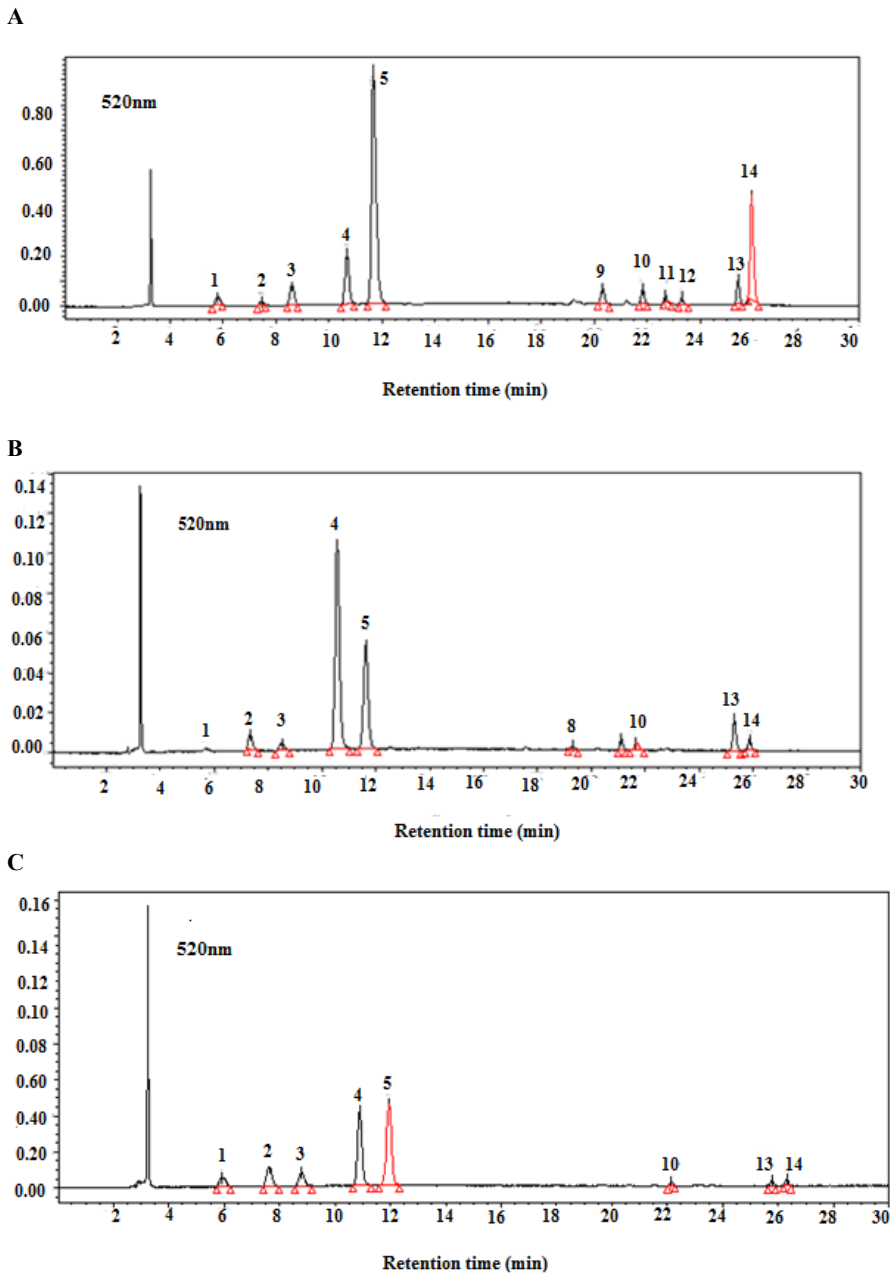


**Figure 1 - Anthocyanin chromatographic pattern of Gros noir (A), Cardinal (B) and Muscat noir (C) skin extract recorded at 520 nm. For list of substances, see Table 2.**


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according to their retention time and UV-visible (UV-vis) spectra. Quantification was carried out from peak areas at 360 and 520 nm using quercetin and malvidin-3-*O*-glucoside as external standards. For quercetin-*O*-glucuronide, a co-injection of a standard with the sample was done in order to validate the identification.

As extractions and injections were done in duplicate, the final result was the arithmetic average of four analyses.

### 5. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the Statistica software version 5.0 (StatSoft, France). Differences between means were first analyzed using the ANOVA test, and the least significant differences (Fisher's LSD) were calculated following significant *F* test ($P \leq 0.05$).

## RESULTS

Polyphenol analysis was carried out on the grape skins of red varieties, because this is the part of the berry that contains the two main classes of polyphenols (anthocyanins and flavonols) (Guerrero *et al*., 2009).

### 1. Anthocyanins identified

Anthocyanins are pigments that give color to many plants including grapes. They occur naturally as glycosides. Berry skin color is an important quality parameter in table grapes (Mizuno *et al.*, 2006). A total of 12 phenolic compounds were identified as anthocyanins on the basis of their retention times and UV-vis spectra, compared with those of standards (Table 2). As can be noted, monoglucoside derivatives were identified, five of which were present in all the red varieties studied including delphinidin-3-*O*-glucoside (peak 1), cyanidin-3-*O*-glucoside (peak 2), petunidin-3-*O*-glucoside (peak 3), peonidin-3-*O*-glucoside (peak 4), and malvidin-3-*O*-glucoside (peak 5). Other peaks were identified as monomeric acylglycosylated anthocyanins: peonidin-3-acetylglucoside (peak 8), malvidin-3-acetylglucoside (peak 9), cyanidin-3-*p*-coumaroylglucoside (peak 10), petunidin-3-*p*-coumaroylglucoside (peak 11), malvidine aglycone (peak 12), peonidin-3-*p*-

**Table 2. Retention time of identified anthocyanins and flavonols in skins of red grape varieties.**

| | Anthocyanins | Retention time (min) |
|---|---|---|
| 1 | Delphinidin-3-*O*-glucoside (Dp3glc) | 5.845 ± 0.080 |
| 2 | Cyanidin-3-*O*-glucoside (Cn3glc) | 7.485 ± 0.109 |
| 3 | Petunidin-3-*O*-glucoside (Pt3glc) | 8.658 ± 0.112 |
| 4 | Peonidin-3-*O*-glucoside (Pn3glc) | 10.717 ± 0.128 |
| 5 | Malvidin-3-*O*-glucoside (Mv3glc) | 11.758 ± 0.130 |
| 6 | Petunidin-3-acetylglucoside (Pt3Acglc) | 16.829 ± 0.072 |
| 7 | Delphinidin-3-*p*-coumaroylglucoside (Dp3Cmglc) | 19.432 ± 0.196 |
| 8 | Peonidin-3-acetylglucoside (Pn3Acglc) | 19.406 ± 0.101 |
| 9 | Malvidin-3-acetylglucoside (Mv3Acglc) | 20.403 ± 0.157 |
| 10 | Cyanidin-3-*p*-coumaroylglucoside (Cn3Cmglc) | 21.889 ± 0.188 |
| 11 | Petunidin-3-*p*-coumaroylglucoside (Pt3Cmglc) | 22.863 ± 0.141 |
| 12 | Malvidin aglycone (MvAgly) | 23.412 ± 0.145 |
| 13 | Peonidin-3-*p*-coumaroylglucoside (Pn3Cmglc) | 25.501 ± 0.188 |
| 14 | Malvidin-3-*p*-coumaroylglucoside (Mv3Cmglc) | 26.055 ± 0.185 |
| | Flavonols | Retention time (min) |
| 15 | Myricetin3-glucoside | 10.236 ± 0.064 |
| 16 | Quercetin3-glucuronide | 13.610 ± 0.180 |
| 17 | Quercetin3-glucoside | 14.150 ± 0.171 |
| 18 | Kaempferol-3-glucoside | 17.726 ± 0.199 |
| 19 | Isorhamnetin3-glucoside | 18.785 ± 0.157 |

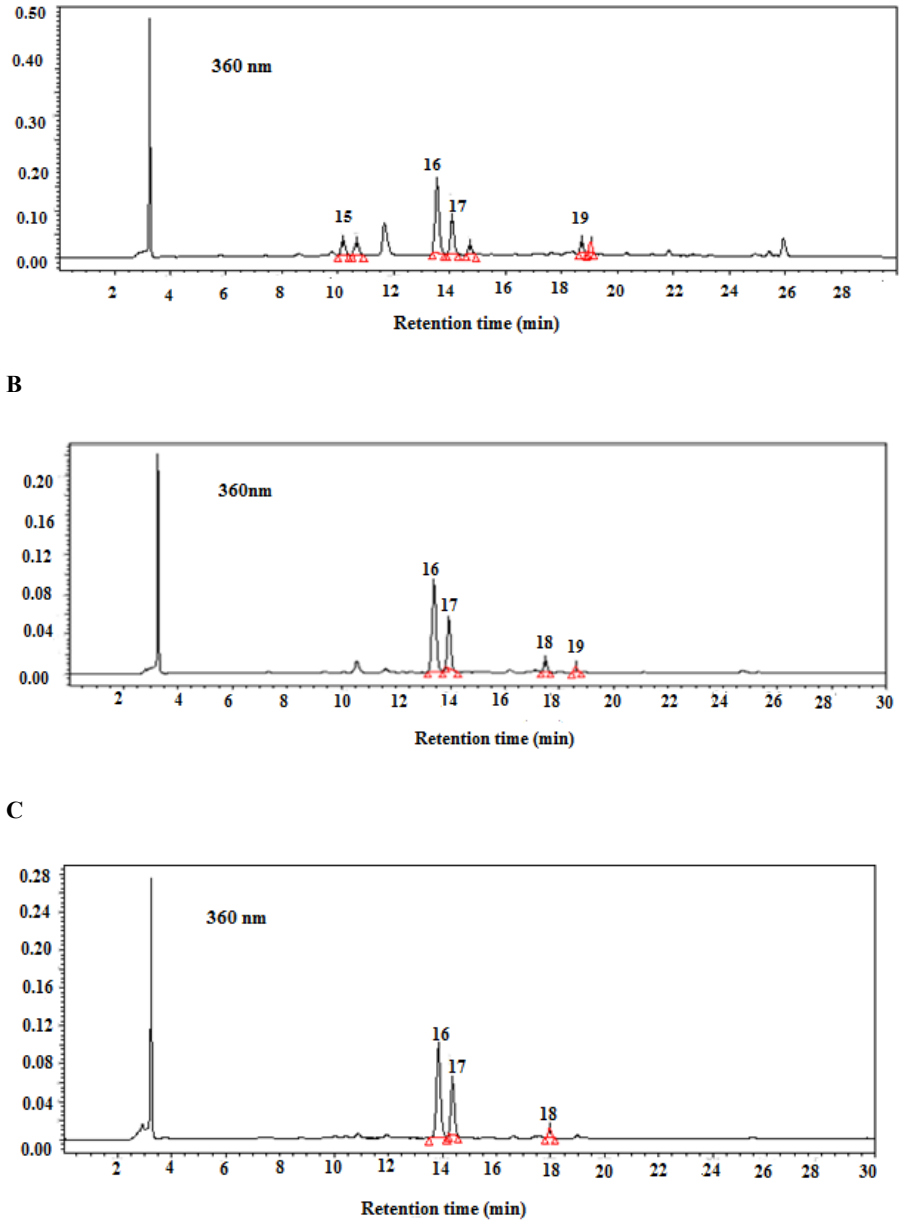


**Figure 2. Flavonol chromatographic pattern of Gros noir (A), Cardinal (B) and Muscat noir (C) skin extract recorded at 360 nm. For list of substances, see Table 2.**

coumaroylglucoside (peak 13) and malvidin-3-*p*-coumaroylglucoside (peak 14) (Fig. 1).

### 2. Flavonols identified

Figure 2 summarizes the flavonol profiles obtained for every red grape cultivar. In general, the results were in agreement with the scarce data available on red grape flavonol profiles (Cheynier and Rigaud, 1986). A total of four phenolic compounds were identified as flavonols on the basis of their retention times and UV-vis spectra, compared with those of standards (Table 2). As can be noted, flavonols detected in the studied red grapes occurred only as the expected 3-*O*-glucosides: myricetin-3-glucoside (peak 15), quercetin-3-glucoside (peak 17), kaempferol-3-glucoside (peak 18) and isorhamnetin-3-glucoside (peak 19), except peak 16 that corresponds to quercetol-3-*O*-glucuronide.

## DISCUSSION

### 1. Anthocyanin profile

The presence of anthocyanidin 3-*O*-monoglucosides along with their acyl derivatives in the berry skin has been demonstrated in different grape cultivars


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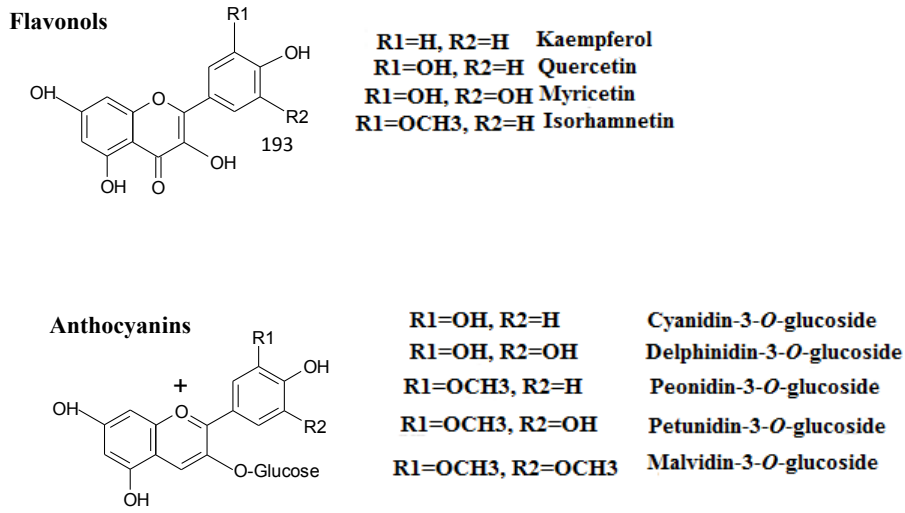


**Figure 3 - Chemical structures of grape flavonols and anthocyanins.**

including Tempranillo, Palomino negro and Cabernet sauvignon (Guerrero *et al.*, 2009), and Syrah (Boss *et al.*, 1996; Marquez *et al.*, 2013). On the other hand, Pinot noir completely lacks acylated anthocyanins (Mattivi *et al.*, 2006). However, not all acylated derivatives were detected in all varieties. In two of the three varieties studied (Cardinal and Muscat noir), malvidin-3-acetylglucoside, petunidin-3-*p*-coumaroylglucoside and malvidin aglycone were not detected, whereas peonidin-3-*O*-acetylglucoside was present in Cardinal but not in Gros noir and Muscat noir.

As shown in Table 3, in the two black cultivars (Gros noir and Muscat noir), malvidin-3-*O*-glucoside was the major anthocyanin component (72.73 and 41.11%, respectively), which is in agreement with the general perception that malvidin-3-*O*-glucoside is the main anthocyanin in grapes (Revilla and Ryan, 2000; Revilla *et al.*, 2001). In agreement with the findings of Brar *et al.* (2008) and Castillo-Munoz *et al.* (2009), peonidin-3-*O*-glucoside was the major anthocyanin found in Cardinal berries, which accounted for 59.27% of total anthocyanins. It has been reported that the cyanidin and peonidin levels are higher in red grape cultivars, whereas delphinidin and malvidin levels are higher in black grape cultivars (Mizuno *et al.*, 2006). Our data indicate that the color of grape berries may be related to the relative concentration of peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside in the grape berry skin. In the biosynthetic pathway it is known that cyanidin is a precursor to the other anthocyanidins and is converted into peonidin by the action of 3′-*O*-methyltransferase or into delphinidin by the action of 3′-hydroxylase. Delphinidin is further converted via petunidin to malvidin by the action of 3′5′-*O*-methyltransferase. The activity of these enzymes increased during grape ripening compared to other activities upstream in the flavonoid pathway (Boss *et al.*, 1996; Pomar *et al.*, 2005; Fournand *et al.*, 2006). So, delphinidin-3-*O*-glucoside is among the minor anthocyanins in the varieties studied, and according to Roggero *et al.* (1986) the concentration of delphinidin-3-*O*-glucoside in grape berry increases during the early stages of berry ripening to reach a maximum value, and subsequently decreases till harvest due to its conversion into petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside by the action of methyl transferases.

It has been suggested that the anthocyanin profile of berry skin may be directly related to the intensity of color, as evidenced from the higher concentrations of anthocyanins produced at the end of the biosynthetic pathway, namely peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside and petunidin-3-*O*-glucoside, in the higher pigmented cultivars (Carreno *et al.*, 1997).

The highest quantity of free anthocyanins (not bounded to tannins) was present in the Gros noir skin extract (214.10 mg/kg of berries), a variety that belongs to the so-called Teinturier varieties. For that reason, it was not surprising that its grapes also showed the highest average content of pigments ($P$<0.05). Lower anthocyanin concentrations found in skins from clusters studied appear to be due to elevated temperature, either through degradation, inhibition of synthesis, or, more likely, both (Spayd *et al.*, 2002).

Non-acylated anthocyanins were the main class of grape anthocyanins, and coumaroylated derivatives were the most important within the minor acylated anthocyanins. The percentage of coumaroylglucoside, acylated and non-acylated anthocyanins in each cultivar was calculated based on the peak area. All the varieties had higher percentages of non-acylated anthocyanins over acetic acid acylated and coumarate anthocyanins. The ratios of these different anthocyanins are also important for grape variety characterization: for instance, Pinot noir has no acylated anthocyanins (Cortell *et al.*, 2007). Non-acylated derivatives were predominant (>70%) but coumaroylglucoside derivatives were also present in quantities >5%, compared to the percentage of acylated anthocyanins which was very low (<2%). Muscat noir presented the highest non-acylated anthocyanin content, followed by Cardinal and Gros noir with 92.83, 88.02 and 70.74%, respectively. The lack of acylation in red grapes is not well understood, and two different hypotheses should be considered: the lack of genes related to the synthesis of acyltransferases or the lack of regulatory genes that module the expression of the first set of genes (Revilla *et al.*, 2012). Further, according to Fournand *et al.* (2006), the degradation rate of coumaroylglucoside derivatives is higher than that of non-acylatedglucoside derivatives or acetylglucoside derivatives at high sugar content in pulp (over 200 g/L). Muscat noir showed the highest content of sugar followed by Cardinal and Gros noir with 21, 17.30 and 17°Brix, respectively (data not shown in this study). Coumaroylglucoside derivatives might be hydrolyzed to restore the non-acylated forms, or the cinnamoyltransferase activity might decrease compared to the other activities involved in anthocyanin biosynthesis. Coumaroylglucoside derivatives may also be more reactive and consequently more involved in the formation of derived pigments than other anthocyanins. Their higher reactivity has been previously observed during fermentation, where the rate of formation of *p*-coumaroylvitisins was estimated to be higher than that of non-acylatedvitisins (Morata *et al.*, 2006). These results are consistent with previous studies performed on berries from different varieties and on the corresponding wines in which the authors systematically observed a higher ratio of non-acylatedglucosides to coumaroylglucosides (He *et al.*, 2010; Zhao *et al.*, 2010).

As stated previously, the presence of the enzyme flavonoid 3-*O*-glucosyltransferase is necessary for anthocyanin biosynthesis. However, the biosynthesis of the different anthocyanin precursors is driven upstream of the enzyme UFGT by the activity of flavonoid-3´-hydroxylase and flavonoid-3´5´-hydroxylase enzymes, which add either a single hydroxyl group or two to dihydrokaempferol. Once converted to dihydroquercetin or dihydromyricetin, these intermediates flow through common downstream enzymes to form disubstituted (cyanidin, peonidin) and trisubstituted anthocyanins (malvidin, delphinidin and petunidin), when UFGT is expressed, and to form other polyphenols (flavanols, flavonols) at different developmental stages (Hernández-Jiménez *et al.*, 2013). It has been demonstrated that the distribution of the ratio of disubstituted/ trisubstituted anthocyanins is associated to the expression of F3´5´H and F3´H, responsible for the hydroxylation of the flavonoid B-ring. Castellarin and Di Gaspero (2007) showed that the F3´5´H/UFGT and F3´5´H/F3´H ratios are strongly correlated to the proportion of trisubstituted and disubstituted anthocyanins in several grape varieties. Gros noir exhibited a higher disubstituted/ trisubstituted ratio than the other varieties. A possible explanation for this is that the enzymes cited above are highly activated in this variety.

The anthocyanin profile is under a strong genetic control, therefore, the profiles of anthocyanins for each variety are relatively stable, while absolute concentrations can vary widely between different vintages, due to both environmental and agronomical factors. The anthocyanin profile can therefore be used as a chemotaxonomic parameter for the classification of red *Vitis vinifera* varieties (Mattivi *et al.*, 1990; Mattivi *et al.*, 2006). For this reason, red berry grapevine cultivars can be classified into groups:

- Cultivars with a higher proportion of disubstituted anthocyanins (Cardinal);

- Cultivars with a higher proportion of trisubstituted anthocyanins (Gros noir); and

- Cultivars with high non-acylated anthocyanins (Muscat noir).

From the description presented above, anthocyanins could be considered useful markers for distinguishing grape varieties, although this characteristic should be used with care since anthocyanin content is heavily influenced not only by agronomical factors such as soil composition, irrigation, light intensity, etc., but also by the year's climatic conditions (Adams, 2006; Conde *et al.*, 2007).

**2. Flavonol profile**

**Table 3 - Anthocyanin compounds in red grape skin extracts.**

| | Cardinal | Gros noir | Muscat noir |
|---|---|---|---|
| Total Free Anthocyanins* | 17.40±0.4[a] | 214.10±1.7[b] | 20.40±0.3[a] |
| Delphinidin (%) | 0.86±0.00[a] | 3.01±0.00[b] | 6.02±0.00[c] |
| Cyanidin (%) | 6.49±0.002[b] | 3.43±0.000[a] | 11.28±0.00[c] |
| Petunidin (%) | 2.22±0.0004[a] | 6.44±0.0000[b] | 7.01±00011[c] |
| Peonidin (%) | 59.27±0.000[c] | 14.44±0.001[a] | 34.58±0.002[b] |
| Malvidin (%) | 31.17±0.004[a] | 72.73±0.002[c] | 41.11±0.008[b] |
| Acylated anthocyanins (%) | 1.29±0.004[a] | 0.68±0.001[a] | 0.43±0.002[a] |
| Coumaroylglucoside anthocyanins (%) | 10.68±0.008[b] | 2858±0.008[c] | 6.35±0.001[a] |
| Coumaroyl-acetylated anthocyanin. (%) | 11.98±0.004[b] | 29.26±0.008[c] | 7.17±0.002[a] |
| Non-acylated anthocyanins (%) | 88.02±0.004[b] | 70.74±0.008[a] | 92.83±0.002[c] |
| Disb/Trisb (%)** | 34.24±0.003[a] | 82.13±0.001[c] | 54.14±0.007[b] |

*expressed as mg/kg of berries ; **ratio of disubstituted anthocyanins/trisubstituted anthocyanins ; Values marked by different letters are significantly different (P<0.05).

The total amount of flavonols found in the grape extracts ranged from 20.37 to 49.13 mg/kg of berries (Table 4). According to Mattivi *et al.* (2006), the flavonol content in the skin of 64 Italian red grape varieties and 27 Italian white grape varieties varied between 3.81 and 80.37 mg/kg of berries and between 1.36 and 30.21 mg/kg of berries, respectively. Guerrero *et al.* (2009) showed flavonol content between 221 and 538 mg/kg of berries in five red grape varieties grown in Andalusia (Spain). In another study, Mélo *et al.* (2006) noted that the levels of flavonols in Italia and Patrícia varieties from Brazil were respectively 2.38 and 8.85 mg/100 g fresh weight.

Differences in flavonol levels compared to those obtained in this study are probably due to several factors, including the extraction technique and/or the sensitivity of the method, varietal differences and geographical origin of the samples analyzed.

Looking at the pattern of the flavonols, the quercetin-3-*O*-glucoside was the major flavonol in grape skin at harvest, accounting for 86.77% of total flavonol content, which is in agreement with previous work on numerous grape tissues in many cultivars (Mattivi *et al.*, 2006; Castillo-Munoz *et al.*, 2007; Hernández-Jiménez *et al.*, 2013). The isorhamnetin-3-*O*-glucoside flavonol was the second in importance (4.90%), followed by kaempferol-3-*O*-glucoside (4.32%), usually occurring as minor compounds and accounting for no more than 5%, and myricetin-3-*O*-glucoside (4%). Kaempferol-3 type flavonols have been found as minor compounds contributing to the flavonol profiles of red grape cultivars (Mattivi *et al.*, 2006; Castillo-Munoz *et al*., 2007). This flavonol was absent in the Gros noir variety; the same observation was made by Mattivi *et al.* (2006) for the red grape variety Tannat. Myricetin-3-*O*-glucoside was not detected in the samples analyzed, with the exception of the black variety Gros noir (5.42 mg/kg). This result does not coincide with that of Mattivi *et al.* (2006), who detected myricetin only in white varieties. By contrast, Tarara *et al.* (2008) did not found myricetin-3-*O*-glucoside in Merlot (black grape), which is consistent with the results of this study where this flavonol was not recorded in the red (Cardinal) nor in the black variety (Muscat noir); the same flavonol was found in trace amounts in pink varieties (Mattivi *et al.*, 2006). This could probably be due to the fact that these varieties contain low amounts of anthocyanins. According to these authors, the varieties having mostly cyanidin-3-glucoside and peonidin-3-glucoside derivatives, such as Cardinal and Muscat noir, are expected to derive from white varieties.

In summary, the red grape cultivars showed remarkable differences in their flavonols, which can be considered as a cultivar characteristic. Gros noir had the highest proportions of myricetin, quercetin and isorhamnetin-3-*O*-glucoside but not kaempferol flavonols. However, the aforementioned results are only preliminary, and confirmation is needed with further analysis. Although the grape samples analyzed were grown in the same viticultural

**Table 4. Flavonol compounds in red grape skin extracts.**

| | Cardinal | Gros noir | Muscat noir |
|---|---|---|---|
| Myricetin3-*O*-glucoside | 0.00±0.00[a] | 5.42±0.00[b] | 0.00±0.00[a] |
| Quercetin3-*O*-glucoside | 18.26±0.59[a] | 39.45±2.26[b] | 36.61±0.01[b] |
| Kaempferol-3-*O*-glucoside | 1.34±0.06[b] | 0.00±0.00[a] | 2.19±0.15[c] |
| Isorhamnetin3-*O*-glucoside | 0.77±0.07[a] | 4.25±0.17[b] | 0.45±0.05[a] |
| Total Flavonols | 20.37±0.71[a] | 49.13±2.98[c] | 39.25±0.19[b] |

Results are expressed as mg/kg of berries
Values marked by different letters are significantly different (P<0.05).

conditions, it is obvious that several unstudied factors (e.g., differences in viticultural practices, soil, or ripening degree) could markedly change the flavonol profile of a red grape cultivar. Other authors have obtained a good differentiation of several red grape cultivars from La Mancha (central southern Spain) on the basis of their total contents of quercetin, kaempferol, and dihydroflavonols (Castillo-Munoz *et al.*, 2007; 2010).

The role of light on flavonol synthesis in grape berries seems well established and it is consistent with the UV-protection function exerted by these compounds. Merlot berries exposed to sunlight showed up to 10-fold more flavonols compared to shaded berries (Spayd *et al.*, 2002; Tarara *et al.*, 2008). Cluster shading caused a significant reduction in the synthesis of flavonols in Shiraz (Downey *et al.*, 2003) and Cabernet sauvignon grapes (Matus *et al.*, 2009). UV radiation also had a positive effect on flavonoid accumulation (Spayd *et al.*, 2002). Moreover, sunlight exposure specifically increased the quantity of quercetin-3-*O*-glucoside in Merlot grapes, while kaempferol-3-*O*-glucoside remained unchanged; however, the authors did not detect myricetin-3-*O*-glucoside (Tarara *et al.*, 2008). Taken together, these results point out that light is the most important factor determining the levels of flavonols in grape berries.

## CONCLUSION

The current study clearly shows that health-promoting compounds exhibit differences, confirming their importance in varietal characterization. The Gros noir variety is characterized by the highest levels of anthocyanins and flavonols. Based on these results, varieties can be classified as Gros noir > Muscat noir > Cardinal. Although the differences in anthocyanin and flavonol content between the varieties analyzed were significant, the levels of these compounds are such that extracting polyphenols from grape skin might be economically viable. Grape skin could then be used (i) as a natural alternative to the synthetic antioxidants used in the food industry to prolong the shelf life of food or (ii) as a good antioxidant source in dietary supplements.

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