PHENOLIC CHARACTERIZATION OF THIRTEEN RED GRAPE CULTIVARS FROM GALICIA BY ANTHOCYANIN PROFILE AND FLAVANOL COMPOSITION

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Aims: Phenolic compounds, extractable from grape skins and seeds, have a notable influence on the quality of red wines. Therefore, in this work the phenolic composition of 13 red grape cultivars, grown in one of the most traditional Spanish vine zones, was studied in order to identify significant varietal differences.

Methods and results: Anthocyanin concentration and profile, total proanthocyanidin and flavanol contents in berry skins and seeds were determined by spectrophotometric and HPLC methods. The highest concentrations of total anthocyanins was found in the Loureira Tinta, Sousón and Ferrol varieties, while Ferrol was rich in proanthocyanidins in berry skin and Caíño Bravo was rich in proanthocyanidins and flavanols in berry seeds. Malvidin-3-monoglucoside was usually the major anthocyanin. Nevertheless, the anthocyanin profile was characterized mainly of di-substituted molecules for Albarello, Brancellao and Caíño da Terra grapes. Ferrol, Loureira Tinta and Sousón grapes showed the highest values of primitive anthocyanins (delphinidin and petunidin derivatives).

Conclusion: Many differences in the phenolic composition of the cultivars studied were found. The results of the phenolic characterization can be utilized in winery in order to apply the most appropriate maceration and winemaking techniques to the processed grapes.

Significance and impact of study: Knowledge of the biodiversity of the grape varieties of Galicia (North-West Spain) is still scarce. This study, on phenolic composition, provides oenological information that can be useful to improve the quality of the wines produced.

Keywords: anthocyanins, total proanthocyanidins, total flavanols, red grape cultivars, spectrophotometry, HPLC

Résumé

Objectif: Puisque les composés phénoliques, que l'on peut extraire des pellicules et des pépins, ont une influence notable sur la qualité des vins rouges, cette étude visait à mieux connaître leur composition dans treize cépages cultivés dans une des régions viticoles les plus traditionnelles d'Espagne et constater les différences variétales éventuelles.

Méthodes et résultats: La concentration et le profil en anthocyanes, la teneur en proanthocyanidines totales et en flavanols ont été déterminées par spectrophotométrie et chromatographie en phase liquide à haute performances (CLHP). La concentration la plus élevée en anthocyanes totaux a été trouvée en « Loureira Tinta », « Sousón » et « Férron »; « Ferrol » était le cépage avec les pellicules les plus riches en proanthocyanidines aussi, tandis que « Caíño Bravo » se signalait pour sa richesse en proanthocyanidines et en flavanols dans les pépins. La malvidine-3-monoglucoside était en général l'anthocyane le plus représenté, sauf en « Albarello », « Brancellao » et « Caíño da Terra », qui avaient un profil marqué plutôt par les anthocyanines disubstituées. Des variations dans la distribution des dérivés de la delphinidine et de la pétunidine ont été observées dans quelques cépages par rapport au climat de la saison.

Conclusion : Plusieurs différences ont été trouvées dans la composition phénolique des raisins des cépages étudiés. Ces résultats pourront être utilisés en œnologie pour améliorer les techniques de macération et de vinification.

Signification et impact de l'étude : Puisque les connaissances sur la biodiversité variétale des cépages de la région galicienne (Nord-Ouest, Espagne) sont encore peu développées, cette étude apporte des informations sur la composition phénolique des raisins qui seront utiles pour améliorer la qualité des vins issus de ces cépages.

Mots clés : anthocyanines, proanthocyanidines, flavanols totaux, cépages noirs, spectrophotométrie, CLHP

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INTRODUCTION

Since the chemical composition of grapes has an important effect on the wine quality, its knowledge is invaluable in winemaking. In addition to the classical technological maturity indexes (sugar concentration, pH, tartaric and malic acid content), the most important parameter to take into account is the polyphenol content (anthocyanins and tannins); this affects both the sensory characteristics of the wine and its health promoting properties (KROON and WILLIAMSON, 2005; VIDAL et al., 2003). Synthesis and concentration of phenols in red grapes depend on the cultivar, fruit ripeness, management vineyard practices, climatic conditions, soil features and crop load (JACKSON and LOMBARD, 1993; DOWNEY et al., 2006).

Anthocyanins, being located in the berry skin, are the main flavonoids responsible for the red colour of grape cultivars (ROLLE and GUIDONI, 2007) and the respective wines (MATEUS et al., 2002; PÉREZ-MAGARÍNÓ and GONZÁLEZ-SAN JOSÉ, 2006). It is well known that the anthocyanin concentration can vary widely among different vintages of a given cultivar, due to both environmental and agronomical factors (GUIDONI et al., 2008; OJEDA et al., 2002). Nevertheless, the distribution of anthocyanins is closely associated with the genetic inheritance of grape cultivars (MAZZA and MINIATI, 1993) and is largely independent of the seasonal conditions or production area. Therefore, the anthocyanin profile has been used as a chemotaxonomic parameter for the classification of red Vitis vinifera varieties (CARREÑO et al., 1997; GARCÍA-BENEYTEZ et al., 2003; LETAIFF et al., 2007; LOVINO et al., 2006; MATTIVI et al., 1990; MATTIVI et al., 2006; NÚÑEZ et al., 2004; ORTEGA-REGULES et al., 2006; POMAR et al., 2005; REVILLA et al., 2001; TAMBORRA et al., 2003; ZEPPA et al., 2001). In Vitis vinifera L. red varieties, the anthocyanins usually present are the 3-O-monoglucosides of malvidin, delphinidin, peonidin, petunidin and cyanidin, along with the corresponding acetylated, p-coumaroylated and caffeoylated derivatives. Some varietal classifications are also based on the relationships between anthocyanins and/or anthocyanin acylated, which are related to enzymatic activity (MATTIVI et al., 2006; NÚÑEZ et al., 2004). The determination of anthocyanins and their ratios has been proposed for validating the identity of the grapes used during winemaking. However, some reports show different anthocyanin profiles as a result of agroecological factors (DOWNEY et al., 2004; GONZÁLEZ-NEVES et al., 2004; ROGGERO et al., 1986; RYAN and REVILLA, 2003).

Flavan-3-ol monomers and proanthocyanidins are very important for red wine quality, because these compounds provide sensory properties like bitterness and astrignency (ROBICHAUD and NOBLE, 1990). Several methods such as thiolysis or phloroglucinolysis were developed for the study of these compounds (RIGAUD et al., 1991; DRINKINE et al., 2007).

Many studies have demonstrated the relationship between grape phenolic composition and maturity and its influence on red wine phenolic composition (CAGNASSO et al., 2008; CASTILLO-MUÑOZ et al., 2007; SUN et al., 1999). A significant proportion of flavanol and proanthocyanidins is extracted from skins and seeds during red wine production, but this quantity varies according to the variety (MATTIVI et al., 2009), to the maceration conditions and alcohol percentages (VIDAL et al., 2002; GONZALEZ-MANZANO et al., 2004; CANALS et al., 2005).

Since only scant literature on the phenolic composition of Galician red grape cultivars is available (POMAR et al., 2005; LETAIFF et al., 2007), the purpose of this work was the phenolic characterization of 13 red grape cultivars, located in the germplasm collection of «Estación de Viticultura e Enoloxía de Galicia», by anthocyanin profile and flavanol content in berry skin and seeds. This study, which completes previous work directed to acquiring knowledge of the berry skin mechanical properties and maturity indexes of these varieties (RIO SEGADE et al., 2008a; RIO SEGADE et al., 2008b), was realized over two consecutive years. The results obtained were statistically analyzed in order to establish a classification.

MATERIALS AND METHODS

1. Grape samples

Grape samples of thirteen red cultivars (Vitis vinifera L.) were collected at harvest (Table 1) from the germplasm collection of the Estación de Viticultura e Enoloxía de Galicia (EVEGA, Leiro, Spain) in two consecutive years (2005 and 2006). This is located in the Ribeiro region, one of the most traditional Spanish vine zones. All the grape varieties, including Albariño (AL), Brancellao (BR), Caño Bravo (CB), Caño Longo (CL), Caño da Terra (CT), Caño Redondo (CR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Mencia (ME), Merenzao (MZA), Mouratón (MR) and Sousón (SO), in this collection have similar characteristics (age and pruning system). The average age of the grapevine was 15 years; grafted onto the 196-17 C rootstock with trellis pruning. Cordon Royat training and planted at 1.2 m x 1.8 m. Each sample consisted of 10 bunches picked randomly from nine different plants. Once in the laboratory, a set of 250 grapes was randomly sampled.
2. Reagents and Standards

Solutions of HPLC-gradient grade and all other chemicals of analytical-reagent grade were purchased from Sigma (Milan, Italy). The solutions were prepared in deionized water produced by a Purelab Classic system (Elga Labwater, Marlow, United Kingdom). Anthocyanin standards (Delphinidin-3-O-glucoside chloride, Malvidin-3-O-glucoside chloride, Peonidin-3-O-glucoside chloride, Cyanidin-3-O-glucoside chloride) were supplied from Extrasynthese (Genay, France). All the standards were stored at -20 °C away from light before use.

3. Sample preparation

a) Total anthocyanin extraction

Two replicates of 50 berries, randomly sampled, were prepared according to the SAINT-CRICQ et al. method (1998), using an acid solution of pH 1.

b) Berry skin preparation

Three replicates of 10 berries, randomly sampled, were prepared according to DI STEFANO and CRAVERO’s method (1991). The berry skins of each replicate were manually separated from pulp and seeds, weighed and quickly immersed in 25 mL of a buffer solution containing 12 % (v/v) ethanol, 2 g/L sodium metabisulphite, 5 g/L tartaric acid; the pH was adjusted to 3.20 by the addition of sodium hydroxide. After homogenization at 8,000 rpm for 1 min with a T25 Ultra-turrax (IKA Labortechnik, Staufen, Germany), the extract was separated by centrifugation in a PK 131 centrifuge (ALC International, MI, Italy) for 5 min at 5,000 rpm. The supernatant liquid was quantitatively transferred into a 50 mL calibrated flask, where it was diluted to volume with the buffer solution. Then, anthocyanins were separated by applying the extract dilute (1:1) with 0.05 M sulphuric acid onto a 1 g Sep-Pak C18 cartridge (Waters Corporation, Milford, MA, USA) and eluting with methanol. The cartridge was previously conditioned with methanol (2 mL) and 0.005 M sulphuric acid (4 mL). The methanolic extract was evaporated to dryness using a R-turrax (IKA Labortechnik, Staufen, Germany), packed with LiChrosphere 100 RP-18 (5 µm) particles supplied by Alltech (Deerfield, IL, USA). Chromatographic separation was carried out using a LiChroCart analytical column (25 cm x 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), which was previously conditioned with ethyl alcohol (10 mL) and degassed under vacuum. The following solvents were used: A=10 % formic acid in water; B=10 % formic acid and 50 % methanol in water. All the solvents were filtered through a 0.20 µm PTFE filter (Millipore Corporation, Bedford, MA, USA).

c) Berry seed preparation

Berry seeds of each replicate were quickly immersed in 50 mL of the buffer solution. Afterwards they were maintained at 25 °C for one week (DI STEFANO and CRAVERO, 1991), the supernatant solution was separated by decantation.

4. Analysis

The analytical parameters involved in technological ripeness were determined in grape must, obtained manually. °Brix was measured by refractometry (Atago model ATC-1), while pH was measured by potentiometry using a Crison electrode combined and titratable acidity was determined by acid-alkali titration with potentiometric detection (Crison automatic titrator model Titromatic 1S, with a combined pH electrode). Tartaric acid and malic acid were determined by HPLC. The analysis was isocratically performed at 0.8 mL/min and 65 °C with a 300 x 7.8 mm i.d. cation exchange column (Aminex HPX-87H) equipped with a cation H+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA) (SCHNEIDER et al., 1987). Mobile phase was 0.0026 N sulfuric acid, prepared by diluting reagent grade sulfuric acid with distilled water, filtered through a 0.45 µm membrane filter (Sartorius, AG, Göttingen, Germany) and degassed under vacuum.

Phenolic compounds were determined using a UV-1601PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). The total anthocyanin concentration was expressed as malvidin-3-O-glucoside chloride while flavanols reactive to vanillin (flavanols vanillin assay) were expressed as (+)-catechin (DI STEFANO and CRAVERO, 1991). The proanthocyanidin content was determined after acid hydrolysis with warming (Bate-Smith reaction) using ferrous salt (FeSO4) as catalyst (VIVAS et al., 2004); it was expressed as cyanidin chloride. The relative standard deviations of phenolic compound determination, based on repeated analyses (n = 20) of ten sample extracts, were 1.14, 2.80 and 1.74 % for total anthocyanins, flavanols and proanthocyanidins, respectively.

Anthocyanin profiles were obtained by HPLC-DAD analyses. They were performed using a P100 instrument equipped with a Spectra Focus Diode Array Detector operating at 520 nm, an AS3000 autosampler and a 20 µL Rheodyne sample loop (Spectra Physics Analytical Inc., San Jose, CA, USA). Chromatographic separation was carried out using a LiChroCart analytical column (25 cm x 0.4 cm i.d.) purchased from Merck (Darmstadt, Germany), packed with LiChrospher 100 RP-18 (5 µm) particles supplied by Alltech (Deerfield, IL, USA). The following solvents were used: A=10 % formic acid in water; B=10 % formic acid and 50 % methanol in water. All the solvents were filtered through a 0.20 µm filter. Solvent flow-rate was 1 mL/min. The following solvent A proportions were used: from 72 to 55 %, 15 min; to 30 %, 20 min; to 10 %, 10 min; to 1 %, 5 min; to 72 %, 3 min. An equilibrium time of 10 min was selected (DI STEFANO and CRAVERO, 1991; LOVINO et al., 2006; ROLLE and GUIDONI, 2007). The data treatment was
carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc., San Jose, CA, USA). The identification of the free forms of anthocyanins in berry skin extracts was performed by comparison with external standards. The acylated forms of anthocyanins were identified by matching DAD spectrum and retention time of each chromatographic peak, and by comparing these with available data in the literature (DI STEFANO et al. 1995; POMAR et al., 2005). Individual anthocyanins were expressed in percentages.

5. Statistical analysis

The differences among years and cultivars were studied by analysis of variance (ANOVA) and principal component analysis (PCA) using the SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The classical parameters of technological maturity indexes were determined in the grape must obtained at harvest in two consecutive growing seasons (table 1). Many differences were found among the cultivars in each year considered. In general, in 2006 year a higher ripeness was reached by all the cultivars, with a higher content of sugars and a lower titratable acidity in comparison with 2005. Moreover, significant differences in tartaric and malic content were found for almost all the cultivars, between these two vintages. High values of malic acid (> 3.5 g/L) were found in some Caíño varieties (CB, CR) and in Ferrol. At the comparable Brix value, Merenzao cultivar revealed higher pH value and lower titratable acidity, tartaric acid concentration and malic acid content than Loureira Tinta cultivar.

Table 1 - Classical parameters determined in grape must at harvest time.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Brix</th>
<th>pH</th>
<th>Titratable acidity (as g/L, tartaric acid)</th>
<th>Tartaric acid (g/L)</th>
<th>Malic acid (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>22.0±0.3a</td>
<td>22.0±0.2a</td>
<td>** 3.34±0.02a,b</td>
<td>3.29±0.04a</td>
<td>5.68±0.06a,b</td>
</tr>
<tr>
<td>BR</td>
<td>21.0±0.6b</td>
<td>22.0±0.4a,b **</td>
<td>3.24±0.09a,b</td>
<td>3.25±0.02a</td>
<td>6.22±0.23a</td>
</tr>
<tr>
<td>CB</td>
<td>-</td>
<td>24.0±0.3c,d</td>
<td>**</td>
<td>3.14±0.08b</td>
<td>-</td>
</tr>
<tr>
<td>CL</td>
<td>-</td>
<td>24.0±0.3e</td>
<td>**</td>
<td>3.35±0.04a,c</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>-</td>
<td>22.2±0.2a,b</td>
<td>**</td>
<td>3.26±0.08a,b,c</td>
<td>-</td>
</tr>
<tr>
<td>CR</td>
<td>-</td>
<td>20.0±0.3c</td>
<td>**</td>
<td>3.26±0.08a,b</td>
<td>-</td>
</tr>
<tr>
<td>FE</td>
<td>21.4±0.4a,b</td>
<td>20.8±0.2d</td>
<td>**</td>
<td>3.11±0.08c</td>
<td>3.31±0.12a,c</td>
</tr>
<tr>
<td>GN</td>
<td>20.0±0.04b</td>
<td>21.0±0.5a,d</td>
<td>**</td>
<td>3.40±0.04b,c</td>
<td>3.65±0.07c **</td>
</tr>
<tr>
<td>LT</td>
<td>21.0±1a</td>
<td>26.0±1f</td>
<td>**</td>
<td>2.90±0.06e</td>
<td>3.17±0.03b **</td>
</tr>
<tr>
<td>ME</td>
<td>-</td>
<td>20.8±0.3d</td>
<td>**</td>
<td>3.7±0.05e</td>
<td>-</td>
</tr>
<tr>
<td>MZ</td>
<td>22.0±0.3a</td>
<td>24.0±0.2a,b **</td>
<td>**</td>
<td>3.46±0.05c</td>
<td>3.61±0.04a,c</td>
</tr>
<tr>
<td>MR</td>
<td>18.8±0.2c</td>
<td>24.1±0.2a,b</td>
<td>**</td>
<td>3.47±0.07a</td>
<td>3.69±0.07a</td>
</tr>
<tr>
<td>SO</td>
<td>18.2±0.6c</td>
<td>21.0±0.5b</td>
<td>**</td>
<td>3.15±0.03c</td>
<td>3.40±0.01c **</td>
</tr>
</tbody>
</table>

All data are expressed as mean value ± standard deviation (n=3).

With regard to total anthocyanin concentration in berry skin and its relative profile, the results obtained are presented in table 2. Each year, the total anthocyanin concentration for Loureira Tinta cultivar (> 2,500 mg/kg grape) was significantly higher than that in other cultivars. In spite of the differences between the two vintages, the lowest total anthocyanin concentration corresponded to the Merenzao cultivar, followed by Albarello and Brancellao cultivars whose anthocyanin contents ranged form 190 to 600 mg/kg. High contents of these red pigments (> 1,400 mg/kg) were also found in each year for Sousón and Ferrol varieties. Therefore, in general, many varieties of ampelographic patrimony of Galicia showed similar or higher anthocyanin concentrations than others cultivated in Spain. In fact, Cabernet Sauvignon and Tempranillo grape varieties, cultivated in Toledo (Spain), reached maximum values of total anthocyanin concentration ranging from 839 to 944 mg/kg and comprised between 719 and 916 mg/kg, respectively (RYAN and REVILLA, 2003). Furthermore, Tempranillo grapes, growing in Andalucía (Southern Spain), showed the same amount of total anthocyanins (944 mg/ kg grapes), while other autochthonous cultivars of this region such as Jean tinto, Palomino negro and Tintilla de Rota were characterized by anthocyanin contents of 906, 1,573, and 2,640 mg/kg grapes, respectively (GUERRERO et al., 2009). In several vineyards located in Jumilla (South-East Spain), Monastrell grapes presented an anthocyanin content which ranged from 750 to 1,100 mg/kg (ORTEGA-REGULES et al., 2008).

Looking at the pattern of anthocyanins, it can be deduced that the major anthocyanin group was the monoglucoside forms, ranging from 49.1 or 50.7 % for

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Mouratón cultivar in 2005 or 2006, respectively, to 97.4 % for Caíño da Terra cultivar in 2006. In the first one, the cinnamoyl glucoside group represented an average of 45.3 % of total anthocyanin content. The presence of acylated anthocyanins may be very important for wine colour since they participate in an intra-molecular copigmentation process, thus increasing the wine colour intensity (ORTEGA-REGULES et al., 2006).

The most abundant anthocyanin compounds in grape skin were malvidin monoglucoside derivative forms (from 40.6 to 84.9 %), except for Albarello, Brancellao and Caíño da Terra cultivars. The cultivars with the highest amount of these compounds were Merenzao and Mouratón (79.1-84.9 %) in 2005, and Mencía and Caíño Longo (79.7-83.5 %) in 2006. On the other hand, the lowest relative amount of malvidin monoglucoside derivative forms (37.3-42.5 %) was found for Brancellao, Albarello, Gran Negro, Ferrol and Loureira Tinta cultivars in 2005, whereas Albarello, Brancellao and Caíño da Terra cultivars contained the lowest values in 2006 (37.1-39.5 %). Nevertheless, significantly different concentrations of these compounds were found in many cultivars between 2005 and 2006.

Peonidin monoglucoside derivative forms, in particular peonidin-3-glucoside, were usually the second most abundant anthocyanin. However, high contents were found for delphinidin-3-glucoside in Caíño Bravo cultivar (13.6%), and for petunidin-3-glucoside (from 12.5 to 20.7 %) and delphinidin-3-glucoside (from 12.0 to 26.7 %) in Caíño Redondo, Ferrol, Loureira Tinta and Sousón cultivars. The accumulation of peonidin derivative forms in grape skin was significantly higher for Gran Negro cultivar in 2005 (53.1%). In contrast, the lowest concentrations were found for Sousón cultivar in both 2005 and 2006 (mean 5.2 %). Furthermore, Brancellao cultivar presented similar values of malvidin and peonidin derivative forms in both years; the same behavior was observed for Caíño da Terra cultivar in the 2006 vintage.

Peonidin-3-glucoside and malvidin-3-glucoside are stable anthocyanins; they are the ultimate forms in the anthocyanin biosynthesis (ROGGERO et al., 1986). The presence of stable molecules, such as tri-substituted anthocyanins like malvidin derivative forms, would give stability to the wine colour during winemaking because these compounds are more resistant to oxidation (RIBÉREAU-GAYON et al., 2003). In the international varieties, such as Syrah, Cabernet Sauvignon and Merlot grapes, cultivated in Murcia (South Spain), the malvidin-3-glucoside content was high, ranging from 67 to 77 % (ORTEGA-REGULES et al., 2006).

Table 2 - Total anthocyanin concentration and relative profile in berry skin.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Total anthocyanins (mg/kg grape)</th>
<th>Simple glucosides</th>
<th>Acetyl-glucosides</th>
<th>Cinnamoyl-glucosides</th>
<th>Σ delphinidin derivative forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>425±4a</td>
<td>432±2a</td>
<td>ns</td>
<td>71.5±0.8</td>
<td>73.8±2.8</td>
</tr>
<tr>
<td>BR</td>
<td>600±23b</td>
<td>457±17a</td>
<td>**</td>
<td>79.0±0.7</td>
<td>75.8±1.0</td>
</tr>
<tr>
<td>CB</td>
<td>882±11b</td>
<td>nd</td>
<td>-</td>
<td>83.6±0.2</td>
<td>nd</td>
</tr>
<tr>
<td>CL</td>
<td>-</td>
<td>1103±0c</td>
<td>nd</td>
<td>83.1±0.6</td>
<td>nd</td>
</tr>
<tr>
<td>GR</td>
<td>775±31d</td>
<td>nd</td>
<td>-</td>
<td>77.7±0.3</td>
<td>nd</td>
</tr>
<tr>
<td>CT</td>
<td>-</td>
<td>788±37d</td>
<td>nd</td>
<td>-</td>
<td>97.4±0.4</td>
</tr>
<tr>
<td>FE</td>
<td>1440±81c</td>
<td>1598±9a</td>
<td>e</td>
<td>70.0±0.4</td>
<td>64.1±1.4</td>
</tr>
<tr>
<td>GN</td>
<td>1962±36d</td>
<td>1902±36e</td>
<td>nd</td>
<td>75.3±1.8</td>
<td>75.2±1.4</td>
</tr>
<tr>
<td>LT</td>
<td>2524±51e</td>
<td>2860±55g</td>
<td>*</td>
<td>71.8±2.3</td>
<td>66.0±1.0</td>
</tr>
<tr>
<td>ME</td>
<td>893±24b</td>
<td>nd</td>
<td>58.2±1.0</td>
<td>nd</td>
<td>18.3±0.1</td>
</tr>
<tr>
<td>MR</td>
<td>335±17f</td>
<td>191±10h</td>
<td>**</td>
<td>69.6±1.2</td>
<td>61.1±2.6</td>
</tr>
<tr>
<td>SO</td>
<td>712±52a</td>
<td>191±10i</td>
<td>**</td>
<td>88.0±5.0</td>
<td>85.5±2.0</td>
</tr>
</tbody>
</table>

All data are expressed as mean value ± type deviation (n=3). Different letters within the same column mean significant differences according to a Tukey test (p<0.01). Non available (nd), non significant (ns), significantly different according to a Tukey test for p<0.05 (*), significantly different according to a Tukey test for p<0.01 (**), Albarello (AL), Brancellao (BR), Caíño Bravo (CB), Caíño Longo (CL), Caíño da Terra (CT), Caíño Redondo (CR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Mencía (ME), Merenzao (MZ), Mouratón (MR) and Sousón (SO).
ratio between malvidin and peonidin derivative forms could be used as varietal markers (MONAGAS et al., 2003). Thus, this ratio was lower for Albarello, Brancellao and Caño da Terra cultivars whereas it was higher for Caño Longo and Mencía cultivars. Furthermore, both anthocyanin monoglucosides increase the probability of the interaction with flavanols and ethanol, leading to a stable red pigmentation of the wine (LOVINO et al., 2006).

Brancellao, Loureira Tinta and Ferrol in 2005, Brancellao, Caño da Terra, Albarello and Loureira Tinta in 2006 were the most abundant cultivars in cyanidin derivative forms with percentages from 5.3 to 8.9 %. Nevertheless, small amounts (1.1 %) were found for Gran Negro, Merenzao and Mouratón cultivars in both 2005 and 2006, as well as Mencía, Caño Redondo and Caño Longo in 2006 year.

During the course of winemaking, grapes rich in cyanidin-3-glucoside and peonidin-3-glucoside showed a remarkable loss of di-substituted anthocyanins, easily extractable from the first phases of maceration, due to the complex processes of combination, oxidation and insolubilisation involving anthocyanin-like substances (ATANASOVA et al., 2002; GERBI et al., 2002; ROLLE et al., 2008; ROLLE et al., 2009).

About the delphinidin derivative forms, the highest concentration was found for Sousón, Ferrol and Loureira Tinta cultivars in 2005 (21.0-26.7 %). On the other hand, the lowest content of these anthocyanins corresponded to Merenzao and Gran Negro cultivars in 2005 (1.2-1.8 %), and to Albarello cultivar in 2006 (1.6 %). Furthermore, the delphinidin-3-glucoside derivative concentration determined for Albarello, Ferrol, Loureira Tinta and Sousón cultivars was significantly different between both 2005 and 2006.

Ferrol, Loureira Tinta and Sousón cultivars contained petunidin derivative concentrations significantly higher in 2005 and 2006 (from 13.8 to 20.7 %). Moreover, the lowest values were associated with Gran Negro cultivar in both 2005 (2.5 %) and 2006 (3.6 %).

Concentrating on the differences found at harvest in the anthocyanin fingerprint among grape cultivars (table 2), principal component analysis (PCA) was performed to confirm the genetic heterogeneity and to establish the relationships among anthocyanin profile and the red grape varieties cultivated in Galicia. In 2005 (figure 1, table 3), two principal components explained 81.1 % of the variance in the original data. Component 1 explained 52.9 %, being mainly associated with the sum of the compounds of delphinidin and petunidin. Component 2 explained 28.2 %, being mainly associated with the sum of the compounds of peonidin. According to some authors (TAMBORRA et al., 2003, NUÑEZ et al., 2004), the percentage of acylated anthocyanins are those that most contribute to varietal differentiation. PCA was again performed to for the year 2006 (figure 2, table 3). Three principal components explained 91.0 % of the variance in the original data. Component 1 explained 45.3 %, being mainly associated with sum of the compounds of peonidin. On the other hand, component

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Two dimensional plot of the two first principal components in PCA of the grape cultivars studied in 2005. Albarello (AL), Brancellao (BR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Merenzao (MZ), Mouratón (MR) and Sousón (SO).
explained 29.5% and it is mainly associated with sum of the delphinidin derivatives. Nevertheless, non-acylated anthocyanins also contributed to varietal characterization, in contrast to that reported by other authors (TAMBORRA et al., 2003).

PCA suggests that varietal differences found in the anthocyanin fingerprint are mainly due to the relative amounts of cyanidin and malvidin forms. This is due to the fact that both anthocyanin derivatives showed values of cumulative variance comprising between 36.1 and 43.0% in both years.

The anthocyanin profile is related to not only to the grape cultivar but also the climatic conditions during the growing season (RYAN and REVILLA, 2003). The results obtained agree with previous work (RYAN and REVILLA, 2003), in which discriminant analysis indicated that the percentages of primitive anthocyanins (delphinidin-3-glucoside and petunidin-3-glucoside) may be significant indicators of weather conditions during ripening probably as a consequence of the modulation of anthocyanin biosynthesis.

Finally, the anthocyanin profiles proposed were also in good agreement with those published by Letaief et al. (2007) for Mencia, Merenzao and Brancellao cultivars, excepting malvidin derivatives for the latter in which a difference of 24% was observed.

Table 4 reports the proanthocyanidin (condensed tannins) and flavanol reactive to vanillin (monomeric and oligomeric tannins) content in berry skins and seeds, obtained by using two different spectrophotometric methods.

Table 4 - Content of total proanthocyanidins and flavanols in grape berries.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Skin flavanols vanillin assay (mg/g grape)</th>
<th>Skin proanthocyanidins (mg/kg grape)</th>
<th>Seed flavanols vanillin assay (mg/g grape)</th>
<th>Seed proanthocyanidins (mg/kg grape)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>2.27±40a</td>
<td>3.1±4a</td>
<td>**</td>
<td>749±52a</td>
</tr>
<tr>
<td>BR</td>
<td>87±5b</td>
<td>32.2±118b,c,d</td>
<td>*</td>
<td>731±89a</td>
</tr>
<tr>
<td>CB</td>
<td>-</td>
<td>305±148b,c,d</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>CL</td>
<td>-</td>
<td>200±148b,e</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>CR</td>
<td>-</td>
<td>125±137b,e</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>-</td>
<td>156±137b,e</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>FE</td>
<td>144±78a,b</td>
<td>365±40c</td>
<td>**</td>
<td>2175±175b</td>
</tr>
<tr>
<td>GN</td>
<td>80±102b</td>
<td>467±53d</td>
<td>*</td>
<td>924±91c</td>
</tr>
<tr>
<td>LT</td>
<td>156±44a,b</td>
<td>97±114a,b,e</td>
<td>ns</td>
<td>1365±73d</td>
</tr>
<tr>
<td>ME</td>
<td>-</td>
<td>117±45e</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>MZ</td>
<td>232±42a,c</td>
<td>345±48c</td>
<td>*</td>
<td>1061±208a,e</td>
</tr>
<tr>
<td>MR</td>
<td>148±194a,b</td>
<td>107±45e</td>
<td>ns</td>
<td>1054±12e</td>
</tr>
<tr>
<td>SO</td>
<td>298±46c</td>
<td>199±208b,c,d</td>
<td>nd</td>
<td>1220±277c,d</td>
</tr>
</tbody>
</table>

All data are expressed as mean value ± deviation (n=10). Differences letters within the same column mean significant differences according to Tukey's test (p<0.01). Non available (nd), non significant (ns), significantly different according to a Tukey test for p<0.05 (*), significantly different according to a Tukey test for p<0.01 (**), Albarrello (AL), Brancellao (BR), Caño Bravo (CB), Caño Longo (CL), Caño da Terra (CT), Caño Redondo (CR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Mencia (ME), Merenzao (MZ), Mouratón (MR) and Sousón (SO).
the concentration of flavan-3-ols in skin tannin extract were lower than in seed tannin extract (CHIRA et al., 2009). In contrast, the opposite was noticed in studies on Italian varieties (CRAVERO and DI STEFANO, 1992; MATIVI et al., 2003).

Total proanthocyanidin content in berry skin was significantly greater for Ferrol and Loureira Tinta cultivars ranging from 1.438 to 2.116 mg/kg grapes (mean values between 2005 and 2006 year), whereas the lowest values corresponded to Caño da Terra cultivar (350 mg/kg). Low amounts of flavanols reactive to vanillin were found in the berry skins of all Galician cultivars (< 467 mg/kg grape). The low flavanols reactive to vanillin and proanthocyanidin ratios, lying between 0.06 and 0.45, indicate that skin tannins are probably highly polymerized. The ratio ranged from 0.39 to 1.12 for seed tannins, with high values for Caño Redondo, Albarello, Caño da Terra, Loureira Tinta, Ferrol and Caño Longo cultivars. The latter cultivars could lead to greater flavanol reactivity due to the low degree of tannin polymerization.

In particular, Caño Bravo cultivar contains the highest total amount of flavanol in berry seeds (8.081 mg/kg grape), while the lowest contents were associated with Mouratón cultivar (mean value of 1.071 mg/kg). Caño Bravo variety also presented total proanthocyanidin concentrations in berry seeds (9,982 mg/kg grapes), statistically higher than those corresponding to other cultivars. Moreover, the lowest total proanthocyanidin concentrations in seeds were found for Gran Negro cultivar (mean value of 1,071 mg/kg). Caño Bravo variety also presented total proanthocyanidin ratios, lying between 0.06 and 0.45, that indicate skin tannins are probably highly polymerized. The ratio ranged from 0.39 to 1.12 for seed tannins, with high values for Caño Redondo, Albarello, Caño da Terra, Loureira Tinta, Ferrol and Caño Longo cultivars. The latter cultivars could lead to greater flavanol reactivity due to the low degree of tannin polymerization.

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In general a high content of flavanol reactive to vanillin and proanthocyanidins characterizes the seeds of all varieties studied. This aspect needs to be considered more seriously by the winemakers in these geographical areas to reduce a possible excess of bitterness and astringency in the wines, by the optimization of maceration and winemaking processes.

Finally, more studies with grapes from different production areas in Galicia should be carried out to complete these observations.

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