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SHORT COMMUNICATION

PHENOLIC CHARACTERIZATION OF GRAPEVINE CULTIVARS FROM GALICIA (SPAIN): BRANCELLAO, MERENZAO AND MENCÌA (*VITIS VINIFERA* L.)

CARATTERIZZAZIONE FENOLICA DI VITIGNI DELLA GALIZIA (SPAGNA): BRANCELLAO, MERENZAO E MENCÌA (*VITIS VINIFERA* L.)

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ABSTRACT

The polyphenolic composition of Brancellao, Merenzao and Mencìa grapes from Galicia (northwestern Spain) was studied using spectophotometric and High Performance Liquid Chromatographic (HPLC) methods. The results showed significant differences between these cultivars. The total anthocyanins ranged from 215 mg/kg berries in Brancellao to 422 mg/kg berries in Mencìa. The three cultivars were characterized by an interesting anthocyanin profile for winemaking

RIASSUNTO

La composizione polifenolica di tre vitigni provenienti dalla Galizia (Nord Ovest, Spagna) è stata valutata per la prima volta con l'utilizzo di metodi analitici spettrofotometrici e HPLC.

I risultati mostrano differenze tra le diverse cultivar. L'indice di antociani totali varia tra i 215 mg/kg uva del Brancellao e i 422 mg/kg uva della Mencìa. Le cultivar sono caratterizzate da un profilo antocianico interessante dal punto di vista tecnologico per la prevalenza di malvidina-3-glucoside. Sia i vi-

- Key words: Anthocyanin profile, flavonoids, Galician grapevine, grape skins, HPLC, seeds -

with a prevalence of malvidin-3-glucoside. The skins and seeds, both had small amounts of flavonoids. In contrast to Brancellao and Mencìa, Merenzao had more flavonoids in the seeds (64%) than in the skins. The most important parameters for differentiating between the cultivars were the peonidin and malvidin derivative forms, and the total amounts of acetyl-glucosides.

naccioli, sia le bucce mostrano un basso contenuto in flavonoidi. Contrariamente al Brancellao e alla Mencìa, il Merenzao possiede più flavonoidi nei vinaccioli (64%) che nelle bucce. I più importanti componenti di differenziazione tra le cultivar studiate risultano le forme esterificate della peonidina e malvidina e le forme acetil-glucosilate.

INTRODUCTION

The phenolic composition of wines depends mainly on the phenolic content of the grapes and on the numerous reactions that occur during juice extraction, winemaking and wine aging. The phenolic content of the berries is of great ampelographic and taxonomic importance in viticulture for classifying grape cultivars (CRAVERO *et al.*, 1994; DI STEFANO, 1996; GUIDONI *et al.*, 2003; MATTIVI *et al.*, 1993) because it varies greatly between species and cultivars (CRAVERO *et al.*, 1994; GONZALEZ-NEVES, 2001; 2005; MATTIVI *et al.*, 2003).

Moreover, the synthesis and concentration of phenols in red grapes depend on a number of environmental factors, as well as vineyard management practices. Of particular importance are fruit ripeness, climatic conditions, soil features and crop load (DI STEFANO *et al.*, 1994; GUIDONI *et al.*, 2002; JACKSON and LOMBART, 1993; PEREZ-MAGARINO and GONZALEZ-SAN JOSE, 2006; ROSON and MOUTOUNET, 1992; ROUBELAKIS-ANGELAKIS and KILEWER, 1986).

Many authors have reported on the anthocyanin profile in the grape skins of several grape cultivars, on the anthocyanin evolution during grape ripening (CLIMENT and PARDO, 1997; HMAMOUCHI *et al.*, 1995; GERBI *et al.*, 2003; MAZZA *et al.*, 1999; TAMBORRA *et al.*, 2003; RYAN and REVILLA, 2003; LOVINO *et al.*, 2006) and on the tannins in skins and seeds (HARBERTSON *et al.*, 2002; ROSON and MOUTOUNET, 1992; TAMBORRA and DI BENEDETTO, 1991). Such information can also be used to evaluate enological potential (CHEYNIER *et al.*, 1997; GERBI *et al.*, 2002; OTTENEDER *et al.*, 2004).

In recent years interest in autochthonous or rare European cultivars and in the unique wines that can be produced from them has increased and many heirloom varieties have been rescued from potential extinction. Many studies on the recovery and qualitative evaluation of minor grapevine cultivars have been carried out in recent years (MARTINEZ and PÉREZ, 2000; SANTIAGO *et al.*, 2005a,b; SCHNEIDER *et al.*, 2001; ZEPPA *et al.*, 2001). There has been particular interest in mountainous viticultural areas. Despite the difficulty of cultivating them because of steep slopes and pedoclimatic conditions, many mountainous areas have preserved a wealth of biodiversity.

A European project, entitled "Sustainable Enhancement of Autochthonous Wine Grapes in Mountain Areas", is aimed at the recovery, conservation and exploitation of the viticultural patrimony of mountain areas. Within the framework of this project, the three indige-

nous cultivars, Mencìa, Brancellao and Merenzao, cultivated in Galicia (northwestern Spain), have been studied (ORRIOLS *et al.*, 2006). The aim of this work was to analyze the phenolic composition of these cultivars in order to assess the most important parameters that characterize and differentiate them.

MATERIALS AND METHODS

Grapes of *Vitis vinifera* cvs. Brancellao, Merenzao and Mencía were collected from nine Ribeira Sacra valley (Galicia, Spain) vineyards located at altitudes over 300 m a.s.l. in 2003 and 2004. The vines had similar characteristics (age, pruning system, amount of buds) and were grown under controlled pedoclimatic conditions.

The samples were harvested at technological ripeness and picked in a statistically representative way from different parts of the bunch. In each vineyard, 300 berries were collected, and three replicates of 10 berries each were then weighed before phenolic extraction.

A total of 18 samples were analyzed for each cultivar; three replicates from each of the three vineyards, were analyzed during the two years.

Samples for analyzing berry skins and seeds were prepared according to the DI STEFANO and CRAVERO (1991) method which reproduces vinification extraction conditions.

The berry skins were removed manually from the pulp and dried with paper. They were then quickly immersed in 25 mL of a buffer solution containing 12% ethanol, 600 mg/L of sodium metabisulfite, 50 mg/L NaN_3, 5 g of tartaric acid and titrated to pH 3.20 by adding 1N NaOH. After homogenization with an Ultraturrax T25 (IKA Labortechnik, Staufen, Denmark), the extract was centrifuged for 10 min at 3,000 rpm at 20°C. The supernatant was then used for analysis.

The seeds were removed from the mesocarp, placed in 50 mL of the same buffer solution used for the skin extraction at pH 3.20 and then put in a temperature-controlled room at 25°C for a week. The extract was then used for analysis.

Analysis

The analytical parameters of technological ripeness (reducing sugars, total acidity, pH) were estimated with official methodologies EC (EEC 1990).

Spectrophotometric methods were used to evaluate the total anthocyanin and flavonoid content in the berry skins and seeds (DI STEFANO, 1996; DI STEFANO and CRAVERO, 1991; DI STEFANO *et al.*, 1994). Analysis of individual anthocyanins was performed after the berry skin extract was placed in a 300 mg SEP-PAK C18 cartridge (Waters Corporation, Milford, MA, USA) and eluted with methanol. The cartridge was preconditioned with methanol (2 mL), water (5 mL) (DI STEFANO *et al.*, 1989) and 0.01N H_2SO_4 (2 mL) before elution. A P100 chromatograph was used that was equipped with an AS3000 auto-sampler (Spectra Physics Analytical, Inc, San Jose, CA, USA) and a 20 mL Rheodyne sample loop.

A LiChroCART analytical column (25x0.4 cm i.d.) from Merck (Darmstad, Germany) was used. It was packed with LiChrosphere 100 RP-18 5-μm particles from Alltech (Deerfield, IL, USA) and was equipped with a Spectra Focus Diode Array Detector (Spectra Physics Analytical, Inc, San Jose, CA, USA) operating at 520 nm.

The following conditions were used: solvent A = 10% formic acid in water. Solvent B = 10% formic acid with 50% methyl alcohol in water. These solvents were filtered through a 0.20 μm filter. Solvent flow rate was 1 mL/min. The solvent program used was 72% A to 55% A over 15 min; to 30% A over 20 min; to 10% A over 10 min; to 1% A over 5 min; to 72% A over 3 min. An equilibri-

um time of 10 min was used (Guidoni *et al.*, 2002). The data treatment was carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc, San Jose, CA, USA).

The anthocyanins in the berry skin extract were identified by matching UV-Vis spectra, the retention times of each chromatographic peak with available data in the literature and using authentic standards purchased from Extrasynthèse (Genay, France).

The single anthocyanin concentrations were determined by comparing the area of the individual peak with the total peak area; the data are expressed in percentages (DI STEFANO and CRAVERO, 1991; HEBRERO *et al.*, 1988).

The statistical differences between the quantities of the various phenolic compounds of the three cultivars analyzed were determined by analysis of variance (ANOVA) using STATISTICA for Windows Release 6.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The average technological ripeness parameter values for the two years in the three vineyards are reported in Table 1. The pH values for the three cultivars were the same. Merenzao had the highest sugar content and the lowest total acidity, while Brancellao had the lowest sugar content and the highest total acidity.

The total amounts of anthocyanins and flavonoids in berry skins and seeds of Brancellao, Mencìa and Merenzao are reported in Table 2.

The results of the analysis of variance showed that the three cultivars differed

Table 1 - Technological ripeness of Brancellao, Mencìa and Merenzao grapes. Values are the means (± standard deviations) of 3 vineyards and 2 years.

	Brancellao	Mencìa	Merenzao
Reducing sugars (g/L)	199±6	200±31	219±14
pH	3.6±0.4	3.6±0.0	3.6±0.1
Total acidity (g/L tartaric acid)	6.5±0.5	4.7±0.4	5.0±0.8

Table 2 - Polyphenolic parameters of the Brancellao, Mencìa and Merenzao berry skins and seeds. The values are the means (± standard deviations) of 18 samples representing three replicates picked from each of the three vineyards and analyzed in the two years. The average values with the same superscript letter are not statistically different at the 5% level.

		Brancellao	Mencìa	Merenzao	Significance
Berry skins	Total anthocyanins (mg malvidin-3-O-glucoside chloride/kg berries)	215^{a}±53	422^{b}±111	$347^{a,b}$±26	*
	Total flavonoids (mg (+) catechin/kg berries)	$3,296^{b}$±511	$2,370^{a}$±168	$1,940^{a}$±140	**
Seeds	Total flavonoids (mg (+) catechin/kg berries)	$2,158^{b}$±189	$1,803^{a}$±188	$3,492^{c}$±136	***
Berry skins and seeds	Total flavonoids (mg (+) catechin/kg berries)	$5,454^{b}$±689	$4,173^{a}$±355	$5,432^{b}$±5.6	**
	% seeds total flavonoids	40	43	64	
	% berry skin total flavonoids	60	57	36	

*: p≤0.05; **: p≤0.01; ***: p≤0.001.

significantly with respect to the flavonoid and anthocyanin contents in both berry skins and seeds.

With regards to the total anthocyanin concentration, Brancellao and Mencìa were completely different while Merenzao had an intermediate behavior. The anthocyanin concentrations in the berries varied from 215 mg/kg in Brancellao to 422 mg/kg in Mencia. The latter value is approaching that of Nebbiolo (550 mg/kg berries) (GERBI *et al.*, 2003), but is low compared to 1,577 mg/kg of Cabernet Sauvignon or 1,541 mg/kg of Sangiovese (TAMBORRA and DI BENEDETTO, 1991).

The total flavonoid concentrations in the berry skins of the three cultivars were higher in comparison with some French and Italian cultivars such as Cabernet sauvignon and Sangiovese (TAMBORRA and DI BENEDETTO, 1991) at harvest but approached that of Nebbiolo (GERBI *et al.*, 2003; GUIDONI *et al.*, 2002). The total flavonoid concentrations in the seeds were also high compared to Cabernet sauvignon (1,479 mg/kg berries) and Sangiovese (1,388 mg/kg berries) which were analyzed by TAMBORRA and DI BENEDETTO (1991).

Based on their flavonoid concentrations, the three cultivars have different skin and seed profiles. Indeed, there were more flavonoids in Brancellao berry skins (3,296 mg/kg berries) than in Mencìa or Merenzao. Whereas Merenzao was the cultivar with the highest flavonoid content in the seed (3,492 mg/kg berries).

The total flavonoid concentrations (sum of berry skins and seeds) for Brancellao and Merenzao were nearly the same as Merenzao (5,454 mg/kg berries and 5,432 mg/kg berries respectively), but the distribution between the berry skins and seeds varied. The flavonoids in Brancellao and Mencìa were primarily located in the skins (60 and 57%, respectively). In contrast, 64% of the flavonoids were located in the Merenzao seeds.

The anthocyanin profiles of Brancellao, Mencìa and Merenzao grapes are shown in Fig. 1. In Table 3, the anthocyanins are grouped according to the acylation (acetyl-glucosides, cinnamoyl-glucosides and free-form monoglucosides) and anthocyanidin (malvidin, delphinidin, petunidin, cyanidin and peonidin) compounds. The cinnamoyl-glucosides included both p-coumaroyl and caffeoyl anthocyanin forms.

The monoglucoside group made up the highest proportion of the anthocyanin forms (72% in Brancellao, 71% in Merenzao and 50% in Mencìa). The anthocyanin distribution in Brancellao was very similar to that in Merenzao, with nearly the same proportions of monoglucosides, acetyl-glucosides (8 and 10% respectively) and cinnamoyl-glucosides (19.5 and 20%, respectively). In contrast, Mencìa was characterized by a smaller proportion of monoglucosides (50%) and higher proportions of acetyl derivatives (21%) and cinnamoyl derivatives (29%) (Table 3).

Malvidin derivative forms were the most abundant anthocyanin group in Brancellao, Merenzao and Mencìa, as in Cabernet sauvignon, Cabernet franc, Merlot and Tempranillo (NAGEL and WOLF, 1979; MAZZA *et al.*, 1999; HEBRERO *et al.*, 1988). Brancellao had the lowest proportion of malvidin derivatives (64%), followed by Merenzao (73%) and Mencìa (85%). The percentages of delphinidin and cyanidin derivative forms in the three cultivars were low (not more than 2%). Merenzao had a slightly higher proportion of petunidin derivative forms (5%), followed by both Brancellao and Mencìa (2.5%). The percentages of peonidin derivative forms, however, were distinctly different among the cultivars: Brancellao (31%), Merenzao (18%) and Mencìa (10%).

Monagas *et al.* (2003) established that in wines made from Graciano, Tempranillo and Cabernet sauvignon grapes, the ratios between cinnamoyl and acetyl-glu-

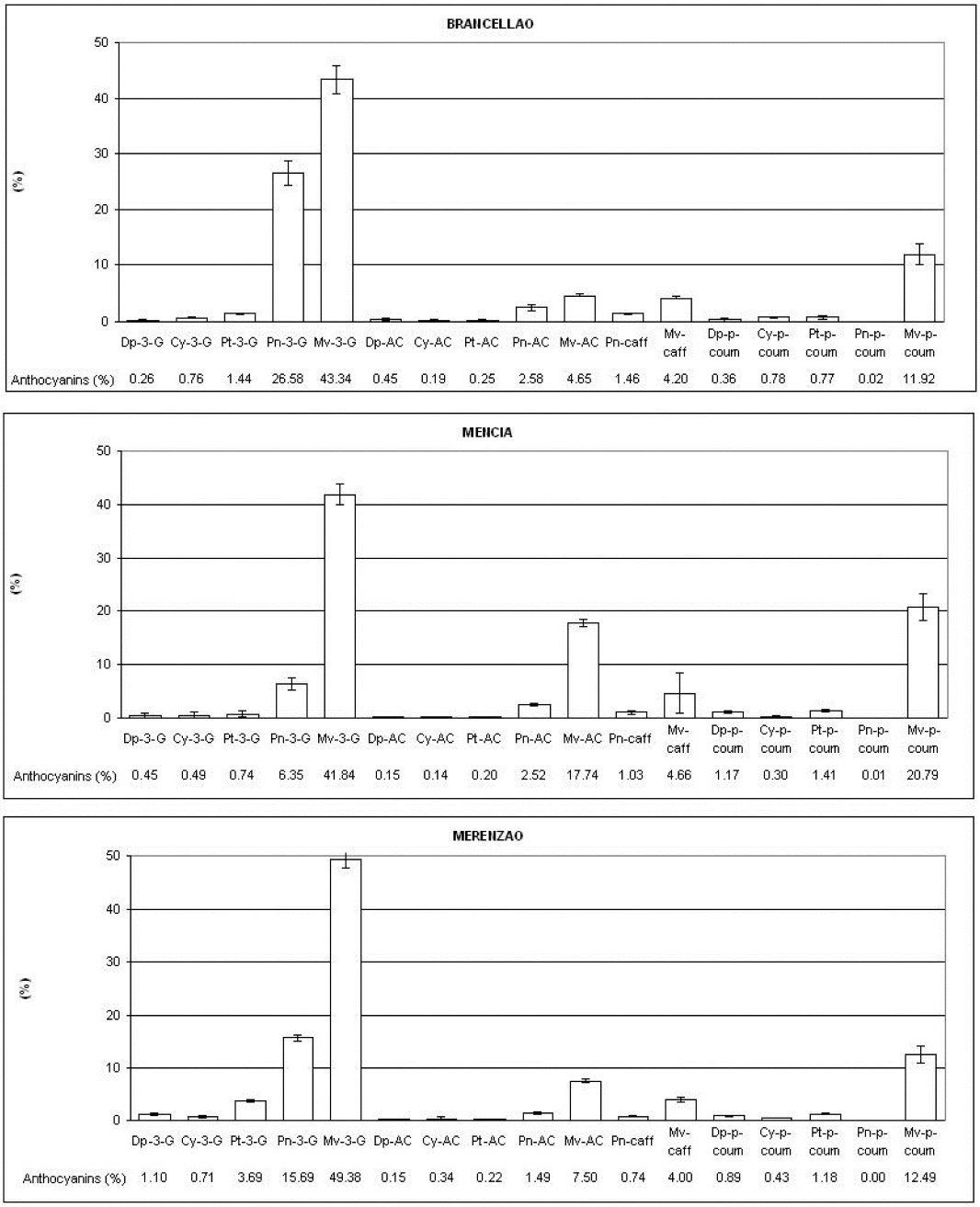

Fig. 1 - Anthocyanin profiles of Brancellao, Mencìa and Merenzao grapes. The amount of each anthocyanin is expressed as the mean of the percentages of the total anthocyanins of 18 samples representing three replicates picked from each of the three vineyards and analyzed in the two years. Key: Dp delphinidin; Cy cyanidin; Pt petunidin; Pn peonidin; Mv malvidin; G glucoside; AC acetyl-glucoside; p-coum p-coumaryl-glucoside; caff o-caffeoyl-glucoside. Vertical bars denote standard deviation computed for each mean value, n = 18.

Table 3 - Percentages and ratios of anthocyanins in Brancellao, Mencìa and Merenzao grapes. The values are the means (± standard deviations) of 18 samples representing three replicates picked from each of the three vineyards and analyzed in the two years. The percentage values with the same superscript letter are not statistically different at the 5% level.

	Brancellao	Mencìa	Merenzao	Significance
Monoglucosides	72.4^{b}± 1.8	50.2^{a}± 2.9	70.6^{b}±1.2	***
Acetyl-glucosides	8.1^{a}± 0.7	20.8^{c}±1.0	9.7^{b}± 0.4	***
Cinnamoyl-glucosides	19.5^{a}± 2.4	29.0^{b}± 3.4	19.7^{a}± 1.5	**
Total anthocyanidins (%)				
Sum of malvidin derivative forms	64.1^{a}± 2.4	85.0^{c}± 1.7	73.4^{b}± 0.7	***
Sum of delphinidin derivative forms	1.1^{a}±0.7	1.8^{a}± 0.4	2.1^{a}± 0.5	ns
Sum of petunidin derivative forms	2.5^{a}± 0.3	2.4^{a}± 0.6	5.1^{b}± 0.2	***
Sum of cyanidin derivative forms	1.7^{a}±0.2	0.9^{a}± 0.5	1.5^{a}± 0.4	ns
Sum of peonidin derivative forms	30.6^{c}±2.8	9.9^{a}± 1.3	17.9^{b}± 0.6	***
Cinn/Ac	2.4±0.5	1.4±0.2	2.0±0.2	
Mvs/Pns	2.1±0.3	8.5±1.3	4.0±0.1	

Cinn: cinnamoyl-glucosides; Ac: acetyl-glucosides; Mvs: malvidin derivative forms; Pns: peonidin derivative forms; Sign: significance; ns: not significant; *: p≤0.05; **: p≤0.01; ***: p≤0.001.

cosides (Cinn/Ac) and between the malvidin derivative forms and the peonin derivative forms proportions (Mv/Pn) could be used as varietal markers. These ratios were calculated for the Brancellao, Mencìa and Merenzao grapes (Table 3); the (Cinn/Ac) ratios for Brancellao and Merenzao were very similar but were different from Mencìa (1.4). On the other hand, the (Mv/Pn) ratio was lower in Brancellao (2.1) than in Merenzao (4.0) and Mencìa (8.5). The presence of more stable molecules, such as the trisubstituted anthocyanins such as malvidin and its acylated forms, would give more stability to the wine color during winemaking. Malvidin is more resistant to oxidation (RIBEREAU-GAYON *et al.*, 2000).

The anthocyanin profile of Cabernet franc was analyzed by MAZZA *et al.* (1999). In Mencìa, delphinidin-3-glucoside accounted for only 0.4% of the anthocyanins while in Cabernet franc it constitutes 15% of the anthocyanins. The cinnamoyl derivatives accounted for 29% of the anthocyanins in Mencìa but only 6.7% in Cabernet franc. Even if malvidin derivative forms were the most abundant anthocyanin group in the two cultivars, the proportion was higher in Mencìa than in Cabernet franc (85 and 35%, respectively). This result is in accord with MARTINEZ and PÉREZ (2000) who found that Mencìa grapes were completely different from Cabernet franc grapes.

The values reported in Table 3 and the analysis of variance of the corresponding data indicated the decreasing order of importance of the acetyl-glucosides, monoglucosides and the cinnamoyl-glucosides. These could be used to differentiate the three Galician varieties. But the best parameters for differentiating were the total derivative forms of anthocyanidins, especially of malvidin-3-glucoside and peonidin-3-glucoside. These results are in accord with CALÒ *et al.* (1994) who showed that they were more important than the petunidin-3-glucoside derivatives in differentiating grapevine cultivars. As GONZALEZ-NEVES *et al.* (2001) reported, the situation in wines differs and is based on petunidin and malvidin glucosides, with the acetyl esters of the petunidin, peonidin and malvidin

glucosides. This indicates the importance of methoxylation in varietal differentiation.

CONCLUSIONS

It can be confirmed that parameters such as total anthocyanins, total flavonoids and the anthocyanin profile show important significant differences between Brancellao, Mencìa and Merenzao grapes. Other indicators such as the cinnamoyl-glucoside/acetyl-glucoside and the malvidin derivative/peonidin derivative ratios, can also be used to differentiate between the three cultivars. However, more studies with grapes from different production areas should be carried out to complete these observations. In addition, a detailed study in the future on the phenolic composition of wines produced from the Brancellao, Mencìa and Merenzao grapes would be interesting to check the differentiating parameters in wine.

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