Spectrophotometric detection of changes in phenol content of red berry skins during grape ripening

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Abstract
Spectrophotometric detection was performed in order to characterize and to quantify phenolic compounds, Hydroxycinnamic Tartaric Acids (HCTAs), Flavonols and Anthocyanins, in berry skin of four different red Vitis vinifera L. varieties, (Alicante, Black Malvasia, Nerello and Prunesta), cultivated in the Reggio Calabria province (South Italy). Five samplings were conducted during ripening, at weekly intervals. The evolution of these phenolic fraction has been monitored by separating the phenols into different classes by SPE. The adsorbing resin Serdolit XAD-2 was employed. This approach provided good performance in terms of linearity, accuracy and precision. Grape skin showed to be rich in phenolic components and especially in anthocyanins whose content increased during ripening, being maximum at commercial ripeness of grapefruit.

Parole chiave: uva, polifenoli, spettrofotometria, Vitis vinifera L.
Keywords: grape, polyphenols, spectrophotometry, Vitis vinifera L.
Introduction

Phenolic compounds contained in grape berry skin can have a simple chemical structure as the phenolic acids or a complex one as the anthocyanins. The importance of the presence of phenols in food has been recognized. Phenols represent the subject of many researches due to their interesting pharmacological properties and applications. They are potential antioxidants and exert a protective action against free radicals, preventing the formation of lipoperoxides as the heart disease factor (Stanley and Mazier, 1999; Shrihande, 2000).

The study of the polyphenolic potential of the grapes and in particular of grape skins is important since it is possible that different winemaking practices may either increase or decrease wine color. The phenolic composition is also established by different viticultural practices (Mattivi et al., 2002b; Mattivi and Valenti, 2003). Moreover, the content of the phenolic compounds and tartaric acids are associated with technological maturity.

Anthocyanins are also proved to be strong radical scavengers comparable to that of quercetin, the prototype of natural polyphenolic antioxidants, and have therapeutic properties which are widely used in the pharmaceutical industry for their capacity to modulate capillary fragility (Saija, 1994). Anthocyanin content in grape berries is related to night temperature condition, when the temperature is low (15°C), the anthocyanin production is higher than when the temperature is high (30°C) (Mori et al., 2005).

No differences in flavonol production are observed between grape berries grown in high or low night temperature condition (Mori et al., 2005). An inverse association between the flavonols and flavones intake and the risk of lung cancer in male smokers was observed (Hirvonen et al., 2001). The flavonol concentration gives an indication of the co-pigmentation potential and it is important in determining wine color and stabilisation (Boulton, 2001).

The phenolic compounds extracted from berry skins and/or from marc after vinification, can be used as a natural antioxidant in food instead of synthetic antioxidants (Bonilla et al., 1999; Negro et al., 2003).

Both the skin natural phenolic compounds as well as their quantitative changes during the ripening period of grapes are described further on. The composition and the evolution of phenolic compounds in grape skins are useful to classify, implement varietal characterization, and to establish the ripening evolution. In fact, these compounds are strictly connected with the technological maturity so, they can be used as markers of maturation (Poudel et al., 2008; Jin et al., 2009; Xu et al., 2010). The concentration, the nature, and the structure of phenols are related to the grape ripening stage and to the type of technology used during winemaking (Mattivi et al., 2002a; Mattivi et al., 2003). The variables traditionally used for determining grape ripening, sugars and acidity, are known as industrial maturity, while tartaric acid and phenolic compounds are associated with technological maturity (Gonzales - San José et al., 1990). Technological maturity is determined by the optimum phenolic composition in obtaining a specific wine style. Furthermore, the concentration and the presence of diverse phenolic compounds in the grape affect directly wine vigor in both the primary and secondary metabolism of the wine. The aim of this paper is to detect hydroxycinnamic tartaric acids, flavonols and anthocyanins in berry skins of four red grapevines of Calabria. In order to employ a rapid and cheap method of phenols determination, the spectrophotometric detection was involved. Solid Phase Extraction (SPE) technique was employed. The XAD-2 adsorbent resin has been used to separate the phenolic compounds in three different classes for a sample preparation in the spectrophotometric analysis (Di Stefano et al., 1989; Di Stefano and Guidoni, 1990; Di Stefano and Cravero, 1992).

Materials and methods

Sampling

Four red grape varieties, Alicante, Black Malvasia, Nerello and Prunesta grown in vineyards at 80 meters above the sea level in the Bagnara Ca-
labra-Scilla area, Reggio Calabria province (Southern Italy), were used in this study. Twenty-five grape plants per variety, 18-year-old in 2009, having similar characteristics in growth and production, were selected in the vineyard. Five samplings were carried out, for each variety, on the following weekly basis, Aug. 26th, Sept. 2nd, Sept. 9th, Sept. 16th, and Sept. 23rd, at the grape harvest, of the year 2009. The fifth harvest was conducted at commercial ripeness.

Two hundred berries/variety were randomly hand-picked from plants and separated into three lots: small, medium, and large according to their size. Out of three lots, ten sublots of 10 berries were formed, each one containing berries small, medium and large in size. At this point, the three most similar sublots in weight were chosen in order to execute analysis in three replicates. Berries were cut with a bistoury in two halves then, peeled within 4 hours of harvest. Berry skins were neither washed nor cleaned before peeling.

**Chemicals**

Reagent, all of pro-analysis grade, were purchased from Merck, Darmstadt (Germany). Serdolit XAD-2 resin was purchased from Serva Co. Ltd. C18 Sep-Pak cartridges were obtained from Millipore. Standards of (+) catechin, caffeic acid and quercetin were of Sigma.

**Extraction of phenolic compounds from berry skin**

Figure 1 shows the scheme of this work. The phenol extract was prepared according to the method of Di Stefano and Cravero (1991) modified as follows. The peeled skins were suspended in a 25 mL buffer solution (pH=3.2) out of a 1L buffer solution consisting of deionized water (200mL), tartaric acid (5g), ethanol (120 mL), sodium metabisulfite (2g), a 1N sodium hydroxide solution (22 mL), and finally deionized water up to the 1L volume.

![Figure 1. Procedure for phenols fractioning](image)
SPE of phenolic compounds

Phenolic compounds were extracted from the skin over 24 h by means of the above-mentioned buffer solution at a temperature ranging from 25°C to 30°C. The extracting solution was separated from the skin by decanting, then it was stored in a freezer until analysis. Extraction, identification and quantification of phenolic compounds of all samples were made in triple sets by detecting the three sublots, each one of 10 berries/varieties. Each result was reported as the average ± S.D. of three analyses. The phenolic content was expressed as milligrams of phenols per kilogram of red berry fresh weight.

Separation of phenolic compounds in different classes was performed by eluting an aliquot of 1.5 mL of extract through column chromatographic, packed with XAD-2 resin and prepared according to the following procedure to eliminate the impurities and the smallest suspended particles. Five grams of resin were suspended in methanol and washed with organic solvents: CH2Cl2, (C2H5)2O, CH3COOC2H5, CH3OH and inorganic solvent, H2O. The resin was acidified using a 12 mL sulphuric acid 0.1 N solution. The sample (1.5 mL of extract) was afterwards applied to the column and eluted with 10 mL of sulphuric acid 0.1 N to remove interfering compounds. The water phase was discarded. The first fraction, containing hydroxycinnamic tartaric acids, was eluted with 50 mL diethyl ether; the second fraction, containing flavonols in glucuronide and monoglucoside forms, was eluted with 50 mL ethyl acetate; the third fraction, containing anthocyanins was eluted with 50 mL chloroform/methanol (2:1, V/V).

Spectrophotometric analysis

After careful purification and fractioning of the sample by SPE, quantitative estimate of the presence of the various classes of phenolic compounds was easily carried out involving spectrophotometric detection. A Perkin Elmer spectrophotometer, mod. Lambda 2, was employed. The methodicals are described in detail by Di Stefano and Cravero in 1991 and were easily employed. Hydroxycinnamic tartaric acids, flavonols and anthocyanins content were measured after eluting of the berry skins extract through XAD-2 resin. Measurements with the spectrophotometer were conducted as follows, hydroxycinnamic tartaric acids at 320 nm, flavonols at 360 nm; anthocyanins at 540 nm. The contents of hydroxycinnamic tartaric acids, flavonols and anthocyanins have been calculated by using the following relations and standards:

HCTAs (mg/L) = E*(10/0.91)*d;
E max at 320 nm, d dilution, 0.9 maximum of absorption at 320 nm of 10 mg/L of caffeic acid standard.

Flavonols (mg/L) = E*15.5*d;
E maximum at 360nm, d dilution and 15.5 = \( \frac{PM}{\varepsilon} \), \( \varepsilon \) of quercetin.

Anthocyanins (mg/L) = Emax vis*(10/0.62)*d;
E maximum of absorption at 540 nm, d dilution and 0.62 maximum of absorption of 10 mg/L of (+) catechin standard solution.

Results and discussion

**HCTAs.** For all the varieties, the concentration of hydroxycinnamic tartaric acids decreased from the first to the third harvest and it increased at the fourth and the fifth harvest (Table I). Nerello had higher HCTAs content than Alicante, Black Malvasia and Prunesta at all five harvests. Prunesta showed the second largest amount.

**Flavonols.** The results showed that there were large differences among varieties in the initial flavonol content (Table II). For all varieties, the flavonol content increased during grape ripening until the fourth harvest and then decreased, Prunesta was the variety with the highest flavonol content for all five harvests whereas Alicante showed the lowest amount.

**Anthocyanins.** Table III summarises the results relative to the anthocyanin content. The anthocyanin content increased during the grape ripening,
Table I. Change in the HCTAs content of berry skins, during grape ripening, value in ppm. Mean of three replicates ± SD

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Alicante</th>
<th>Black Malvasia</th>
<th>Nerello</th>
<th>Prunesta</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 Aug.</td>
<td>100 ± 3.21 k</td>
<td>77 ± 1.53 e</td>
<td>122 ± 3.21 n</td>
<td>100 ± 3.00 k</td>
<td>**</td>
</tr>
<tr>
<td>2 Sept.</td>
<td>76 ± 2.00 e</td>
<td>58 ± 1.00 b</td>
<td>105 ± 2.52 l</td>
<td>82 ± 2.00 gh</td>
<td>**</td>
</tr>
<tr>
<td>9 Sept.</td>
<td>67 ± 1.73 c</td>
<td>47 ± 1.00 a</td>
<td>84 ± 4.16 h</td>
<td>79 ± 2.52 efg</td>
<td>**</td>
</tr>
<tr>
<td>16 Sept.</td>
<td>79 ± 1.15 efg</td>
<td>71 ± 1.53 d</td>
<td>112 ± 2.00 m</td>
<td>97 ± 1.15 ij</td>
<td>**</td>
</tr>
<tr>
<td>23 Sept.</td>
<td>95 ± 2.52 i</td>
<td>81 ± 2.08 fgh</td>
<td>121 ± 3.06 n</td>
<td>98 ± 2.65 ij</td>
<td>**</td>
</tr>
</tbody>
</table>

Significance
*Significance at P<0.05; **Significance at P<0.01.
Data followed by different letters are significantly different by Duncan’s multiple range test.

Table II. Change in the Flavonols content of berry skins, during grape ripening, value in ppm. Mean of three replicates ± SD

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Alicante</th>
<th>Black Malvasia</th>
<th>Nerello</th>
<th>Prunesta</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 Aug.</td>
<td>15 ± 1.15 a</td>
<td>41 ± 2.08 f</td>
<td>17 ± 2.65 ab</td>
<td>57 ± 2.00 h</td>
<td>**</td>
</tr>
<tr>
<td>2 Sept.</td>
<td>24 ± 2.08 c</td>
<td>47 ± 1.15 g</td>
<td>20 ± 1.00 b</td>
<td>63 ± 1.53 ij</td>
<td>**</td>
</tr>
<tr>
<td>9 Sept.</td>
<td>33 ± 1.53 d</td>
<td>60 ± 2.65 hi</td>
<td>25 ± 1.15 c</td>
<td>73 ± 3.21 kl</td>
<td>**</td>
</tr>
<tr>
<td>16 Sept.</td>
<td>38 ± 1.53 ef</td>
<td>70 ± 0.58 k</td>
<td>49 ± 1.53 g</td>
<td>76 ± 1.00 l</td>
<td>**</td>
</tr>
<tr>
<td>23 Sept.</td>
<td>35 ± 0.58 de</td>
<td>65 ± 2.52 j</td>
<td>46 ± 2.08 g</td>
<td>65 ± 2.08 j</td>
<td>**</td>
</tr>
</tbody>
</table>

Significance
*Significance at P<0.05; **Significance at P<0.01.
Data followed by different letters are significantly different by Duncan’s multiple range test.

Table III. Change in the anthocyanin content of berry skins, during grape ripening, value in ppm. Mean of three replicates ± SD

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Alicante</th>
<th>Black Malvasia</th>
<th>Nerello</th>
<th>Prunesta</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 Aug.</td>
<td>85 ± 3.51 b</td>
<td>71 ± 2.52 a</td>
<td>2106 ± 9.64 m</td>
<td>476 ± 5.13 h</td>
<td>**</td>
</tr>
<tr>
<td>2 Sept.</td>
<td>173 ± 3.06 c</td>
<td>91 ± 3.61 b</td>
<td>2218 ± 7.64 n</td>
<td>499 ± 3.61 i</td>
<td>**</td>
</tr>
<tr>
<td>9 Sept.</td>
<td>285 ± 6.08 e</td>
<td>190 ± 3.00 d</td>
<td>2709 ± 7.00 o</td>
<td>508 ± 6.43 j</td>
<td>**</td>
</tr>
<tr>
<td>16 Sept.</td>
<td>336 ± 4.00 f</td>
<td>198 ± 2.89 d</td>
<td>3098 ± 8.50 p</td>
<td>606 ± 6.51 k</td>
<td>**</td>
</tr>
<tr>
<td>23 Sept.</td>
<td>463 ± 4.51 g</td>
<td>277 ± 2.08 e</td>
<td>3966 ± 6.66 q</td>
<td>618 ± 2.65 l</td>
<td>**</td>
</tr>
</tbody>
</table>

Significance
*Significance at P<0.05; **Significance at P<0.01.
Data followed by different letters are significantly different by Duncan’s multiple range test.
The highest concentration of anthocyanins in the grape skin at all harvests has been found in Nerello (2,106-3,966 ppm) with a level about four-six times higher than in Prunesta, the second cultivar in quantity and 14-30 times higher than in Black Malvasia, the cultivar with the lowest amount. In Alicante, the level of anthocyanins increased more than 5 times from the first to the fifth harvest (85-463 ppm). In Black Malvasia, at the same period, the increase was less than 4 times. The lowest rates of increase were in Nerello, 1.9 times and in Prunesta, 1.3 times. Data revealed that the accumulation of anthocyanins and the different value obtained are depending on grape varieties.

Conclusions

The understanding of polyphenolic potential in grapes is particularly important in obtaining valuable wines during a medium or long maturing process. Due to the different polyphenolic potentials in each variety, it becomes necessary that the style for winemaking will be flexible in order to adapt itself to the various compositions of the raw materials. The measure of the polyphenolic compounds serves also to classify the grapes by quality, optimising the maceration technology. The polyphenolic potential related to each grape variety can be used by agronomists as an additional instrument of appraisal for the potentiality of the vineyards in a viticultural area. The evaluation of phenolic compounds together with other parameters may be useful to characterize appropriate agronomic operations necessary to improve the quality level of the grapes. Further studies are required to achieve more information on the technological consequences of the phenolic composition in order to exalt Calabria's wines. The calabrian region lacks data on polyphenolic potentiality of the different varieties, including those considered as “minor”, which are used in constituting a “regional data bank”. In addition, the evaluation technology is necessary to exalt the potentiality of the phenolic quality of wines. According to the data obtained, it has been demonstrated that in the course of grape ripening, the quantitative phenolic composition has changed considerably for anthocyanins. However, the polyphenolic potential needs to be monitored in order to identify the right period for collecting grapes, as well as the characteristics of the wine produced within a given year. Although quantity of polyphenols within a variety does not determine the bias toward the maturing of a wine, yet the quality plays an important role in the process itself. Spectrophotometer detection has demonstrated to be a rapid, precise and cheap method for total phenol determination, especially useful in medium or small winemaking industries, where expensive methods of analysis cannot be applied.

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References


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