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A preliminary study of non-coloured phenolics in wines of varietal white grapes (códega, gouveio and malvasia fina): effects of grape variety, grape maturation and technology of winemaking

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Abstract

The effects of cultivar, grape maturation and technology of winemaking on the levels of phenolic acids were evaluated. In all varieties, 3,4-dihydroxybenzoic, *p*-coumaric and caffeic acids were the predominant acids. Among the three varieties, significant differences were observed in the concentration of *p*-coumaric acid. The grape maturation only influenced the concentration of 3,4-dihydroxybenzoic acid. According to the technology of winemaking, significant differences could be observed in syringic, and *p*-coumaric acids by the induction of fermentation temperature. Comparisons of the phenolic composition, when the fermentations were conducted in stainless steel tanks and/or in oak barrels, showed differences in the concentrations of 3,4-dihydroxybenzoic, ellagic and caffeic acids.

1. Introduction

Several achievements have recently promoted an increasing interest in polyphenolic research of cultivars of *Vitis vinifera* and some varietal types of wines. In fact, the recognized benefits of some polyphenols for human health, the effective rise of the total blood antioxidant activity after consumption of wines (Frankel, Kanner, German, Parks, & Kinsella 1993; Kanner, Frankel, Granit, German, & Kinsella, 1994) and also making the authentication of commercial wines are notable. This has motivated study of polyphenolics in an attempt to establish the phenolic fingerprinting of cultivars and elemental wines (Soleas, Dam, Carery, & Goldberg, 1997). Such achievements would facilitate the selection of better enological techniques for enrichment of wines with these beneficial compounds and through them guarantee the genuiness of commercial wines and their production (Gil, Garcia-Viguera, Brittle, & Tomás-Barberán, 1995).

Although the biosynthesis of polyphenols is complex, in *Vitis vinifera* and is predominantly of genetic expression, the geographic regions where cultivars are grown, the level of ripening of grape berries and practised winemaking techniques are determining factors in the modulation of phenolic types or their contents (Badea & Tudorache, 1998; Konyek, Kontek, & Radulescu, 1998; Sanjose, Izcara, Perez-Magarino, & Revilla, 1998; Simon, Hernandez, Estrella, & Gomez-Cordoves, 1992; Stoian, Avramescu, & Varga, 1998; Vrhovsek, & Wendelin, 1998). Furthermore, due to the enormous varieties of grapes that exist in each country and the fact that the biosynthesis of polyphenols can proceed by a number of interrelated biochemical systems, including the shikimate, cinnamate, chalcone, and stilbene pathways, the results of these recent studies have been difficult to interpret and need to be continued.

Phenolic acids in grape berries are located primarily in the skin where, in general, they are present at much lower concentrations than anthocyanins. In white grape varieties, the concentration of phenolic compounds is much lower in pulp and in the must whereas benzoic

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and cinamic acids are predominant. Catechins and procyanidins are located in solid parts of the berry, particularly in the seeds (Simon et al., 1992).

Several publications can be found for the determination of phenolic compounds in grapes, musts and wines, and particulary red wines (Garcia-Viguera & Bridle, 1995). In contrast to the general practice in portwine grape varieties (Andrade, Seabra, Ferreira, Ferreres, & Garcia-Viguera, 1998), little attention seems to have been paid to the non-coloured phenolic acids in white varietal grapes that grow in Portugal. Thus, this work examines the changes of eight phenolic acids in white wines manufactured with cultivars of three important white varieties (Códega, Gouveio and Malvasia Fina) that grew in 1996 in the Douro region. Preliminary data on the variation of these compounds due to effects of the level of maturation of the grapes and of conditions of fermentation are also reported.

2. Material and methods

2.1. Wine samples

Three white wine varieties of grapes (Códega, Gouveio and Malvasia Fina) from the Douro Region in north-east of Portugal were picked at 2 levels of maturation, characterized by 158 and 192 g l^{-1} sugar content, respectively. In the winery (Caves Transmontanas), they were directly crushed in a membrane press with a pressure that varied from 0 to 2 bar. The juice obtained was divided into 2 fractions: one that resulted from the pressing period, 0–0.4 bar (first cut), and the other from the period 0.4–2 bar (second cut). After static clarification at 10°C and inoculation with a commercial yeast (*S. cerevisiae*), each fraction was divided into stainless steel tanks. The fermentation occurred at spontaneous (19°C) or regulated (14°C) temperature by internal plates placed within the tanks. In some trials of Códega and Gouveio varieties, the fermentation also occurred in wooden barrels (new and 2 usage years) with spontaneous temperature.

2.2. Sample preparation

Fifty millilitres of wine were extracted with 50 ml of diethyl ether (three times). The ether fraction was separated and concentrated to dryness using a rotary evaporator and redissolved in 0.5 ml of methanol for HPLC analysis.

2.3. HPLC analysis of phenolic compounds

The separation of phenolic compounds was achieved with an analytical HPLC unit (Gilson), using a reversed-phase ODS-Hypersil (20×0.21 cm, 5 μm size particle) after injecting the samples (20 μl). The solvent system used (Garcia-Viguera & Bridle, 1995) was a gradient of water/formic acid (19:1) (A) and methanol (B); 0–2% B and 60–62% B at a solvent flow rate of 0.3 ml min^{-1}. Detection was achieved with a diode array detector and chromatograms were recorded at 280 and 320 nm. Phenolic compounds were identified by comparison of retention times and UV-vis spectra in the 200–400 nm range with those of standard solutions of pure substances and quantified by the external standard method. All the analyses were done in duplicate and the results expressed as the mean value.

2.4. Statistics

The results were statistically analysed by analysis of variance (ANOVA) methodology, followed by Fishery's PLSD test, with a significance level of 5%.

3. Results and discussion

The compounds identified and quantified by HPLC were gallic acid (RT 2 min 48 s), 3,4-dihydroxybenzoic acid (RT 5 min 29 s), caffeic acid (RT 17 min 58 s), syringic acid (RT 20 min 28 s), *p*-coumaric acid (RT 22 min 45 s), ferulic acid (RT 26 min 17 s), sinapic acid (RT 27 min 20 s) and ellagic acid (RT 37 min 56 s). Besides these compounds, quercetin was identified in one sample (data not presented).

In spite of no qualitative differences found, there were quantitative differences (Table 1) among the concentrations of individual phenolic compounds. The 3,4-dihydroxybenzoic acid presented the greatest values followed by *p*-coumaric acid and caffeic acid.

3.1. Influence of grape variety

The highest mean concentration of 3,4-dihydroxybenzoic acid was observed in Malvasia Fina (1216 μg l^{-1}), *p*-coumaric acid in Gouveio (726 μg l^{-1}) and caffeic acid in Códega (372 μg l^{-1}) (Fig. 1). Significant differences ($p = 0.002$) were reported for levels of *p*-coumaric acid among the 3 grape varieties assayed. According to the conclusions of Soleas et al. (1997) our results may suggest that these acids are exemplary of the phenolic acid pattern of these grape varieties.

3.2. Influence of grape maturation

Only the concentration of 3,4-dihydroxybenzoic acid was significantly affected ($p < 0.001$) by the level of grape maturation (Fig. 2). The difference between the two original groups of musts (158 and 192 g l^{-1} sugar content) corresponded to a discrepancy of 15 days in the date of harvest. This fact, associated with the terminus

Table 1
Phenolic acid contents of experimental varietal wines (($\mu g l^{-1}$)[a]

Local	Variety	Level of maturation[b]	Crushing pressure[c]	Reservoir of fermentation	Temperature of fermentation[e]	Gallic acid	3,4-DiOH-benzoic acid[g]	Syringic acid	Ellagic acid	Ferulic acid	Sinapic acid	*p*-Coumaric acid	Caffeic acid
A	Gouveio	1°	0–0.4	Stainless steel	14	140.3 (2.1)	1001 (17.9)	14.4	145 (5.1)	0.00	156 (3.3)	1643 (91.7)	457 (41.2)
A	Gouveio	1°	0–0.4	Stainless steel	19	68.3 (1.8)	872 (40.8)	0.00	127 (4.5)	22.8 (0.3)	222 (0.8)	843 (67.8)	296 (28.0)
A	Gouveio	1°	0.4–2.0	Stainless steel	14	Nq[f]	346 (6.6)	nq	58.3 (4.2)	20.6 (0.2)	169 (3.7)	848 (41.8)	244 (25.0)
A	Gouveio	°	0.4–2.0	Stainless steel	19	3.37 (0.0)	447 (3.3)	nq	40.3 (3.0)	0.00	94.5 (0.8)	615 (11.5)	203 (5.0)
B	Gouveio	1°	0–2.0	Stainless steel	14	120 (11.3)	699 (65.5)	22.2 (1.3)	179 (4.7)	nq[f]	188 (1.9)	1027 (24.4)	291 (11.5)
B	Gouveio	1°	0–2.0	Stainless steel	19	nq	662 (46.8)	19.3 (1.3)	61 (1.6)	0.00	114 (2.8)	720 (56.9)	255 (15.5)
B	Gouveio	1°	0–2.0	Barrel used[d]	19	nq	1020 (71.9)	25.0 (0.7)	119 (0.3)	32.4 (1.0)	137 (8.8)	510 (42.1)	271 (18.8)
C	Malvasia	1°	0–0.4	Stainless steel	14	nq	786 (66.8)	nq	0.00	0.00	98.4 (2.8)	600 (21.8)	92.0 (2.0)
C	Malvasia	1°	0–0.4	Stainless steel	19	1200 (16.5)	0.00	30.01 (1.1)	96.4 (0.8)	nq	96.4 (0.8)	255 (13.2)	98.9 (0.70)
C	Malvasia	1°	0.4–2.0	Stainless steel	14	14.8 (0.9)	867 (41.4)	0.00	62.4 (1.1)	18.2 (0.1)	86.0 (0.7)	592 (25.6)	794 (11.4)
C	Malvasia	1°	0.4–2.0	Stainless steel	19	nq	735 (24.2)	nq	43.4 (0.5)	0.00	38.6 (3.4)	362 (114.7)	103 (4.1)
D	Malvasia	1°	0–0.4	Stainless steel	14	nq	571 (14.2)	0.00	6.64 (0.1)	nq	107 (4.9)	629 (69.4)	136 (1.3)
D	Malvasia	1°	0–0.4	Stainless steel	19	nq	663 (5.8)	nq	nq	0.00	48.34 (5.3)	135 (10.1)	144 (9.5)
E	Códega	1°	0–0.4	Stainless steel	14	nq	595 (10.5)	9.08 (0.1)	0.00	0.00	85.9 (10.2)	372 (21.2)	132 (3.4)
E	Códega	1°	0–0.4	Stainless steel	19	nq	425 (14.3)	0.00	7.27 (0.6)	0.00	47.0 (9.4)	258 (35.9)	173 (4.7)
F	Códega	1°	0.4–2.0	Stainless steel	14	nq	759 (0.9)	35.8 (0.8)	44.5 (2.8)	17.2 (0.3)	131 (8.9)	457 (23.6)	200 (3.1)
F	Códega	1°	0.4–2.0	Stainless steel	19	57.6 (0.1)	785 (10.6)	0.00	7.65 (0.1)	nq	112 (1.1)	228 (11.2)	167 (14.5)
G	Gouveio	2°	0–0.4	Stainless steel	14	57.1 (0.3)	886 (8.8)	0.00	19 (8.9)	nq	157 (13.0)	632 (19.0)	251 (18.4)
G	Gouveio	2°	0–0.4	Stainless steel	19	58.1 (3.3)	601 (9.5)	nq	72.7 (3.2)	0.00	133 (9.2)	267 (23.5)	196 (6.5)
G	Gouveio	2°	0–0.4	Barrel new	19	181 (2.8)	2477 (147.0)	50.9 (1.6)	573 (37.4)	259 (7.6)	756 (69.2)	293 (8.7)	292 (3.0)
G	Gouveio	2°	0–0.4	Barrel used	19	81.7 (6.1)	1133 (7.3)	43.7 (3.6)	188 (0.1)	0.00	3.31 (0.2)	414 (12.9)	216 (6.9)
H	Gouveio	2°	0–0.4	Stainless steel	14	60.4 (5.3)	1208 (159.1)	18.8 (0.8)	84.7 (1.6)	0.00	81.3 (2.0)	362 (71.2)	272 (1.0)
H	Gouveio	2°	0-0.4	Stainless steel	19	nq	909 (30.7)	nq	63.0 (1.0)	0.00	91.7 (1.6)	303 (17.4)	272 (23.4)
I	Malvasia	2°	0-0.4	Stainless steel	14	nq	605 (4.6)	6.96 (0.2)	23.7 (0.5)	28.1 (0.5)	106 (0.5	526 (12.6)	126 (9.5)
I	Malvasia	2°	0–0.4	Stainless steel	19	nq	1085 (6.5)	0.00	0.00	0.00	78.6 (3.0)	252 (2.9)	92.9 (1.4)
J	Malvasia	2°	0–0.4	Stainless steel	14	110 (1.2)	2614 (17.6)	14.4 (0.5)	44.5 (0.2)	0.00	7.57 (0.3)	363 (15.1)	144 (14.1)
J	Malvasia	2°	0–0.4	Stainless steel	19	71.0 (1.6)	4786 (319.5)	25.6 (0.7)	61.3 (0.9)	nq	214 (30.3)	928 (108.5)	270 (18.6)
K	Malvasia	2°	0–0.4	Stainless steel	14	nq	615 (5.7)	56.6 (1.8)	29.8 (0.7)	0.00	138 (5.1)	788 (28.6)	134 (4.0)
K	Malvasia	2°	0–0.4	Stainless steel	19	43.9 (0.2)	1072 (6.4)	0.00	nq	nq	132 (7.1)	748 (39.9)	180 (35.6)
L	Códega	2°	0–0.4	Stainless steel	14	57.6 (19.5)	1010 (45.7)	26.5 (0.9)	18.6 (0.6)	0.00	98.6 (5.1)	367 (17.6)	198 (12.6)
L	Códega	2°	0–0.4	Stainless steel	19	nq	1219 (176.8)	13.2 (2.0)	32.5 (0.8)	nq	96.8 (1.9)	364 (4.9)	1359 (30.2)
L	Códega	2°	0–0.4	Barrel new	19	53.8 (1.8)	847 (32.1)	30.6 (2.7)	83.8 (17.1)	0.00	41.2 (1.2)	127 (3.5)	1244 (2.4)
L	Códega	2°	0-0.4	Barrel used	19	nq	593.46 (30.9)	nq	73.4 (0.9)	0.00	80.6 (1.7)	2101 (26.5)	137 (0.0)

[a] Values are expressed as mean (standard deviation) of two determinations for each sample.
[b] 1°: Juice with 158 gl^{-1} of sugar content; 2°: 192 gl^{-1}.
[c] Pressure (bar).
[d] Usage of 2 years.
[e] °C
[f] nq: not quantified.
[g] 3,4-Dihydroxybenzoic acid.

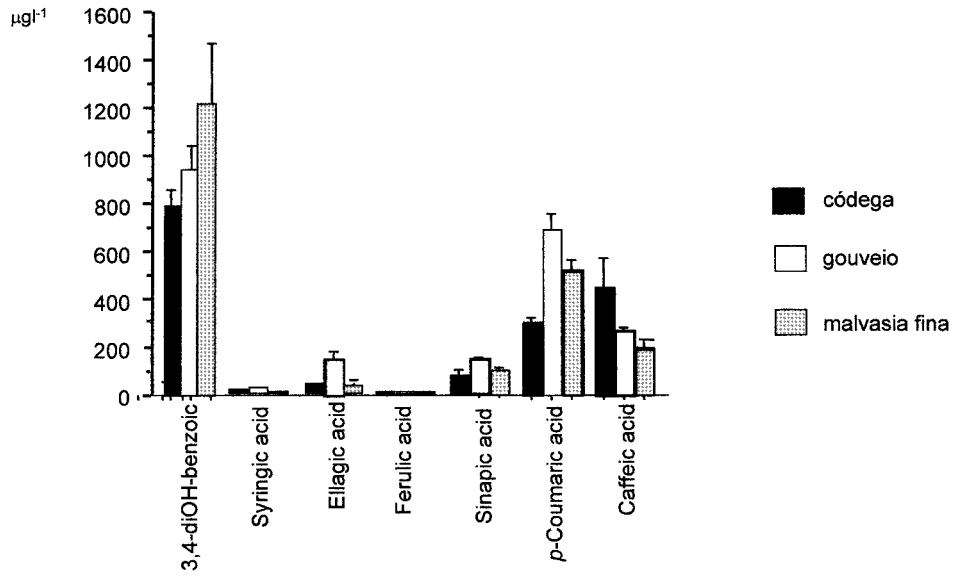

Fig.1. Comparison of phenolic acid mean concentration for each white varietal wine. Vertical bars represent the standard error.

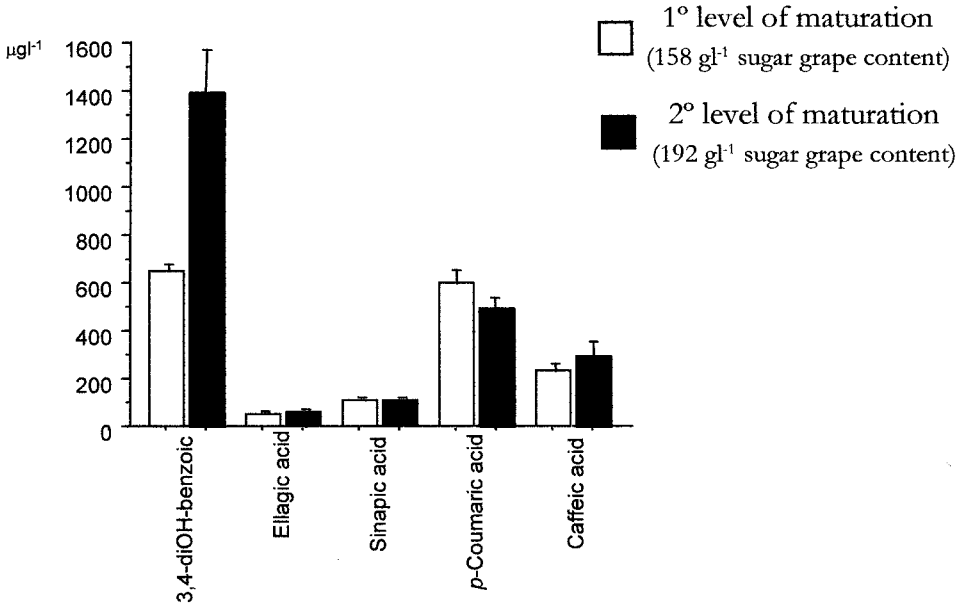

Fig.2. Comparison of phenolic acid mean concentration for each level of grape maturation. Vertical bars represent the standard error.

of grape maturation, may possibly explain the reduced differences found.

3.3. Influence of fermentation temperature

The mean fermentation temperatures (14 and 19°C) induced significant differences in syringic acid ($p = 0.0196$) and *p*-coumaric acid ($p = 0.013$). A decrease in the fermentation temperature, from 19 to 14°C caused an increase of syringic and *p*-coumaric acids in all varietal wines (Fig. 3). The concentrations of ellagic, sinapic and 3,4-dihydroxybenzoic acids in Códega and Gouveio wines also registered an increase associated with the drop in fermentation temperature, whereas Malvasia Fina wine gave an inverse relationship.

3.4. Influence of wooden barrels

The fermentation of both varietal wines in new wooden barrels (Fig. 4) leads to significantly higher concentrations of 3,4-dihydroxybenzoic, and ellagic acids that in other materials where the fermentation took place

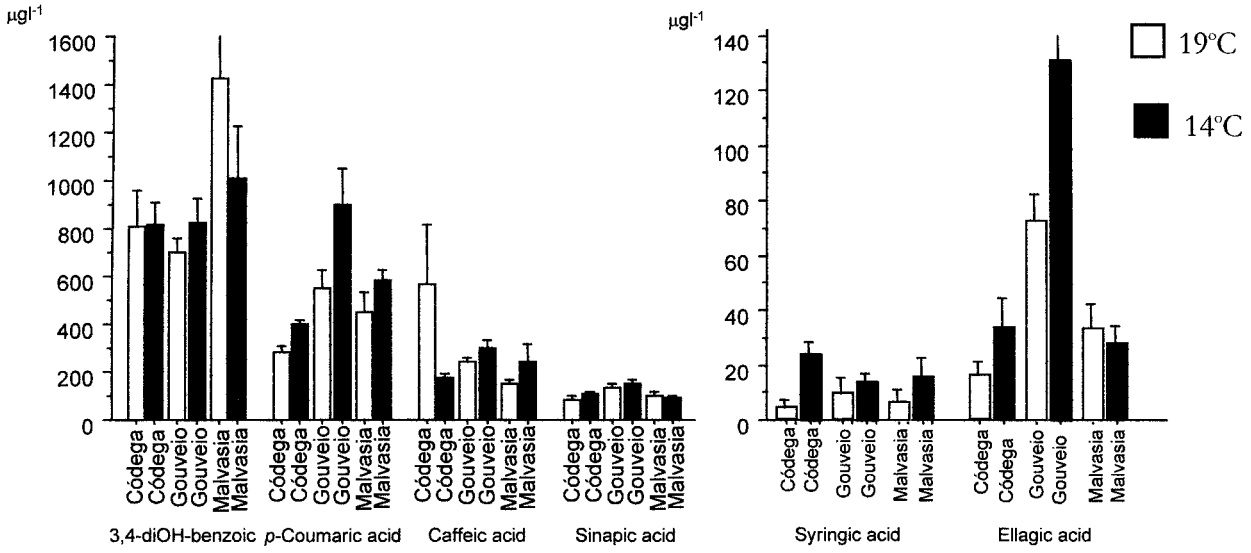

Fig.3. Comparison of phenolic acid mean concentration for each fermentation tempreature. Vertical bars represent the standard error.

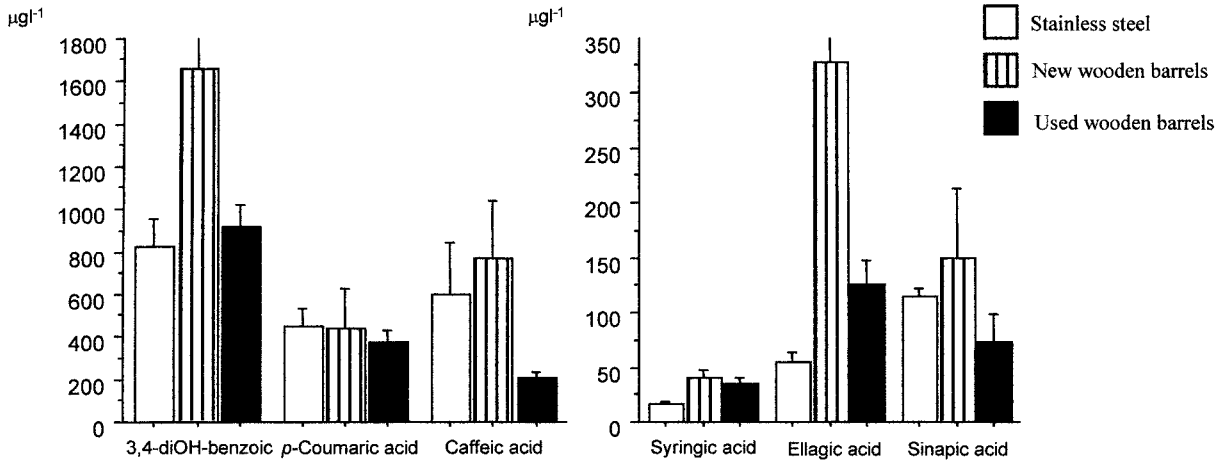

Fig.4. Comparison of phenolic acid mean concentration for each reservoir of fermentation. Vertical bars represent the standard error.

(stainless steel or used wooden barrels). The used wooden barrels did not significantly affect the concentration of the phenolic acids, with the exception of caffeic acid which showed a significant decrease.

In terms of total phenolic compounds under study, varietal Gouveio wines fermented in wooden barrels presented 3223 µ g l^{-1}, followed by Códega wines with 1734 µg l^{-1}. In Gouveio wines, an increase of all phenolics compounds was detected. On the other hand, in Códega wines the main phenolic acids decreased with the fermentation in wooden barrels.

This preliminary study shows that phenolic analysis can be useful for studying effects of grape composition and winemaking technology in the quality of white varietal wines.

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