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

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Abstract

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

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-MAGARIÑO and GONZÁLES-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; LETAIEF *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 acetyl, *p*-coumaroyl and caffeoyl 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 astringency (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 wine grape cultivars is available (POMAR *et al.*, 2005; LETAIEF *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 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), 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

Solvents 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 Extrasynthèse (Genay, France). All the standards were stored at -20 ℃ 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-200 rotating evaporator (Büchi, Flawil, Switzerland) under reduced pressure at 35 ℃ and resuspended in the solvent B used in HPLC analysis. All the extracts 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 ℃ 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. ºBrix 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 sulphuric acid, prepared by diluting reagent grade sulphuric 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 (Shimazdu 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 LiChrosphere 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 ChromQuestTM 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 ºBrix value, Merenzao cultivar revealed higher pH value and lower titratable acidity, tartaric acid concentration and malic acid content than Loureira Tinta cultivar.

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 ampelografic 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

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

Cultivar	Brix			pH			Titratable acidity (as g/L tartaric acid)			Tartaric acid (g/L)			Malic acid (g/L)		
	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign
AL	22,0±0,3a	22,6±0,2a	ns	3,35±0,02a,b	3,29±0,04a	ns	5,88±0,06a	5,70±0,07a	*	7,1±0,1a	7,3±0,2a,b	ns	1,0±0,2a	1,4±0,1a,b	*
BR	21,0±0,0b	22,0±0,4a,b	**	3,24±0,09a,c	3,29±0,02a	ns	6,22±0,23b	5,76±0,07a	**	7,5±0,1b	7,3±0,1a,b	ns	1,1±0,1a	1,2±0,1a	ns
CB	-	20,4±0,3c,d	nd	-	3,14±0,08b	nd	-	9,14±0,33b	nd	-	7,1±0,0a	nd	-	4,6±0,1c	nd
CL	-	24,0±0,3e	nd	-	3,35±0,04a,c	nd	-	6,38±0,28c	nd	-	6,0±0,2c,d	nd	-	3,0±0,1d	nd
CT	-	22,2±0,2a,b	nd	-	3,26±0,08a,b	nd	-	7,38±0,30d	nd	-	7,7±0,1e	nd	-	2,9±0,2d	nd
CR	-	20,0±0,3c	nd	-	3,26±0,08a,b	nd	-	7,50±0,33d	nd	-	6,2±0,2c,d	nd	-	3,9±0,2e	nd
FE	21,4±0,4a,b	20,8±0,2d	ns	3,11±0,08c	3,31±0,12a,c	ns	15,12±0,30c	8,85±0,24b	**	8,8±0,1c	7,0±0,1a	**	7,4±0,1b	4,4±0,1c	**
GN	20,6±0,4b	21,0±0,5b,d	ns	3,40±0,04b,d	3,55±0,07d	*	4,90±0,05d	4,54±0,15e	**	6,8±0,1d	6,3±0,1c	**	1,2±0,1a	0,9±0,0f	**
LT	21,8±0,1a	26,0±0,1f	**	2,90±0,06e	3,17±0,03b	**	12,12±0,10e	7,28±0,23d	**	10,2±0,0e	7,6±0,3b,e	**	2,0±0,1c	2,3±0,1g	*
ME	-	20,8±0,3d	nd	-	3,71±0,05e	nd	-	4,27±0,27e,f	nd	-	5,7±0,3d	nd	-	2,3±0,1g	nd
MZ	22,0±0,3a	24,2±0,3e	**	3,46±0,05d	3,61±0,04d,e	**	4,15±0,03f	4,01±0,08f	*	5,6±0,0f	5,9±0,2d	ns	1,0±0,0a	1,2±0,1a	*
MR	18,8±0,2c	22,4±0,1a,b	**	3,47±0,07d	3,69±0,07d,e	**	5,22±0,04g	4,23±0,06f	**	4,6±0,1g	5,9±0,1d	**	2,2±0,1c	2,0±0,1h	ns
SO	18,2±0,4c	21,8±0,5b	**	3,16±0,03c	3,40±0,01c	**	10,94±0,71h	5,32±0,44a	**	8,3±0,3h	5,8±0,2d	**	3,0±0,2d	1,7±0,3b,h	**

All data are expressed as mean value ± standard deviation (n=3).
Different letters within the same column mean significant differences according to a Tukey test (p<0.01). Not 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).

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

Cultivar	Total anthocyanins (mg/kg grape)			Simple glucosides			Acetyl-glucosides			Cinnamoyl-glucosides			∑ delphinidin derivative forms		
	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign
AL	425±44a	432±29a	ns	71,5±0,8a	73,8±2,6a	ns	8,7±0,3a	5,5±0,8a	**	19,8±0,5a	20,7±2,1a,b	ns	11,3±0,9a	1,6±1,0a	**
BR	600±23b	457±17a	**	79,0±0,7b	75,8±1,0a	ns	5,3±0,4b	6,1±0,5a	ns	15,6±0,4b	18,1±1,2a,c	ns	6,9±0,3b	5,5±0,2b	ns
CB	-	882±11b	nd	-	83,6±0,2b	nd	-	4,2±0,1a,b	nd	-	12,2±0,1d,e	nd	-	13,6±2,3c,d	nd
CL	-	1103±20c	nd	-	83,1±0,6b	nd	-	4,1±0,2a,b	nd	-	12,9±0,4d,e,f	nd	-	3,7±0,1a,b	nd
CR	-	775±31d	nd	-	77,7±3,9a	nd	-	4,2±0,4a,b	nd	-	18,1±3,5a,c	nd	-	12,0±1,9c	nd
CT	-	788±37d	nd	-	97,4±0,4c	nd	-	0,5±0,1d	nd	-	2,1±0,3g	nd	-	5,8±0,7b	nd
FE	1446±81c	1589±64e	ns	70,0±0,8a	64,1±1,4d,e	**	19,3±0,4a	19,7±2,1c	ns	10,7±0,6c,d	16,3±0,9c,f	**	25,0±0,9c	13,1±1,0c,d	**
GN	1062±36d	1005±39f	ns	78,3±1,8b	75,2±1,4a	ns	2,9±0,4c	1,3±0,2b,d	ns	18,9±1,4a	23,5±1,5b	**	1,2±0,3d	2,3±0,2a	ns
LT	2524±51e	2660±53g	*	71,8±2,3a	66,0±1,0e	**	17,1±0,4d	18,8±0,6c	ns	11,1±2,0c	15,2±0,4c,d,f	**	26,7±2,9c	15,5±0,6d,e	**
ME	-	893±24b	nd	-	58,2±1,0f	nd	-	18,3±0,1c	nd	-	23,5±1,1b	nd	-	5,9±1,1b	nd
MZ	335±17f	191±10h	**	66,6±1,2c	61,1±2,3d,f	**	9,1±1,1a	5,6±2,9a	**	24,3±1,8e	33,3±1,4h	**	1,8±0,2d	4,2±0,3a,b	ns
MR	1033±37d	821±11d	**	49,1±0,2d	50,7±1,5g	ns	4,2±0,3e	5,4±0,2a	ns	46,7±0,2f	43,9±1,4i	ns	5,5±0,2b	5,8±0,5b	ns
SO	1732±5g	1913±5i	**	88,0±0,8e	85,5±0,2b	ns	3,8±0,2c,e	4,3±0,0a,b	ns	8,2±0,7d	10,3±0,2e	ns	21,0±1,2e	17,9±1,1e	**

	∑ cyanidin derivative forms			∑ petunidin derivative forms			∑ peonidin derivative forms			∑ malvidin derivative forms		
	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign
AL	2,1±0,3a	6,9±0,2a	**	12,2±0,6a	4,8±0,7a	**	37,3±2,0a	49,6±1,8a	**	37,2±1,7a	37,1±1,9a	ns
BR	6,9±0,7b	7,6±0,3a	ns	8,7±0,3b	7,2±0,4a,b	ns	40,2±3,1a	40,2±0,6b	ns	37,2±3,1a	39,5±0,9a	ns
CB	-	1,4±0,2b,c	nd	-	8,8±6,3a,b,c	nd	-	10,6±0,7c,d	nd	-	65,5±3,5b	nd
CL	-	0,5±0,2b	nd	-	6,3±0,3a	nd	-	6,0±2,2c,d	nd	-	83,5±2,1c	nd
CR	-	0,9±0,3b,c	nd	-	12,5±1,2b,c,d	nd	-	5,8±0,8c,d	nd	-	68,8±3,4b,d	nd
CT	-	8,9±0,8d	nd	-	7,5±0,4a,b	nd	-	40,6±2,9b	nd	-	37,2±2,4a	nd
FE	6,7±0,3b	2,8±0,2e	**	18,7±0,1c	14,5±0,7d	**	8,2±0,1b	7,8±1,1c,d	ns	41,4±1,1a	61,8±1,3b	**
GN	0,7±0,1a	0,8±0,2b,c	ns	2,5±0,2d	3,6±0,2a	ns	53,1±2,4c	31,1±5,5e	**	42,5±2,3a	62,2±5,8b	**
LT	5,9±1,9b	5,3±0,6f	ns	20,7±0,7e	15,3±0,1d	**	5,9±0,8b	11,1±0,7c,d	ns	40,6±6,2a	52,9±1,0e	**
ME	-	0,6±0,1b	nd	-	7,5±0,9a,b	nd	-	6,4±0,6c,d	nd	-	79,7±1,6c,f	nd
MZ	0,4±0,1a	0,9±0,4b,c	ns	4,0±0,6f	7,9±1,1a,b	ns	9,0±1,4b	11,8±2,3c	ns	84,9±1,6b	75,2±1,2d,f	**
MR	0,7±0,0a	1,1±0,2b,c	ns	7,9±0,2b	9,1±0,7a,b,c	ns	6,8±0,7b	9,6±1,5c,d	ns	79,1±0,3b	74,5±2,8d,f	ns
SO	2,0±0,4a	1,7±0,4c	ns	15,1±0,5g	13,8±0,7c,d	ns	5,2±0,4b	5,3±0,7d	ns	56,7±2,2c	61,3±2,8b	ns

All data are expressed as mean value ± typic 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).

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.

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).

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

Table 3 - Component principal matrix

	2005		2006		
	Component		Component		
	1	2	1	2	3
Simple glucosides	0,468	-0,727	-0,715	0,376	-0,583
Acetyl-glucosides	0,731	0,451	0,512	0,383	0,640
Cinnamoyl-glucosides	-0,804	0,444	0,538	-0,702	0,297
∑ of delphinidin derivative forms	0,914	0,302	0,348	0,866	-0,104
∑ of cyanidin derivative forms	0,813	-0,045	-0,825	0,256	0,446
∑ of petunidin derivative forms	0,897	0,349	0,429	0,790	0,171
∑ of peonidin derivative forms	-0,251	-0,874	-0,911	-0,273	0,274
∑ of malvidin derivative forms	-0,682	0,593	0,864	-0,263	-0,375

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 Caíño da Terra cultivars whereas it was higher for Caíño 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, Caíño 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, Caíño Redondo and Caíño 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, NÚÑEZ *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
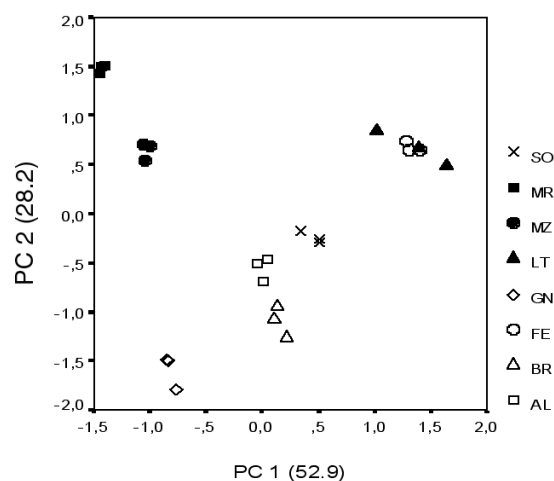

Figure 1 - Two dimensional plot of the two first principal components in PCA of the grape cultivars studied in 2005.
Albarello (AL), Brancellao (BR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Merenzao (MZ), Mouratón (MR) and Sousón (SO).

2 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 Mencía, 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.
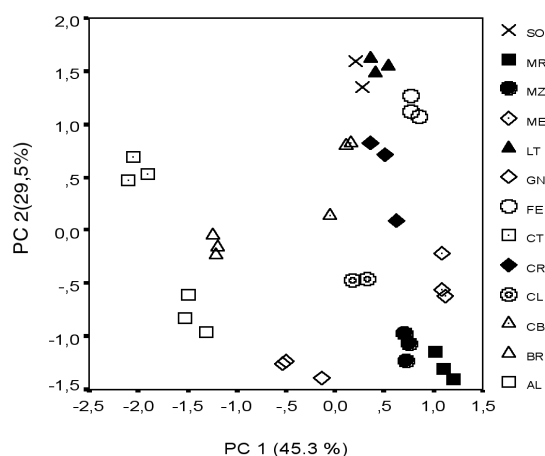

Figure 2 - Two dimensional plot of the two first principal components in PCA of the grape cultivars studied in 2006.
Albarello (AL), Brancellao (BR), Caíño Bravo (CB), Caíño Longo (CL), Caíño da Terra (CT), Caíño Redondo (CR), Ferrol (FE), Gran Negro (GN), Loureira Tinta (LT), Mencía (ME), Merenzao (MZ), Mouratón (MR) and Sousón (SO).

No studies about both the classes of flavanols in red Galician grape cultivars were previously available. Furthermore, regarding Spanish varieties, little information on these parameters has been published in the literature (CANALS et al., 2005; ORTEGA-REGULES et al., 2008; RODRÍGUEZ MONTEALEGRE et al., 2006). Flavan-3-ol monomers were not investigated in this study.

In Galician vine, lower concentrations of tannins, namely proanthocyanidins and flavanols reactive to vanillin, were found in skins compared to seeds. In Cabernet-Sauvignon and Merlot grapes from Bordeaux,

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

Cultivar	Skin flavanols vanillin assay (mg/kg grape)			Skin proanthocyanidins (mg/kg grape)			Seed flavanols vanillin assay (mg/kg grape)			Seed proanthocyanidins (mg/kg grape)		
	2005	2006	sign	2005	2006	sign	2005	2006	sign	2005	2006	sign
AL	227±40a	31±4a	**	749±82a	525±2a	**	7936±1106a	4118±600a,b	**	7607±1590a,b	5412±1395a,b,c,d,e	ns
BR	87±95b	322±118b,c,d	*	731±80a	1249±103c,d	**	2599±286b	2970±166c	ns	4709±799c	4602±147a	ns
CB	-	305±148b,c,d	nd	-	1388±189c,d	nd	-	8081±262d	nd	-	9982±287f	nd
CL	-	200±48b,e	nd	-	617±50e	nd	-	4009±129a	nd	-	4804±311a	nd
CR	-	125±87b,e	nd	-	830±134f	nd	-	5979±737e	nd	-	5346±959a,c,d	nd
CT	-	156±89b,e	nd	-	350±14g	nd	-	5001±1254a,b,e	nd	-	5055±313a,c	nd
FE	144±78a,b	366±40c	**	2175±175b	2057±69h	ns	6519±1431a,c	4755±225b	**	7512±1184a	6804±358b	ns
GN	80±102b	467±53d	**	924±81c	1355±198c,d	**	1643±152d	1916±694f	ns	1913±126d	3373±757e,g	**
LT	158±44a,b	97±114a,b,e	ns	1363±73d	1513±267c	ns	4942±660c,e	4972±340b,e	ns	5933±247b	5383±141c	**
ME	-	117±45e	nd	-	1220±71c,d	nd	-	2694±299c,f	nd	-	3659±306g	nd
MZ	232±42a,c	345±48c	*	1061±208c,e	943±280d,e,f	ns	3842±874e,f	4504±585a,b	ns	6092±374a,b	6759±834b,d	ns
MR	148±104a,b	107±43e	ns	1054±12e	854±133f	**	1193±110g	949±122g	*	3060±154e	2440±1132g	ns
SO	338±64c	199±209a,b,c,e	ns	1228±277c,d,e	1119±159c,d	ns	3525±630f	2401±402c,f	**	6481±871a,b	4759±445a	**

All data are expressed as mean value ± typic 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).

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; MATTIVI *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 Caíño 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 Caíño Redondo, Albarello, Caíño da Terra, Loureira Tinta, Ferrol and Caíño Longo cultivars. The latter cultivars could lead to greater flavanol reactivity due to the low degree of tannin polymerization.

In particular, Caíño 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). Caíño 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 in 2005 vintage (1,913 mg/kg grapes) and Mouratón cultivar in 2006 (2,440 mg/kg grapes).

CONCLUSION

The study determined the phenolic composition and anthocyanin profile of the most frequently grown red winegrapes in Galicia.

Notable differences among the cultivars studied were found in both parameters, confirming their importance in the varietal characterization as well as in the assessment of potential oenological value.

The highest concentrations of total anthocyanins corresponded to Loureira Tinta, Sousón and Ferrol varieties, malvidin-3-monoglucoside being usually the major anthocyanin. Nevertheless, anthocyanin profile was made up mainly of peonidin derivative forms for Albarello, Brancellao, Gran Negro and Caíño da Terra grapes.

In general a high content of flavanol reactive to vanillin and of 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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