PHENOLIC COMPOUNDS PRESENT IN NATURAL HAZE PROTEIN OF SAUVIGNON WHITE WINE•

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1. INTRODUCTION

The appearance of protein haze in wines remains as problem for wineries (Ribéreau-Gayon *et al*., 2006). In addition to the proteins, other factors can participate and even trigger the appearance of hazes (Esteruelas *et al*., 2009. Phenolic compounds are probably one of the non-protein factors potentially involved in protein haze formation (Siebert, 1999). The aim of this work was the identification and quantification of polyphenols present in natural precipitate of a Sauvignon wine.

2. MATERIALS AND METHODS

2.1. Sample preparation.

A Sauvignon blanc wine, just after alcoholic fermentation, was centrifuged (10 min; 12,000 g), filtered (0.45 μm), bottled (375 mL) and stored at room temperature until natural protein precipitation appeared. The precipitate was homogeneously distributed in 8 centrifuge tubes. The tubes were then centrifuged (10 min at 12,000g) and the pellets were transferred to a previously weighed microcuvette. This sample was then lyophilized, weighted to determine the dry weight of the precipitate and conserved at -20 ºC. Subsequently, the samples were directly dissolved with a total volume of 2 mL of 50 mM disodium phosphate solution (pH 9.23).

2.2. Acidic hydrolysis

600 μL of HCl 12 M were added to 300 μL of dissolved precipitate. The samples were boiled for 30 min to develop the Bate-Smith reaction (Bate-Smith, 1954) to verify the presence of proanthocyanidins by means of the HPLC detection of cyanidin. The sample was cooled to room temperature and 100 μL of ethanol were added. The sample was lyophilized and solved in 500 μL of methanol.

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2.3. CG/MS

100 µl of dissolved precipitate were lyophilized and the residue was dissolved in 100 µl of 1:1 BSTFA/pyridine (v/v). A Hewlett Packard 6890 Series gas chromatograph, equipped with a split/splitless injection port, interfaced to a 5973 mass-selective detector was used. An HP-5MS fused silica capillary column was used. The carrier gas-line pressure was set at 50 psi, column head pressure at 7 psi, and the septum purge was set at 0.8 ml min^{-1}. The injector temperature was 300 ºC, and the volume injected was 1 µl in the splitless mode. The column temperature program was: oven equilibration time 1 min; initial temperature 120 ºC for 3 min, then raised to 320 ºC at a rate of 5 ºC min^{-1}, with a final isotherm of 5 min. Identification of each phenolic compound was established by comparing their gas chromatographic retention times and sylylated derivative mass spectra to those of the derivatized standards. The concentration of the identified phenolic compounds was performed by relating the peak areas of the compound.

2.4. HPLC/ESI-TOF

An HPLC 1200 Series coupled with a MSD 6210 Time of Flight (Agilent Technologies, Madrid, Spain) equipped with electrospray ionization (ESI) was used, using a Eclipse-Plus C18 column 4.6 x 250 mm. A gradient of solvent A (formic acid, 0.2 % v/v) and solvent B (acetonitrile, solvent A, 80/20, v/v) was used. The elution conditions were: flow rate 1.2 mL min^{-1}, temperature 30 °C; volume injection 50 µL. The following proportions of solvent B were used: 0-5 min, 2 %; 5-10 min, 6 %; 10-15 min, 12 %; 15-30 min, 22 %; 30-35 min, 34 %; 35-40 min, 100 %; 40-45 min, 0 %. Positive-ion mode electrospray ionization mass spectrometry (ESITOF) spectra was recorded. ESI was performed using: 3.5 kV of capillary voltage; 350 ºC of drying gas temperature and 12 L min^{-1} of drying gas flow at 60 psi of Nebulizer gas pressure. The fragmentor voltage was 150 V, 65 V the skimmer voltage and 250 V the octapol voltage, the mass was scanned from m/z 50 to 2400. Identification of each phenolic compound was established by comparing their retention times to those of the standards.

3. RESULTS AND DISCUSSION

The phenolic compounds chromatogram obtained by gas chromatography-mass spectrometry (GC-MS) analysis in selected ion monitoring (SIM) and the quantification of these data (fig. 1) indicate that tyrosol, p-coumaric acid and caffeic acid are the most abundant phenolic compounds present in the protein precipitate. Other phenolic compounds, vanillic acid, protocatechuic acid, syringic acid, gallic acid, ferulic acid, (+)-catechin and shikimic acid were also present but in minor amount.

The extract ion chromatogram of the natural precipitate directly and after applying the Bate-Smith reaction (fig. 2 and tab. 2) shows that when the dissolved precipitate was directly injected, only three peaks were identified: vanillic acid and trans-p-coumaric acid, which were

also detected in the precipitate by GC/MS, and ethyl coumaric acid ester, which was not detected by GC/MS. Finally, quercetin and cyanidin were detected after the acid hydrolysis of the precipitate. The presence of cyanidin suggests that proanthocyanidins are involved in the natural precipitate.

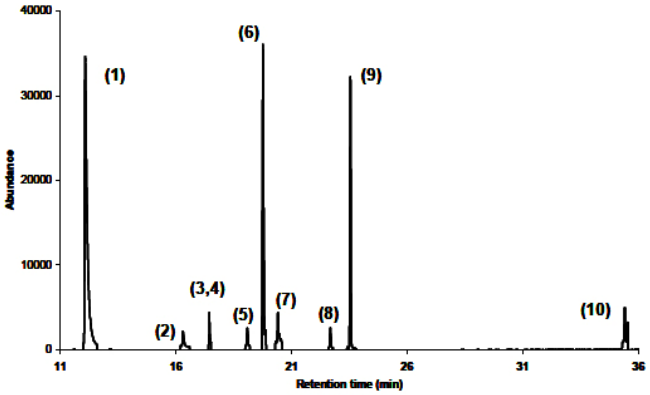

Table 1. CG/MS

Compound	Amount (µg)	% (w/w)
1. Tyrosol	116 ± 2	0.95 ± 0.01
2. Vainillic acid	1.27 ± 0.08	0.011 ± 0.001
3. Protocatechuic acid	24.4 ± 0.3	0.204 ± 0.002
4. Shikimic acid	16 ± 1	0.13 ± 0.01
5. Syringic acid	7.3 ± 0.6	0.06 ± 0.01
6. t-p-Coumaric acid	172 ± 7	1.40 ± 0.06
7. Gallic acid	6.9 ± 0.5	0.06 ± 0.01
8. Ferulic acid	39 ± 2	0.33 ± 0.01
9. t-Caffeic acid	380 ± 18	3.13 ± 0.06
10. (+)-Catechin	6.2 ± 0.3	0.05 ± 0.01
TOTAL	**769 ± 3**	**6.32 ± 0.15**

Fig. 1 – Identified phenolic compounds in natural precipitate by GC/MS.

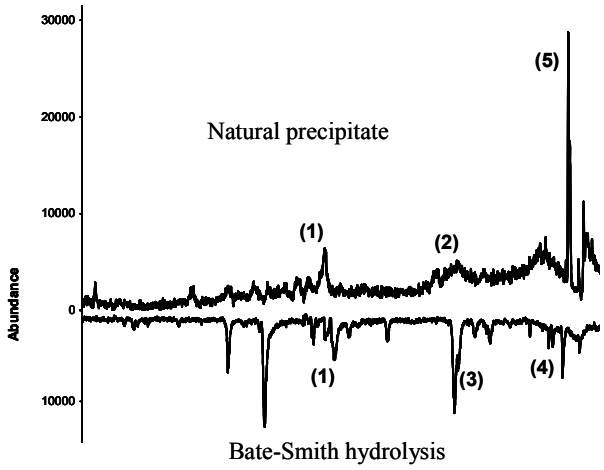

Fig. 2 - Identified phenolic compounds in natural precipitate by HPLC/ESI-TOF.

According to the data concerning the quantification of these compounds (tab. 2), the total amount of phenolic compounds represented 6.3 % of the dry weight of the natural protein precipitate. In an earlier study (Esteruelas *et al*., 2009), we found that the percentage of phenolic compounds present in a natural precipitate, by the colorimetric method of Folin-

Ciocalteu, was 7.2 ± 0.4 %. Both values are very concordant and confirm that phenolic compounds are a non-negligible component of protein precipitate, thus suggesting also that they may play a role in the appearance of protein haze.

Tab. 2 - The quantification of compounds by HPLC/ESI-TOF.

Compound	Direct analysis		After Bate-Smith Reaction	
	Amount (μg)	% (w/w)	Amount (μg)	% (w/w)
Vanillic acid	2.02 ± 0.07	0.017 ± 0.001	9.9 ± 0.4	0.082 ± 0.003
t-p-Coumaric acid	68 ± 1	0.57 ± 0.01	nd	nd
Cyanidin	nd	nd	47 ± 2	0.39 ± 0.02
Quercetin	nd	nd	16 ± 2	0.13 ± 0.02
Coumaric acid ethyl ester	99 ± 6	0.82 ± 0.05	nd	nd
TOTAL	168 ± 7	nd	72 ± 1	0.60 ± 0.01

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Abstract

The aim of this work was the identification and quantification of polyphenols present in natural precipitate of a Sauvignon wine. Phenol analysis in wine precipitate was based on acid hydrolysis, CG-MS after derivatization, and LC-MS. Several phenolic compounds were identified, and total phenols reached 6.3% DW of the total precipitate. These results confirm that phenolic compounds are a non-negligible component of wine protein precipitates.

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