Phenolic characterization of four different red varieties with "Caíño" denomination cultivated in Northwestern Spain

Caractérisation phénolique de quatre cépages rouges appelés 'Caiño' cultivés dans le Nord-Ouest de l'Espagne

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Abstract

In this work, these four red varieties were characterized in terms of phenolic composition. Thus, the anthocyanin accumulation and the extractability evolution during ripening were compared. The extractability assays were carried out using similar pH conditions than those involved in winemaking process. Furthermore, seed phenolic maturity was determined in order to obtain information about the tannin aggressiveness. These parameters are of great importance not only for the varietal differentiation but also for the planning and management of winemaking process. On the other hand, the anthocyanin distribution was determined because it permits the assessment of varietal differentiation, being it considered as a taxonomic characteristic.

Total anthocyanin concentration was significantly greater for Caíño Longo variety, while extractable anthocyanin content for this variety was similar than that corresponding to Caíño Astureses variety. Furthermore, the lowest total and extractable anthocyanin concentrations were associated with Caíño Redondo and Caíño da Terra cultivars. Thus, the extraction facility showed good skin ripeness grade only for Caíño Astureses variety. Furthermore, the seed ripeness resulted to be particularly incomplete for Caíño Astureses and Caíño Redondo varieties, which indicates their worse adaptation to the "terroir". Malvidin glucoside was the majority anthocyanin in all the varieties studied, excepting the Caíño da Terra variety. The Caíño Longo cultivar showed results statistically higher, while the Caíño da Terra cultivar presented the lowest values of this compound.

Keywords*:* Caíño; phenolic characterization; anthocyanin; accumulation; extractability.

Introduction

In Galicia, the growing interest of the winery for the elaboration of high quality red wines, with particular sensory characteristics, requires a greater implication in the study of the potential offered by the minority red varieties. Caíño has potential for producing quality wines. The first differentiation of red varieties with Caíño denomination cultivated in Northwestern Spain was reported in 1997, identifying Caíño Bravo and Caíño Gordo by morphology. Furthermore, other authors described Caíño Blanco, Caíño do Freixo, Caíño Redondo and Caíño Longo as Galician cultivars. On the other hand, Caíño from Galicia is recorded in the *Registro de Variedades Comerciales* as Caíño Tinto, being synonyms Caíño Bravo or Cachón (BOE, 2005). The combination of molecular markers and ampelometric methods was considered important in order to identify synonymies and homonymies.

The phenolic profile, as determined by the relationship between different compounds, is characteristic of each variety and it is considered as a chemotaxonomic parameter for the classification and differentiation of red varieties of *Vitis vinifera*. However, their concentration may vary significantly depending on the environmental and agronomic conditions. In this regard, some studies recently published also show differences in the anthocyanin profiles as a result of agroecological factors (Downey et al., 2004; Gonzalez-Neves et al., 2004; Guidoni et al., 2002 ; Ryan and Revilla, 2003).

In this sense, the investigation of the grape varieties with "Caíño" denomination is very important because of those seem to be Galician native varieties. The four cultivars characterized in relation to phenolic ripeness and anthocyanin profile were Caíño Redondo, Caíño Longo, Caíño Astureses and Caíño da Terra. They were cultivated in the Estación de Viticultura e Enoloxía de Galicia (EVEGA), located in Ribeiro region; one of the Galician zones with great viticole tradition. Therefore, these grape varieties were cultivated under the same soil and climatic conditions.

Materials and methods

Location and vegetal material

The experiment was carried out in the Estación de Viticultura e Enoloxía de Galicia (EVEGA); located in the municipality of Leiro (Ourense) and included in the geographical area of Denomination of Origen Ribeiro. The cultivars studied were Caíño Redondo (CR), Caíño Longo (CL), Caíño Astureses (CA) and Caíño da Terra (CT). Average age of grapevine is 15 years old; grafted onto the 196-17 C rootstock with trellis pruning and Cordon Royat training. They were planted at 1.2 m x 1.8 m with a height of 60 cm.

Sample collection and preparation

Each sample consisted of 10 bunches picked randomly from 10 different plants. Once in the laboratory, a set of 300 grape berries of each variety was randomly hand picked without detaching the pedicel. The berries were visually inspected before analysis and those with damaged skins were discarded.

Afterwards, the evolution of phenolic maturity during ripening was performed following the methodology proposed by Saint-Cricq et al. (1998). So, 100 grape berries of each cultivar were weighed and triturated using a conventional blender. Then, 50 g sample homogenized were introduced in two erlenmeyer flasks. An identical volume of an aqueous solution at pH 1 was added to the first erlenmeyer flask, while the same volume of an aqueous solution at pH 3.2 was added to the second one. After handly shaking every flask, they were left to stand for 4 h. Finally, the suspensions were centrifuged at 4000 rpm for 5 min and the supernatant fluid was after filtered with a paper filter of pore diameter comprised between 20 and 25 μm. The procedure was performed in duplicate.

Moreover, 10 berries of each cultivar were weighed being skins separated, which were also weighed. Then, skins were introduced in 25 mL of buffer dissolution, facilitating the extraction of phenolic compounds by crushing and homogenization with Ultraturrax for 1 min (Di Stefano and Cravero, 1991). The buffer dissolution contains 12 % ethanol (v /v), 2 g/L of sodium metabisulphite, 5 g/L tartaric acid and pH value was adjusted to 3.2 with sodium hydroxide. The supernatant solution obtained from the centrifugation at 4000 rpm for 5 min was diluted to 50 mL with the buffer dissolution. Then, the resulting solution, previously diluted 1:1 with 0.1 N sulphuric acid, was submitted to solid phase extraction procedure, using 1 g Sep-Pak C_{18} cartridges previously activated with 2 mL of methanol and 4 mL of 0.01 N sulphuric acid. The eluent used was methanol. The red fraction was collected, which was evaporated to dryness in a vacuum system (Universal Vacuum System Thermo UVS400A model) to 35 °C. Finally, the residue obtained was dissolved in the mobile phase. This procedure was performed in triplicate.

Analytical methodology

The classic parameters ºBrix, pH and total acidity were determined in the must obtained by pressing and subsequent filtration, using refractometry (Atago, model ATC-1), potentiometry (Crison electrode combined of pH), acid-alkaline titration with potentiometric detection (Crison automatic titrator, model Titromatic 1S, with electrode combined of pH) and redox titration, respectively. Concentrations of malic and tartaric acids were determined using an enzymatic-spectrophotometric and colorimetric method, respectively (automatic analyzer LISA 200).

The determination of total and extractable anthocyanins was carried out by ultraviolet-visible spectrophotometry (Perkin-Elmer, model Lambda 20) using the method based on the discoloration with metabisulphite. Anthocyanin concentration was expressed as mg/L malvidin. The concentration of total anthocyanins was determined in the aqueous solution of pH 1, while the concentration of extractable anthocyanins was determined in the aqueous solution of pH 3.2. The first ones

corresponded to the total anthocyanins present in the grape, being the last ones those extracted during winemaking process.

On the other hand, the contribution of the tannins present in seeds to the wine astringency or phenolic maturity index of seeds (MP) was calculated from the extractable anthocyanin concentration (g/L) and total phenolic compounds (A280) as follows:

$$MP\ (\%) = (A280 - (ApH3.2 * 40)) / 100 * A280$$

where A280 is the absorbance, measured in quartz cuvettes of 1 cm optical path, of the aqueous solution of pH 3.2, previously diluted 100 times with distilled water.

Afterwards, the separation and determination of anthocyanin compounds in skins by high performance liquid chromatography (HPLC) with diode array detection (DAD) was performed. The chromatographic column consisted of a SunfireTM C_{18} (5 μm, 4.6 x 250 mm) and the mobile phase flow selected was 0.5 mL/min. The chromatographic separation required heating of the column at 40 º C and the use of three mobile phases, with the aim of increasing the acidity and polarity of the mobile phase: 50 mM ammonium dihydrogen phosphate at pH 2.6 (mobile phase A), acetonitrile (mobile phase B) and 0.2 M phosphoric acid at pH 1.5 (mobile phase C). The determination of individual anthocyanins was performed at 520 nm.

Results and discussion

In this work, all red varieties were cultivated in the same experimental parcel and, therefore, the evolution of phenolic composition of grape berries during ripening did not depend on the climatic and soil conditions. Nevertheless, the adaptation degree of a certain variety to these conditions can be a differentiating factor in relation to the accumulation of phenolic compounds in grapes.

The accumulation of sugar in grape must was gradual during ripening until achieving a maximum value and, then, it remained practically stable. The loss of titrable acidity, tartaric acid and malic acid, during ripening, was less intense for Caíño da Terra cultivar and, therefore, the least increment of pH value corresponded to Caíño da Terra and Caíño Astureses cultivars. Nevertheless, Caíño Redondo cultivar presented the most accused diminution of titrable acidity and malic acid. Caíño Astureses cultivar showed a higher ripeness degree in both years, as can be observed in table 1.

Parameter	CA	CR	CL	CT
2005 year				
ºBrix	16,5	19,3	20,8	22,0
pH	2,88	3,00	3,03	3,12
Total acidity (g/L)	14,97	11,10	10,90	7,90
Malic acid (g/L)	6,8	4,6	3,5	1,1
Tartaric acid (g/L)	7,8	7,7	9,3	8,1
2006 year				
ºBrix	20,4	20,0	24,0	22,2
pH	3,14	3,26	3,35	3,26
Total acidity (g/L)	9,14	7,50	6,38	7,38
Malic acid (g/L)	4,6	3,9	3,0	2,9
Tartaric acid (g/L)	7,1	6,2	6,0	7,7

Table 1 Classical parameters

Total anthocyanin concentration increased during the ripening step until reaching a maximum value, from which it began to diminish (figure 1). However, this parameter increased continuously for Caíño

Longo variety in 2005 year. On the other hand, Caíño da Terra cultivar presented the earliest anthocyanin accumulation, achieving the maximum total anthocyanin concentration in the first days of September. Moreover, the greatest accumulation of total anthocyanins corresponded to Caíño Longo cultivar, followed by Caíño Astureses cultivar in both 2005 and 2006 years.

The easiness for the anthocyanin extraction, using smooth winemaking conditions, depends fundamentally on the cellular wall degradation of the berry skin. The greatest extractable anthocyanin concentration corresponded to Caíño Longo cultivar, followed by Caíño Astureses cultivar (figure 2). However, the best cellular maturity index was obtained for Caíño Astureses and Caíño da Terra cultivars.

Although Caíño Longo cultivar showed a cellular maturity index, or difficulty of extracting anthocyanins, higher than the value recommended of 30%, its seed phenolic maturity index was 30 % in 2005 year and slightly higher in 2006 year whereas the values determined for Caíño Astureses, Caíño Redondo and Caíño da Terra were higher than 60 %, as can be seen in figure 3. The seed phenolic maturity index diminished along ripening in accordance with the evolution previously published by other authors for different varieties.

The anthocyanin profile is shown in figure 4, where it can be observed that Caíño da Terra presented a profile significantly different from that corresponding to the other cultivars. So, cianidin-3-glucoside and peonidin-3-glucoside contents were statistically higher, while malvidin-3-glucoside, malvidin-3-acetylglucoside and malvidin-3-coumarylglucoside amounts were significantly lower. The profile obtained for Caíño Longo cultivar also showed significant differences, being its content of malvidin-3-glucoside statistically higher but that corresponding to delphinidin-3-glucoside and petunidin-3-glucoside was statistically lower.

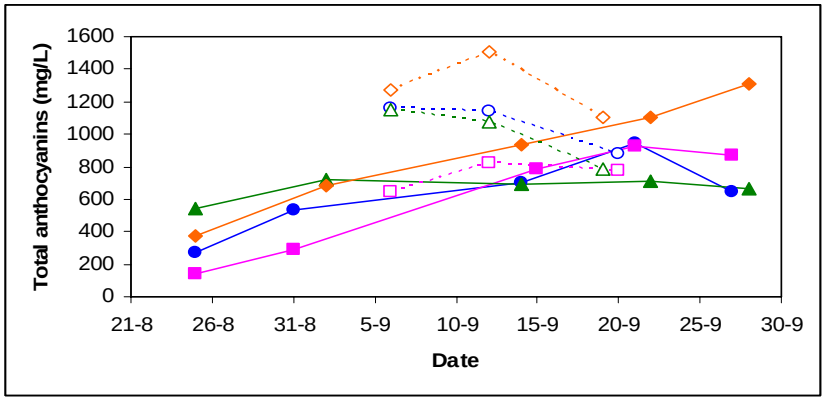

Figure 1 Evolution of total anthocyanin concentration during ripening

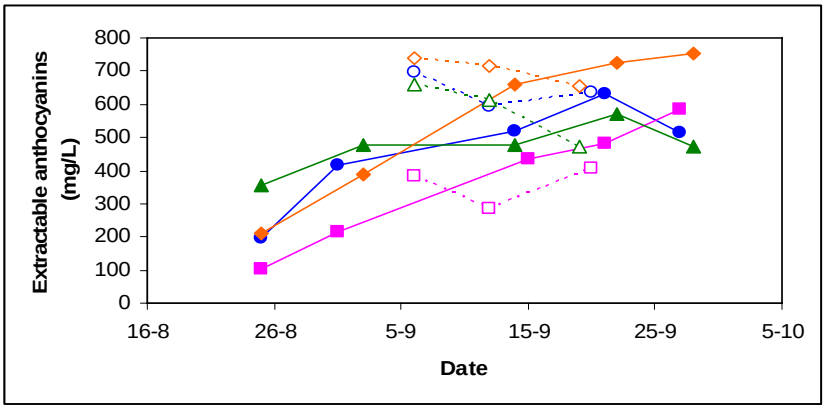

Figure 2 Evolution of extractable anthocyanin concentration during ripening

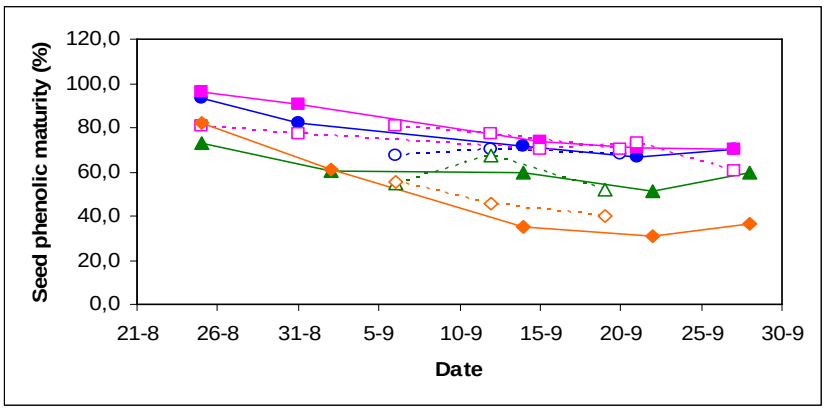

Figure 3 Evolution of seed phenolic maturity index during ripening¨

Caíño Astureses (●), Caíño Redondo (■), Caíño da Terra (▲), Caíño Longo (♦) in 2005 year. Caíño Astureses (○), Caíño Redondo (□), Caíño da Terra (Δ), Caíño Longo (◊) in 2006 year.

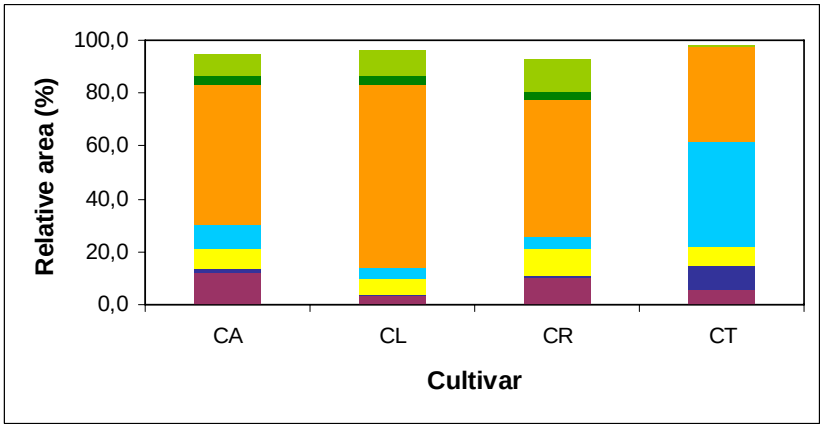

Figure 4 Anthocyanin profile: Delphinidin-3-glucoside (■), cyanidin-3-glucoside (■), petunidin-3-glucoside (■), peonidin-3-glucoside (■), malvidin-3-glucoside (■), malvidin-3-acetylglucoside (■) and malvidin-3-coumarylglucoside (■).

Conclusions

The extractable anthocyanin amount and seed phenolic maturity index are important factors that determine the harvest date and the selection of the most suitable winemaking methodology. Furthermore, the elaboration of high quality red wines requires grape berries containing high anthocyanin concentration and low content in seed tannins with strong astringent character. In this sense, the Caíño Longo cultivated in EVEGA showed the best phenolic characteristics. The anthocyanin profile reported the highest malvidin-3-glucoside amount in grape berries for this cultivar.

Acknowledgements

S. Rio, S. Cortés and E. Díaz acknowledge to the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria the contract for doctors in INIA-CCAA system, co-financed with European Social Fund.

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