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trans-Resveratrol Analysis: Comparison of Methods and Contents in 1 Muscatel Fortified Wines from Setúbal Region in Portugal 2

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12 Abstract

13 Liquid chromatography and mass spectrometry conditions were developed in order to identify 14 trans-resveratrol in sweet fortified Muscatel wines from Setúbal region (Portugal). Diode array, 15 fluorescence and electrochemical detectors were used for quantitation purposes. The detection and quantitation limits for each detection mode were: 0.02 and 0.06 mgL⁻¹ for UV-Vis, 0.01 and 16 0.03 mgL^{-1} for fluorescence and 0.45 and 1.35 mgL⁻¹ for electrochemical. Repeatibility (n=6) 17 18 expressed as the relative standard deviation of peak areas for a standard solution of trans-19 resveratrol (1.75 mgL⁻¹) was 0.5% for fluorescence and 1.5 % for UV-Vis and electrochemical 20 detection modes. Samples collected at one representative producer, during winemaking process, 21 were injected without pre-treatment and the quantitation of trans-resveratrol was carried out 22 using fluorimetric detection. The trans-resveratrol content decreased slightly along the 23 winemaking process and the concentrations ranged from 0.22±0.02 to 0.16±0.02 mgL⁻¹. 24 After maturation stages, trans-resveratrol contents in wines collected at different producers were compared: values obtained range from 0.13±0.02 mgL⁻¹ to 0.38±0.03 mgL⁻¹. The trans-25 26 resveratrol contents in commercially available wines from the same producers were lower.

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29 Keywords: Muscatel wine; trans-resveratrol; Liquid chromatography; fluorescence detector; 30 mass spectrometry

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- 33

34 1. Introduction

35 Stilbenes belong to the non-flavonoid class of phenolic compounds and resveratrol is 36 the major stilbene component present in white and red grapes and in grape products 37 such as wines. Resveratrol occurs in two isomeric forms (*trans* and *cis*): the *trans*-38 resveratrol or 3,5,4'-trihydroxystilbene (Fig.1.) is the most abundant form and is mainly 39 located in grape skins (Monagas et al., 2005; Wang et al., 2002). This compound can 40 also be detected as glucosides: *trans* and *cis* piceid.

41 trans-Resveratrol has an important role in human health as it presents antibacterial, antifungal and antioxidant properties (Kolouchová-Hanzlíkova et al., 2004) and has 42 43 been reported as responsible, in part, for the lower mortality by cardiovascular diseases observed in moderate and regular wine-consuming populations (Vitrac et al., 2002; 44 45 Urpí-Sardà et al., 2005; Lamuela-Raventós et al., 1995). This compound has also the 46 capacity to protect against global cerebral ischemic injury and to protect from oxidative 47 damage and inhibits tumour initiation, promotion and progression (Sánchez et al., 2005; 48 Moreno-Labanda et al., 2004).

49 Resveratrol in grapes is synthesized almost entirely in the grape skin and its maximum 50 concentration occurs just before the grapes reach maturity. The stilbene synthase is the 51 enzyme used in the biosynthesis of resveratrol and the concentration in grapes varies 52 considerably, depending on the grape variety (Threlfall et al., 1999; Gürbüz et al., 2007) 53 and abiotic factors, such as hydric stress (Abril et al., 2005; Moreno-Labanda et al., 54 2004) or UV irradiation (Sun et al., 2006; Sánchez et al., 2005; Piñeiro et al., 2006). The 55 content of *trans*-resveratrol in red and white grape skins (Kammerer et al., 2004) ranged from 11.1 to 123.0 mgKg⁻¹ of dry matter, but a large variation was observed for the 56 57 same cultivars analysed in different years. The resistance of the plant against fungal 58 diseases as downy mildew and grey mould caused by *Plasmopara viticola* or *Botrytis*

cinerea respectively (Adrian et al., 2000; 37-41; Gerogiannaki-Christopoulou et al., 2006) has been related with the occurrence of resveratrol. Jeandet et al. (1995), refer that higher concentrations of resveratrol should be expected in wines made with grapes that suffered a *Botrytis* infestation.

63 The process used in winemaking can also induce significant changes in the content of 64 trans-resveratrol (Castellari et al., 1998, Jeandet et al., 1995; Clare et al., 2004, Stervbo 65 et al., 2007). Wines obtained using traditional winemaking technologies (skin 66 fermentation with or without stems) have higher content of resveratrol than those obtained by carbonic maceration techniques (Sun et al., 2006). The extraction of 67 68 resveratrol from grape skins seems to be completed before or by the end of alcoholic 69 fermentation and a prolonged maceration after alcoholic fermentation may not affect the total content of resveratrol in finished wines. The use of bentonite treatment can be 70 71 responsible for a decrease (2-5%) in the amounts of trans-resveratrol (Castellari et al., 72 1998) and the effect of other parameters have also been referred: a decrease in the temperature during maceration, precipitation and adsorption on marc and/or pH 73 74 increasing (Stervbo et al., 2006; Gambuti et al., 2004).

75 In table 1 are summarized data on *trans*-resveratrol contents in wines.

The *trans*-resveratrol can be analysed directly (Abril et al., 2005; Vitrac et al., 2002) or after pre-treatment of wine samples in order to increase its content. Liquid-liquid extraction (Wang et al., 2002) and solid-phase extraction (SPE) (Rodríguez-Delgado et al., 2002; Domínguez et al., 2001; Malovaná et al., 2001; Gamoh et al., 1999) have been used in sample preparation for concentration and cleaning purposes.

In order to determine the concentration of *trans*-resveratrol in wine, separative methods have been reported. Gas chromatography-mass spectrometry (GC-MS), (Jeandet *et al*, 1995; Flamini et al., 2003) requires derivatization to enhance the

volatility of resveratrol. Liquid chromatography (LC) is the most used technique 84 85 (Mattivi et al., 1995; 43, Pour Nikfardjam et al., 2006; Dourtoglou et al., 1999; Flamini 86 et al., 2003) but capillary electrophoresis (CE) techniques have also been reported and 87 sensitivity is similar to LC methods (Xuelin et al., 2000). Different detection modes 88 have been used. The chomatograms obtained using UV detection are very complex due 89 the enormous variety of compounds present in wines, making difficult the data 90 treatment. Other detectors, more specific and sensitive, have been used recently, as mass spectrometry (MS) (Wang et al., 2002; Callemien et al., 2005), electrochemical (ED) 91 (Zhu et al., 2000; Kolouchová-Hanzlíková et al., 2004; Bravo et al., 2006) and 92 fluorescence (FD) (Rodríguez-Delgado et al., 2001; Vinas et al., 2000; Vitrac et al., 93 94 2002). The excitation and emission wavelengths reported in the literature and some 95 conditions of analysis with fluorescence detection are included in table 1.

96 The aim of the present study was to determine the concentration of *trans*-resveratrol 97 in Muscatel fortified wines from different producers from Setúbal region in Portugal. 98 During the winemaking process the grape seeds and the skins of the white Muscatel 99 grape vine varieties, stay in contact with wine for several months, after the addition of 100 spirit to stop the fermentation process. As far as we know there are no reports about 101 trans-resveratrol content in this type of portuguese wines. LC-MS was used to identify 102 the compound in the samples analysed. The performances of other detection modes 103 (UV, FD and ED) were compared in order to assess their possible contributions for the 104 purpose of quantitation or simple comparison of samples. Among these detection modes 105 fluorescence was chosen to monitor the content of *trans*-resveratrol present in samples 106 collected from one producer, during vinification process. The same methodology can be 107 used to analyse resveratrol in other types of wines provided there is attention to eventual 108 matrix effects.

110	2. Materials and methods
111	2.1. Reagents
112	Acetonitrile and methanol gradient grade were from Lab-Scan (Dublin, Ireland), the
113	acetonitrile (LC-MS) was purchased from Riedel-de-Häen (Seelze, Germany) and the o-
114	phosphoric acid 85% p.a. was from Panreac (Barcelona, Spain).
115	Deionised water (0.050 μ Scm ⁻¹) used in all experiments was obtained from Milli-Q
116	system (Millipore, Molsheim, France).
117	The <i>trans</i> -resveratrol was obtained from Sigma (Steinheim, Germany).
118	G
119	
120	2.2. Sampling
121	All Muscatel wines were produced during 2004 harvest at three different wineries
122	(identified as A, B and C) located at Setúbal Denomination of Controlled Origin
123	(D.O.C.) region. During the vinification process, spirit (77% alcohol content) was added
124	to the must in order to stop the fermentation. The proportion of spirit added was among
125	25-30L for 100 Kg of must. After a maceration (M) period (4, 5 and 7 months for
126	producers A, B and C respectively) the wines were separated from the pomace and the
127	liquid resulting from the pressing (LP) was mixed with the wine and a maturation stage
128	(Mt) was started. Producer A transferred the wine into wooden barrels, but the other
129	producers kept the wine in cement tanks with an epoxy coating. After 18 months of
130	maturation, producer B transferred the wine and blended it with wines from others
131	tanks. The wine samples studied in this work are described in table 2. In the text,
132	samples will be identified by the letter corresponding to the producer and a number in
133	subscript corresponding to the month when collection was held.

Commercially available samples from producers A, B and C were purchased at a local
supermarket: wines were analysed immediately after opening the bottles, using the same
analytical conditions.

137

138 2.3. Sample preparation

In order to concentrate samples to be analysed by LC-MS, wine sample C_1 (10 mL) was extracted four times with 7 mL of ethyl acetate. The ethyl acetate extracts were combined and evaporated to dryness under vacuum. The residue was redissolved in 250 µL of methanol/water (6:4 v/v) (Wang et al., 2002). For the analysis by LC-DAD-FD-ED the samples were injected without pre-concentration.

144 The extracts and the wines were filtered through 0.45 µm membranes (Millipore).

145

146 2.4. Standards solutions preparation

A standard stock solution of *trans*-resveratrol (1000 mgL⁻¹) was prepared in methanol and stored in darkness at -20°C. Different standard solutions of *trans*-resveratrol were prepared depending on the detection method sensitivity: 0,075-5.0 mgL⁻¹ for the UV-Vis and FD detection and 1.35-8.0 mgL⁻¹ for ED.

In order to obtain the absorption and emission spectra of *trans*-resveratrol, a working standard solution was prepared (1.0 mgL⁻¹) by diluting the stock solution with the solvent corresponding to the chemical composition of the mobile phase used to elute the compound during chromatographic separation: 35 % of phosphoric acid solution 0.1 % and 65 % of phosphoric acid:acetonitrile:water (1:400:599). For MS analysis a 120 mgL⁻¹ standard working solution of *trans*-resveratrol in methanol was prepared.

157

158 2.5. Equipments and conditions of analysis

159 2.5.1. Liquid chromatography with mass spectrometry

160 Analyses by LC were performed with a Surveyor equipment from Thermo Finnigan. 161 The mass spectrometry system was an LCQ ion trap mass spectrometer (Thermo 162 Finnigan, San Jose C.A., USA) equipped with an ESI (Electrospray ionisation) or APCI 163 (Atmospheric pressure chemical ionization) sources. The LC-MS system was run by 164 Excalibur version 1.3 software (Thermo Finnigan – Surveyor, San Jose, USA). ESI and 165 APCI were tested as ionization sources in negative and positive polarity modes. The analytical column was a RP-18 (100×2.1 mm i.d., 5 µm particle size) from Thermo 166 with a guard column of the same type. The samples were injected using a 20 µL loop; 167 the separations were carried out with a flow rate of 250 µLmin⁻¹. The column 168 169 temperature was 35° C. The mobile phase used for resveratrol identification consisted of 170 a mixture of eluent A (formic acid 0.5%) and eluent B (acetonitrile). The analyses were 171 performed using the following program of eluents: 0-4 min from 0 until 15% eluent B; 4-20 min, from 15 until 40% eluent B; 20-30 min, from 40 until 55% eluent B. 172

In the mass spectrometer experiments the following conditions were used for the APCI source in negative and positive modes: vaporizer temperature, 470° C; discharge current, 5 μ A; temperature of the heated capillary 175° C. ESI ionization source in positive and negative mode was also tested in this work with the following conditions: temperature of the heated capillary 280° C, source voltage 3.70 kV. Nitrogen was used as sheath gas and auxiliary gas with a gas flow rate of 80 and 20 arbitrary units, respectively.

180 LC-MS was performed in the selected ion monitoring mode (SIM) for m/z 229. In the 181 fragmentation experiments, a collision energy of 35% was used with scanning range 80-182 2000 amu.

183

184 2.5.2 Absorption and emission spectra of trans-resveratrol

185 In order to evaluate the effect of the mobile phase composition in the absorption and 186 emission spectra of *trans*-resveratrol and to optimize fluorescence detection, the spectrum of a standard solution (1.0 mgL^{-1}) was determined in a mixture of eluents A 187 and B described in section 2.4. The UV spectra of the standard working solution of 188 trans-resveratrol (1.0 mgL⁻¹) was recorded with the spectrophotometer (DU-70, 189 190 Beckman Instruments, Inc., Fullerton, USA) using a 1cm path length quartz cell. The 191 value of absorbance measured at 306 nm (room temperature) was used to calculate the 192 molar absorptivity.

The fluorescence emission spectra of *trans*-resveratrol was recorded in a spectrofluorometer (SLM Aminco), equipped with a Xenon lamp (Muller Elektronik Optic). The excitation wavelength was set at 300 and 306 nm. The excitation slits were set to provide 8-nm band passes slit and a 1 cm path length quartz cell was used. Data acquisition was performed with the software Model 8100 Spectrofluorometer version 4.20 1998 Spectronic Instruments, Inc.

199

200 2.5.3. Liquid chromatography with diode array, fluorescence and electrochemical
201 detection

Surveyor equipment from Thermo Finnigan with a diode array, fluorescence (Thermo
Finnigan FL 3000) and an electrochemical (Dionex, ED40) detectors were used.

The analytical column was a RP-18 ($250 \times 4 \text{ mm i.d}$, 5 µm particle size) from Merck with a guard column of the same type. The samples were injected using a 20 µL loop; the separations were carried out with a flow rate of 700 µLmin⁻¹ and column temperature of 35° C. The conditions of analysis were previously optimized and reported (Bravo et al., 2006).

- 209 All samples were analysed as duplicates. Mean values and confidence intervals are 210 reported.
- 211

212 **3. Results and discussion**

213 3.1. Identification of trans-resveratrol by LC-MS

Analysis of trans-resveratrol at a flow rate of 700 µLmin⁻¹, in APCI and ESI positive 214 215 modes SIM at m/z= 229 were compared: the detection limit (LOD) for APCI was lower (10 mg/L) than for ESI (100 mg/L). Using a flow rate of 250 µLmin⁻¹ (Urpí-Sardà et al., 216 2005, Silva et al., 2005) and the same APCI analytical conditions the LOD was 217 improved to 1.0 mg/L. The chromatograms (SIM m/z= 229) of the Muscatel wine 218 extract from producer C after one month maceration and a standard solution of trans-219 resveratrol 120 mgL⁻¹ are compared in Fig. 2. For *trans*-resveratrol standard a peak was 220 221 detected at 9.59 min (Fig. 2A.) and a peak eluting at the same retention time as the standard (Fig. 2B) was detected in the analysis of the Muscatel wine extract. For 222 identification purpose, the mass spectra obtained by MS^2 fragmentation of ion m/z 229 223 [M+H]⁺ for a standard solution and a wine sample extract are compared in Fig. 3. This 224 225 fragmentation originated several ions: at m/z 107.0, 118.9, 134.9, 183.0 and 211.1. These fragments are in agreement with published data (Callemien et al., 2005): 226 fragment at m/z 211.1 corresponds to the neutral loss of 18 mass units equivalent to a 227 228 molecule of water and proposed structures for ions with m/z 107.0, 118.9, 134.9 are also 229 presented in Fig. 3.

230 These experiments confirmed the presence of trans-resveratrol in the wine samples.

231

232 3.2. Absorption and emission spectra of trans-resveratrol

The UV spectra of a *trans*-resveratrol (1.0 mgL^{-1}) in eluent solution (35% eluent A and 65% eluent B), recorded from 200 to 400 nm presents a maximum ranging from 300 to 320 nm and these values are in accordance with data published by Trela et al. (1996). The molar absorptivity for trans-resveratrol at 306 nm in this solvent (32000 mol⁻¹Lcm⁻¹) is similar to the values reported by the same authors (29452 mol⁻¹Lcm⁻¹ in methanol) at the same wavelength and for López et al. (2001) at 308 nm (30000 mol⁻¹Lcm⁻¹ in ethanol).

Fig. 4. shows the emission spectra of *trans*-resveratrol in eluent solution using 300 and 306 nm as excitation wavelengths. The intensity of emission with excitation at 300 nm was slightly higher and therefore was chosen as excitation wavelength. The following conditions were chosen in order to perform fluorescence detection: excitation wavelength (λ_{Ex}) at 300 nm and the emission wavelength (λ_{Em}) at 390 nm.

245

246 3.3. Analysis by LC-DAD-FD-ED of trans-resveratrol standard solutions

In order to perform quantitation of *trans*-resveratrol in samples, standard solutions were analysed and peak areas were measured using UV-Vis absorption at 306 nm, fluorescence ($\lambda_{Ex} = 300 \text{ nm} / \lambda_{Em} = 390 \text{ nm}$) and electrochemical detection. Results from calibrations performed are shown in table 3. Each point of the calibration curve corresponds to the mean value obtained from two independent area measurements.

Limits of detection (LOD) and quantitation (LOQ) were determined considering the concentrations of *trans*-resveratrol that gave a signal to noise ratio (S/N) of 3/1 and 10/1, respectively. Noise was measured in the chromatogram of a blank run in the range near the retention time of the analyte using the Root Mean Square (RMS) calculation. The lowest detection and quantitation limits were achieved with a fluorescence detector. For electrochemical detector the LOD and LOQ obtained were higher (Table 3). These

limits were confirmed through the repeated injections (n=6) of the correspondingstandard solutions in the concentrations indicated for the three detectors.

The repeatability of peak areas using this analytical method was tested by the repeated injections (n=6) of a standard solution (1.75 mgL⁻¹⁾) of *trans*-resveratrol. The repeatability of peak area, expressed as relative standard deviation, for fluorescence detector is lower (0.5%) than the results obtained with UV-Vis and electrochemical detectors (1.5%).

265

266 3.4. Analysis by LC-DAD-FD-ED of Muscatel fortified wines

In previous work (Bravo et al., 2006) several phenolic compounds were already identified in this type of wine using HPLC-DAD-MS. The chromatographic profiles obtained with DAD are very complex due to the diversity of the phenolic compounds present in wine samples but there is still a good resolution among a high number of peaks.

Wine samples from the three producers collected 3 months after the beginning of the vinification process (A_3 , B_3 , C_3), during the maceration of seeds and skins in contact with wine, were analysed using the three detection modes.

The chromatogram of the wine sample (B_3) is shown in Fig. 5. The UV spectrum of the peak with the retention time of trans-resveratrol is compared with the spectrum of the trans-resveratrol standard. The observed differences in spectra may be explained by the co-elution of an interfering compound in the sample. Therefore quantitation of transresveratrol with UV is a difficult task and can lead to incorrect results.

280 Chromatographic profiles obtained with electrochemical detection present a lower 281 number of detected peaks since this technique is more selective (Fig. 6.). This detection 282 mode was useful for the detection of phenolic compounds electrochemically active, but

283 *trans*-resveratrol content in the samples of Muscatel wines analysed were lower than
284 LOD determined for electrochemical detection. In order to use this detector for
285 quantitation, it would be necessary to prepare concentrated extracts.

The fluorimetric detection allowed analysis without sample pre-treatment, even in wines with low trans-resveratrol content. The chromatographic profiles obtained for wine B_3 and a standard solution are compared in Fig. 7. The identification of transresveratrol was confirmed by analysis of samples spiked with a standard.

290

291 3.5. trans-Resveratrol content of Muscatel wines

The content in *trans*-resveratrol was compared in wines obtained from different producers at different vinification stages as well as commercial samples from the same producers and results were estimated from chromatograms obtained with fluorescence detection.

Analysis of samples collected along 20 months of the winemaking at producer B are 296 297 discussed as an example of the evolution of *trans*-resveratrol content. During the maceration process (4 months) the concentration of *trans*-resveratrol had no significant 298 changes (mean value 0.22 mgL⁻¹). The results obtained are in accordance with published 299 300 data which suggest that prolonged maceration after alcoholic fermentation does not 301 increase the resveratrol content (Sun et al., 2006). After the maceration stage, the wine 302 was separated from the pomace, transferred into a cement tank with an epoxy coating. 303 and the liquid resulting from the pressing of the pomace was added to the wine. There is 304 no significant difference in *trans*-resveratrol content between this sample and the ones previously analysed. The wine was then blended with other wines from the same 305 vinification: the content of *trans*-resveratrol was higher (0.24 mgL⁻¹) probably due to 306 phenolic composition differences in the blended wines. During the maturation process 307

there was a decrease in the *trans*-resveratrol content ($0.14\pm0.02 \text{ mgL}^{-1}$ after 18 months). As SO₂ or other antioxidant was not added to wine, *trans*-resveratrol content could decrease due to an oxidation process.

311 The contents of *trans*-resveratrol in wines at different vinification stages are presented 312 in table 4. For wines collected at equal periods of maceration or maturation, the results 313 show that the concentration of *trans*-resveratrol of wine from the producer A is lower 314 than the concentrations found in wines from other producers. Lower temperatures 315 during maceration can be responsible for lower concentrations in wines from producer A, as the maceration process occurred in inox tanks protected at the top by a metallic 316 317 roof, while the other producers used cement tanks located inside buildings. The pH value (4.42) found for sample A is higher than the values obtained for samples B and C 318 (3.65 and 3.60 respectively) and this higher value of pH and lower content of trans-319 320 resveratrol may be related.

Each producer has brand name wines made by careful blending of wines produced with grapes of the company vineyards. The *trans*-resveratrol contents of wines commercially available from producers B and C (0.16 ± 0.01 and 0.15 ± 0.02 respectively) were similar and for producer A it was lower than LOD.

Comparison of results obtained with those presented in table 1 show that values obtained for Muscatel wines are higher than the concentrations reported for white table wines using fluorescence detection but lower than contents of fortified wines quantified using UV detection. The maceration with grape skins used in Muscatel wine production may contribute to a higher content of *trans*-resveratrol in wines. The use of a UV detector may explain higher values for the contents of this compound if there are coeluting peaks in the analysis by HPLC.

4. Conclusions

HPLC with fluorescence detection is more adequate than DAD for the analysis of *trans*-resveratrol in wine samples because some compounds may co-elute at the same retention time of *trans*-resveratrol. An electrochemical detector could also be used but the concentrations of this compound in the samples of Muscatel wines analysed were lower than LOD.

The use of fluorescence detection ($\lambda_{ex} = 300 \text{ nm}$, $\lambda_{em} = 390 \text{ nm}$) was suitable to quantify low concentrations of *trans*-resveratrol in Muscatel wines, allowing the quantitation of the compound by direct injection of wine samples with LOD = 0.010 mgL⁻¹ and LOQ=0.030 mgL⁻¹.

The concentration of *trans*-resveratrol decreased during the winemaking process of the Muscatel wine. Although grapes are grown in the same geographic region and processing conditions are similar, a higher concentration of *trans*-resveratrol was observed for one of the produced wines. The results obtained in commercial wines analyses from the same producers, confirmed the results observed for samples collected at production stages.

348

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Fig. 1. Structure of the *trans*-resveratrol

Fig. 2. SIM m/z=229 for a (**A**) standard solution of *trans*-resveratrol (120 mgL⁻¹); (**B**) extract of Muscatel wine from Setúbal region (C_1) , when analysis was performed at positive mode using

APCI source

Fig. 3. MS^2 of ion m/z=229 and the proposed fragments: (A) standard solution of trans-resveratrol (120 mgL⁻¹); (**B**) extract of Muscatel wine from Setúbal region (C_1)

Fig. 4. Emission spectra of a solution of *trans*-resveratrol (1.0 mgL⁻¹) in eluent solution : (A) excitation at 300 nm (B) excitation at 306 nm

Fig. 5. Chromatograms at 300 nm and UV spectra of (A) trans-resveratrol solution (0.45 mgL⁻ ¹); (B) Muscatel wine from Setúbal region (B₃). Identification: 1. gallic acid; 2. 5-HMF; 3. protocatechuic acid; 4. furfural; 5. caftaric acid; 6. catechin; 7. caffeic acid; 8. fertaric acid; 9. procyanidin dimer; 10. epicatechin; 11. ethyl gallate; 12. ferulic acid; 13. trans-piceid; 14. quercetin-3-glucuronide; 15. quercetin-3-glucoside; 16. cis-piceid, kaempferol-3-glucoside; quercetin-3-rutinoside; 17. trans-resveratrol; 18. cis-resveratrol, ethyl caffeate; 19. quercetin

Fig. 6. Chromatograms using electrochemical detection of Muscatel wines from Setúbal region (producers A₃, B₃ and C₃) and standard solution (3.75 mgL⁻¹). Identification: 1. gallic acid; 3. protocatechuic acid; 5. caftaric acid; 6. catechin; 7. caffeic acid; 14. quercetin-3-glucuronide; 15. quercetin-3-glucoside; 17. trans-resveratrol; 19. cis-resveratrol, ethyl caffeate

Fig. 7. Chromatograms using fluorescence detection (300/390 nm) of Muscatel wine from producer B_3 and standard solution (0.60 mgL⁻¹). Identification: 1. gallic acid; 5. caftaric acid; 6. catechin; 7. caffeic acid; 13. trans-piceid; 14. quercetin-3-glucuronide; 15. quercetin-3-glucoside; 16. quercetin-3-rutinoside; 17. trans-resveratrol; 19. cis-resveratrol Accel

553 **Table 1** Contents of *trans*-resveratrol (mgL⁻¹) in different wines analysed by HPLC and 554 corresponding detection (LOD) and quantitation (LOD) limits reported in the literature

- 554 corresponding detection (LOD) and quantitation (LOQ) limits reported in the literature

Table 2 Samples of Muscatel wines from Setúbal region collected during the vinification
 process

Table 3 Parameters for the quantitation of *trans*-resveratrol

Table 4 Concentrations of *trans*-resveratrol in Muscatel wines from Setúbal region (mean 562 value±confidence interval, mgL⁻¹)

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Table 1.

Wine origin	Win	e content (mgL ⁻¹⁻		Detection mode	λ _{abs} (nm)	A _{Exe} /A _{Emi} (nm)	LOD (mgL ⁻¹)	LOQ (mgL ⁻¹)	Eluents
	Red	White	Rosé						
Portugal mainland ^{(Baptista} et al. 2001)	1.56 ^a	C		diode array	306		0.010		 0.4% (v/v) o-phosphoric acid (pH 2.3) 80% (v/v)acetonitrile and 20% eluent A
Azores Island (Baptista et al, 2001)	3.36 ^a			1					
(Gerogiannaki-Christopoulou et al, 2004)		0.015 ^b		diode array	308			0.015	water: methanol (5:5)
		0 ^{.009°}						610.0	• water: acetonitrile (7:3)
Spain (Abril et al , 2005)	0.32 - 4.44		0.12-2.80	diode array	306				 glacial acetic in water (pH 2.4) glacial acetic in water (pH 2.4) / acetonitrile (20:80)
Azores Island (Ribeiro de Lima et al. 1999)		0.3-2.1		diode array	306		5 ^d		• glacial acetic in water (pH 2.4) • glacial acetic in water (pH 2.4) / acetonitrile (20:80)
e (Vinas et al, 2000)	n.d. – 2.35	p.u		diode UV-vis	300		0.06	0.22	
Span	n.d. – 2.36	0.06		fluorescence		324/370	0.003	0.01	
French (Vitrac et al, 2002)	2.3 ^a	0.06 ^a		fluorescence		300/390	0.01	0.03	• H ₂ O (99%) / trifluoro acetic acid (1%) • acetonitrile (80%) / eluent A (20%)
Spain (Rodriguez-Delgado et al, 2002)	2.89 ^a	0.095^{a}		fluorescence		360/374	0.003		 methanol (10%)/ acetic acid (2%) / water (88%) methanol (90%)/acetic acid (2%) / water (8%)
Turkey (Gürbüz et al. 2007)	0.176 - 4.403	0.116-1.931		fluorescence		324/370			 acetonitrile (9%)/ 5% aqueous acetic acid (91%) acetonitrile (25%) / 5% aqueous acetic acid (75%) acetonitrile (70%) / 5% aqueous acetic acid (30%)
Czech republic (Kalouchová-Hanzlíkova et al, 2004)	0.7-11			electrochemical			0.003		• acetonitrile (25%)/ 0.1% H ₃ PO ₄ and NaCl (5mmolL ⁻¹)
^a Mean value ^b Muscat of A	Alexandria	W,	iscat of Samos	^d ng calculated a	ccording	to IUPAC n	iles (25 μL)		

Table 2.

Producer	Maceration (M)	Maturation (Mt)	Ageing in wood
	(time in months)	(time in months)	(time in months)
А	3	10	18
В	0, 2, 3 and 4	5, 6, 7, 8 and 10, 18 and 20	
С	1 and 3	10 and 20	

604 Table 3.

Detector	Range (mgL ⁻¹)	Equation	r ²	LOD ^a (mgL ⁻¹)	LOQ ^b (mgL ⁻¹)
Uv-vis (306 nm)	0.075 - 5.0	y=744531x - 92367	0.9920	0.02	0.06
Fluorescence	0.075 - 5.0	y=329.77x - 36.149	0.9953	0.01	0.03
Electrochemical	1.35 - 8.0	y=3.9928x - 0.6105	0.9973	0.45	1.35

^a; LOD, limit of detection (S/N=3); ^bLOQ, limit of quantitation (S/N=10);

610 Table 4.

0	10			
			Vinification	
	Producer	Maceration	Maturation	Ageing in wood
	A	0.15 ± 0.014 (A ₃)	$0.13 \pm 0.02 \ (A_{10})$	$0.13 \pm 0.01 (A_{18})$
	В	$0.22 \pm 0.023 (B_3)$	$0.21 \pm 0.01(B_{10})$	
			$0.16 \pm 0.01 (B_{20})$	
	C	0.35 ± 0.014 (C ₃)	$0.38 \pm 0.03 \ (C_{10})$	
			0.28 ± 0.03 (C ₂₀)	
6	11	6		
6	12			
6	13			
6	14			

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