

# ANTHOCYANINS, FLAVONOLS AND HYDROXYCINNAMATES OF EIGHT *VITIS VINIFERA* CULTIVARS FROM THE BALEARIC ISLANDS<sup>1</sup>

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## 1. INTRODUCTION

Since the 1990s in the Balearic Islands wine production has become one of the most profit-bearing sector in agriculture. In 1990 and 1999 two Designations of Origin (DO) were appointed, Binissalem and Pla i Llevant. Binissalem wines can be produced using at least 50 % of 'Manto Negro' in red wines and 50 % of 'Moll' (also called 'Prensal blanc') in white wines. Pla i Llevant DO wines can be produced by a wider range of cultivars such as 'Manto negro', 'Callet', 'Fogoneu', 'Tempranillo', 'Monastrell', 'Cabernet sauvignon', 'Merlot', 'Syrah' and 'Pinot noir' for red wines and 'Moll', 'Chardonnay', 'Macabeo', 'Moscatel de grano menudo', 'Parellada', 'Sauvignon blanc' and 'Viognier' for white wines. Among these cultivars, only four are genuine autochthonous cultivars: the coloured cultivars 'Manto negro', 'Callet' and 'Fogoneu', and the white cultivar, 'Moll' (or 'Prensal blanc'). Other minor cultivars, well adapted to Balearic Islands' local environmental conditions and which could contribute to the production of typical wines, are diffused in Mallorca and have previously been characterized through some morphological and physiological parameters (Escalona *et al.*, 2006). There are few studies about flavonoid characterisation of Balearic cultivars (Medrano *et al.*, 2003; Escalona *et al.*, 2008). In 2008 we characterized the anthocyanin, flavonol and hydroxycinnamate (HCT) contents of the skins of five coloured berry cultivars ('Escursac', 'Esperó de Gall', 'Galmeter', 'Valent negre' and 'Vinater negre'), of two white cultivars ('Argamussa' and 'Prensal blanc') and of one weakly rose one ('Giró ros').

## 2. MATERIALS AND METHODS

The vines were grown in the experimental vineyard of the Conselleria d'Agricultura i Pesca located in Palma (Mallorca, Spain); vines had been planted in 1997, grafted onto 'R 99' and trained to the spur-pruned cordon bilateral system. The vineyard was managed according to the standard practices of the region.

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In 2008 the anthocyanin, flavonol and hydroxycinnamate quantities and profiles were assessed; at harvest the production of six plants per cultivar was collected and weighted. About 100 berries were collected from the bunches, picking them alternatively from the upper, the middle and the distal part of the bunch. For the analysis of anthocyanins three groups of ten berries each were extracted in methanol 100 % and successively extracted again with methanol:water; the final extract was injected into a Waters 717 HPLC/DAD detector (Waters, 34 Maple Street, Milford MA 01757 USA); anthocyanins were separated on a Nova-pak C18 (150 x 9.9 mm, i.d.5 micron, Waters, Milford MA, USA) (Garcia-Beneytez *et al.* 2002). For the analysis of flavonols and HCTA three groups of ten berries each were extracted at 30 °C for 72 hours in a pH 3.2 ethanolic buffer. Extracts were diluted 1/1, filtered, injected into a 200-L HPLC/DAD (Perkin Elmer, 45 William St Wellesley, MA 02481, USA) and separated on a Lichrosphere 100 RP-18 (25 x 0.4 cm, i.d. 5 micron, Merck) column equipped with a pre-column (LichroCART 4-4. Merck). Flavonols were detected at 360 nm and expressed as mg of quercetin 3-O-glucoside equivalents per kg of berries. HCTAs were detected at 320 nm and expressed as caffeic or *p*-coumaric acid equivalents, respectively for caffeoyl tartaric and *p*-coumaroyltartaric acids (Ferrandino, Guidoni, 2010).

### 3. RESULTS AND DISCUSSION

At harvest the total anthocyanin content varied from 388.4 mg kg<sup>-1</sup> of berries (‘Esperó de Gall’) to 1067.2 mg kg<sup>-1</sup> (‘Valent negre’). The ratio between tri and di-hydroxylated anthocyanins varied from 3.5 of ‘Vinater negre’, to 12.3 of ‘Escursac’ where the percentage of di-hydroxylated cyanidin and peonidin 3-O-glucoside was very low, accounting for only 2.8 % of total anthocyanin. All the examined cultivars were characterized by the prevalence of malvidin 3-O-glucoside except ‘Valent negre’ where delphinidin 3-O-glucoside was prevalent (28 % of total amount). In ‘Valent negre’ the acylation of anthocyanins with acetic acid prevailed over caffeoyl and *p*-coumaroyl derivatives. In all the other examined cultivars *p*-coumaroyl derivatives were prevalent (Tab. 1). These results are in accordance with previous studies (Escalona *et al.*, 2008).

Tab. 1 - Total anthocyanins (TA as mg kg<sup>-1</sup> of grape), ratio between tri- and di-hydroxylated anthocyanin contents, percentages of anthocyanin acetyl (ac. der.), *p*-coumaroyl (*p*-coum. der.) and caffeoyl (caff. der.) derivatives in the skins of 5 native cultivars of the Balearic Islands.

Cv	TA	tri/di	ac.der	p-coum.der.	caff.der
<b>Escursac</b>	610.5 ± 11.4	12.3	4.8	50.6	0.1
<b>Esperó de Gall</b>	388.4 ± 15.9	5.4	3.7	21.7	-
<b>Galmeter</b>	800.3 ± 70.3	3.8	7.3	33.2	0-9
<b>Valent negre</b>	1067.2 ± 53.0	3.8	<b>21.2</b>	17.7	-
<b>Vinater negre</b>	493.2 ± 12.7	3.5	6.0	18.2	0.1

In coloured berry cultivars the total flavonol content ranged from 55.4 mg kg<sup>-1</sup> in ‘Galmeter’ to about 178 mg kg<sup>-1</sup> in ‘Esperó de Gall’ and ‘Vinater negre’. Quercetins (3-O-glucuronide and 3-O-glucoside) were the prevalent flavonols in all cultivars except in ‘Valent negre’, where myricetin 3-O-glucoside (39.2 % of flavonol total amount) prevailed,

and in ‘Galmeter’ where kaempferol 3-O-glucoside accounted for 32.4 % of flavonol total amount (tab. 2). In not coloured cultivars the total flavonol content ranged from 10.1 mg kg<sup>-1</sup> of ‘Argamussa’ to 52.1 mg kg<sup>-1</sup> of ‘Giró ros’. As expected myricetin 3 O-glucoside (myr. gl.) was totally absent in white cultivars whereas it was detected in ‘Giró ros’ which is a pale-rose cultivar (tab. 2).

Tab. 2 - Total flavonols (TF as mg kg<sup>-1</sup> of grape) and percentages of individual flavonols: myricetin 3-O-glucoside (myr. gl.), quercetin 3-O-glucuronide (q. glcr.), quercetin 3-O-glucoside (q. gl), kaempferol 3-O-glucuronide (k. glcr.) and kaempferol 3-O-glucoside (k. gl) of 8 native cultivars of the Balearic Islands.

Cv	TF	myr.gl	q.glcr.	q.gl.	k. glcr.	k.gl.
<b>Escursac</b>	64.3 ± 11.4	21.7	19.2	46.7	0.3	12.1
<b>Esperó de Gall</b>	178.7 ± 14.0	20.2	18.3	45.2	2.7	13.6
<b>Galmeter</b>	55.4 ± 6.5	23.0	12.7	31.9	-	<b>32.4</b>
<b>Valent negre</b>	86.8 ± 11.3	<b>39.2</b>	30.3	26.0	3.5	0.2
<b>Vinater negre</b>	177.8 ± 11.9	9.2	22.3	51.1	2.9	13.5
<b>Argamussa</b>	10.1 ± 2.4	-	21.5	71.5	-	7.0
<b>Moll</b>	45.5 ± 6.7	-	22.0	54.8	-	23.3
<b>Giró ros</b>	52.1 ± 8.4	0.8	35.8	53.2	2.3	17.8

The skin total content of hydroxycinnamates was very low in all the studied cultivars, ranging from 1.5 mg kg<sup>-1</sup> of the white ‘Argamussa’ to 11.4 mg kg<sup>-1</sup> of ‘Escursac’ (tab.3), similarly to what it has been assessed by other authors in wines produced from vines grown in hot and dry climatic conditions (Kallithraka *et al.*, 2006). *Trans* caffeoyltartaric acid was the predominant hydroxycinnamate in the skins of ‘Esperó de Gall’, ‘Galmeter’, ‘Vinater negre’ and ‘Moll’ whereas in ‘Escursac’, ‘Valent negre’ and ‘Giró ros’ *p*-coumaroyltartaric acid prevailed; ‘Argamussa’ accumulated equal quantities of the two main hydroxycinnamates (tab. 3). Hydroxycinnamate concentration was generally higher in pulps respect to skins; in pulps caffeoyl tartaric acid (mainly in the *trans* form) was prevalent over *p*-coumaroyltartaric acids (*cis* and *trans* forms) (tab. 3).

Tab. 3 - Total hydroxycinnamates in skins (SThcta as mg kg<sup>-1</sup> of grape) and pulps (PThcta as mg kg<sup>-1</sup> of grape) and ratio between *p*-coumaroyl tartaric acid and caffeoyl tartaric acid contents of 8 native cultivars of the Balearic Islands.

Cv	SThcta (mg kg <sup>-1</sup> )	<i>p</i> -coumT/cT (skin)	Pthcta (mg kg <sup>-1</sup> )	<i>p</i> -coumT/cT (pulp)
<b>Escursac</b>	11.4 ± 0.7	21.7	19.2	46.7
<b>Esperó de Gall</b>	11.3 ± 0.6	20.2	18.3	45.2
<b>Galmeter</b>	7.8 ± 0.4	23.0	12.7	31.9
<b>Valent negre</b>	7.6 ± 0.9	<b>39.2</b>	30.3	26.0
<b>Vinater negre</b>	10.0 ± 1.1	9.2	22.3	51.1
<b>Argamussa</b>	1.5 ± 0.2	-	21.5	71.5
<b>Moll</b>	4.3 ± 0.3	-	22.0	54.8
<b>Giró ros</b>	3.4 ± 0.8	0.8	35.8	53.2

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## Abstract

In 2008 the anthocyanin, flavonol and hydroxycinnamate (HCT) contents of the skins of five coloured berry cultivars ('Escursac', 'Esperó de Gall', 'Galmeter', 'Valent negre' and 'Vinater negre'), of two white cultivars ('Argamussa' and 'Prensall blanc') and of one weakly rose cultivar ('Giró ros'), native from Balearic Islands, were characterized. The total anthocyanin content varied from 388.4 mg kg<sup>-1</sup> berry weight ('Esperó de Gall') to 1067.2 ('Valent negre'). The ratio between tri and di-hydroxylated anthocyanins varied from 3.5 of 'Vinater negre' to 12.3 of 'Escursac'. In 'Valent negre' delphinidin 3-O-glucoside was the prevalent anthocyanin (28 % of total amount). The total flavonol content ranged from 10.1 mg kg<sup>-1</sup> in the white 'Argamussa' to about 178 mg kg<sup>-1</sup> in 'Esperó de Gall' and 'Vinater negre'. Quercetins were the prevalent flavonols in most cultivars except 'Valent negre', characterized by the prevalence of myricetin 3-O-glucoside and 'Galmeter' where kaempferol 3-O-glucoside accounted for 32.4 % of flavonol total amount. The skin total content of hydroxycinnamates was low in all cultivars (from 1.5 to 11.4 mg kg<sup>-1</sup>) probably because of the very hot and dry climatic conditions of the cultivation site.

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