Microchemical Journal 153 (2020) 104383

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Polyphenols, biogenic amines and amino acids patterns in Verdelho wines according to vintage

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ARTICLE INFO

Keywords:
Verdelho
Vintage
Polyphenols
Amino acids
Biogenic amines

ABSTRACT

Verdelho wine grape variety is an emblematic cultivar of Madeira Island. In this study, Verdelho wines, from six successive vintages (2010–2015) were characterized in terms of individual polyphenols, biogenic amines and their precursor's amino acids by reversed-phase high-performance liquid chromatography (RP-HPLC). In addition, the total phenolic content, total tannins and antioxidant capacity were evaluated. Twenty-six polyphenols, four biogenic amines and four amino acids were identified. Verdelho wines from 2011, 2014 and 2015 vintages showed the highest concentration of polyphenols (145 mg/L), amino acids (52.0 mg/L) and biogenic amines (7.27 mg/L), respectively. Principal component analysis (PCA)) was used for classification and identification of compounds related to vintage effect. Epicatechin, gallic acid, phenylethylamine, histidine, caftaric acid, phenylalanine, histamine and kaempferol-3-O-rutinoside were the main compounds responsible for Verdelho wines classification according to vintage. Finally, it was observed that vintage did not promote the formation of biogenic amines and amino acids.I Independently of vintage the total concentration of biogenic amines in Verdelho wines are lower than the legal limits set by some European Union countries (< 8 mg/L).

1. Introduction

Wine is a broadly consumed beverage worldwide and unquestionably represents an significant food commodity of relatively high commercial value [1]. Since seventies, the Madeira wine industry has expanded its production to table wines, with Protected Designation of Origin "Madeirense" and wines with Protected Geographical Indication "Terras Madeirenses", besides the famous worldwide known Madeira wine. The Protected Designation of Origin "Madeirense" should fulfill several criteria, such as agro-climatic conditions, grape varieties, vinification techniques, yield/hectare, organoleptic properties, bottle type, labeling, among others. If one of these criteria are not fulfilled, the wines are categorized as Protected Geographical Indication "Terras Madeirenses". From the noble varieties, Verdelho grape variety typically makes a delicate and profoundly aromatic while yet being full-bodied wine characterized by smokey and citrus notes and high acidity. Epidemiological and clinical studies show that moderate wine consumption, up to one drink per day for women and up to two drinks per day for men, have potential biological effects, namely on cardiovascular system (reduction of overall cardiovascular risk), preservation of endothelial health, modulation of vascular smooth muscle cells, modulation of platelet aggregation, lower systolic blood pressure, diabetes and osteoporosis [2]. However, harmful effects can arise depending on the consumer profile and consumed amount. In this sense, several studies have been performed on the determination of antioxidant capacity, as well as on the establishment of polyphenols profile, which are mainly responsible for the antioxidant action [3–6]. These effects are attributable to the wide range of wine polyphenols. On the other hand, the polyphenols play an important role on the wine sensorial properties, being responsible for some of organoleptic characteristics, such as aroma, color, bitterness and astringency [7]. The content of polyphenols in wines depends on grape variety, *terroir*, vineyard location, vine cultivation practices, ripeness, winemaking process and phenolic reactivity during winemaking and ageing [4,8]. Wine polyphenols are usually categorized into two main groups: flavonoids and non-flavonoids. The polyphenols content in white wines is lower than red wines, being hydroxycinnamates the most dominant polyphenols class. The esters of tartaric acid (e.g., caftaric acid, coutaric acids) combined with some flavanols (e.g., (epi)catechin) are considered the major oxidation substrates and browning precursors in white wines, to form yellow-brown products due to the polymerization of *o*-quinones [4,9].

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https://doi.org/10.1016/j.microc.2019.104383
Received 23 September 2019; Received in revised form 28 October 2019; Accepted 28 October 2019
Available online 31 October 2019

Biogenic amines are of worldwide importance as they are naturally formed by the microbial decarboxylation of amino acids, and may be used as markers for quality and freshness of food products and alcoholic beverages [10], although, at high concentrations can result in allergic reactions, characterized by difficulty in breathing, rash, vomiting, and hypertension [5]. The pattern of biogenic amines in wines has been reported in several studies [5,10,11]. Up to 20 biogenic amines have been identified in wines, with concentration ranging from few to 50 mg/L, depending on several parameters, such as winemaking conditions, fermentation process and ageing, being the most dominants histamine, tyramine, phenylethylamine and putrescine [5,10,11].

Regarding to Madeira table wines the investigation is scarce and the few studies reported in literature are related to the establishment of volatile composition [1], antioxidant capacity and total phenolic content [12]. As far as we know, the establishment of polyphenols, biogenic amines and their amino acids precursors have not been well investigated in Verdelho table wines. Thus, the aim of the current study was to establish and compare the pattern of polyphenols, biogenic amines and their amino acids precursors for white wines obtained from Verdelho *Vitis vinifera* L. grape variety from six successive vintages (2010–2015), using reversed-phase high-performance liquid chromatography (RP-HPLC). Furthermore, the total phenolic content, total tannins and antioxidant capacity, were also determined. Multivariate statistical analysis (e.g., analysis of variance (ANOVA), principal components analysis (PCA)) was used for classification and identification of compounds related to vintage.

2. Materials and methods

2.1. Chemicals

Methanol (HPLC grade), acetonitrile (HPLC grade), formic acid (50%, LC–MS grade), sodium carbonate (Na_2CO_3, ≥ 99%), iron(III) sulfate hydrate ($Fe_2(SO_4)_3$•$5H_2O$, > 97%), *n*-butanol (BuOH, 99.9%), hydrochloric acid (HCl, 37%), α,α-diphenyl-β-picrylhydrazyl (DPPH, ≥95%), potassium dihydrogen phosphate (KH_2PO_4, ≥ 99%), sodium 1-heptanesulfonate monohydrate (≥ 99%), phosphoric acid (≥ 99%) and octylamine (99%) were purchased from Sigma–Aldrich (Milan, Italy). Water (HPLC grade) was obtained by ELGA PURELAB Ultra system (M-medical, Cornaredo, Milano, Italy). All the other polyphenol, amino acid and biogenic authentic standards were of analytical grade and purchased from Sigma–Aldrich (Milan, Italy).

2.2. Wine samples

Healthy mature-state of *Vitis vinifera* L. Verdelho grapes were collected, from the same vineyard, at maturity state based on maximum sugar content and minimum titratable acidity in Região Autónoma da Madeira Appellation (RAM), Portugal. The grapevines were 16 years old (in 2010), planted 2.5 m (row) × 1.8 m (vine); on own roots, spur pruned, with a single wire trellis and sprawling canopies. The vinification process was performed based on the technologies currently employed in Madeira wine production, from the whole grape until the clarified wine. First, the harvested grapes were crushed and destemmed with the addition of a solution containing sulfur dioxide (SO_2) generator, around 50 ppm. Then, the mixture was placed in an appropriate stainless steel chamber, where the fermentation take place (200 mg/Kg *Saccharomyces cerevisiae*) during seven days in a temperature-controlled room at 15 °C. Then, the mixture was pressed and the liquid part was collected (first pressing wine). The remaining solid phase is further pressed mechanically to yield the last liquid-phase collection (press wine). Wines were then racked off gross lees, and 60 ppm SO_2 added, and were acid-adjusted to pH around 3.3. Wines were cold-stabilized at 0 °C for a minimum of 21 days, and thereafter racked off fining lees. The final SO_2 content was adjusted to a total of 80 ppm (free around 40 ppm). Wines were filtered through a 0.8 μm membrane and bottled in 750 mL bottles under screw-cap.

A total of 18 monovarietal wines from Verdelho *Vitis vinifera* L. grape variety, Protected Designation of Origin "Madeirense", three wine samples per year, produced in six successive vintages (2010–2015) were considered in the current study. In order to obtain representative samples for each vintage, all samples analyzed were collected from three independent tanks produced with the same technological treatment and from the same winery. The ethanol content of the Verdelho wines under study ranged from 12 to 13% (v/v). The samples were kindly provided by Quinta do Barbusano, Madeira Island, Portugal, and analyzed one year after wine production.

2.3. Physical-chemical characterization of Verdelho wines

2.3.1. Total phenolic content

The estimation of total phenolic content (TPC) was performed by the Folin–Ciocalteu (F-C) assay according to Paixão et al. [12]. The results were expressed as milligrams of gallic acid equivalent per liter of sample (mg(GAE)/L). Spectrophotometric measurements were performed with using a Kontron UVIKON 930 Spectrophotometer (Kontron Instruments, Milan, Italy), using 1 cm quartz cells.

2.3.2. Total tannins

The *n*-butanol/HCl method was used to measure the content of condensed tannins in wine samples, as previously reported by Bate-Smith [13]. Wine samples were 50-fold diluted with distilled water; then, 1 mL of diluted wines were added to 3 mL of *n*-butanol reagent (0.31 mM $Fe_2(SO_4)_3$•$5H_2O$ in 50:50 (*v/v*) n-BuOH/HCl conc.). The samples were heated in a water bath (at 95 °C for 30 min in screw cap tube) and then cooled in ice water; the corresponding blank solutions were maintained in the dark at room temperature (25 ± 1 °C). Absorbance at 550 nm was recorded (Kontron UVIKON 930 spectrophotometer); the total tannin (TT) content was determined using 0.1736 as conversion factor.

2.3.3. Antioxidant capacity

The DPPH free radical scavenging assay was performed according to the method reported by Locatelli et al. [14]. Briefly, 700 μL of sample (wine samples diluted 1:500 with MeOH) or MeOH (control) was added to the same volume of methanolic solution of $DPPH^{\bullet}$ (100 μM). The antioxidant capacity of wines was expressed as inhibition percentage (I %) of the radical.

2.4. Polyphenols analysis

A Shimadzu LC-20A Prominence chromatographic system equipped with a diode array detector (DAD detector SPD-M20A) was used. Separation was performed on a reversed-phase Synergi™ 4 μm Max-RP 80 Å, LC Column (250 × 4.6 mm i.d., with particle size of 4 μm; Phenomenex, Torrance, CA, USA), protected by a pre-column packed with the same phase, at 30 °C. The mobile phase consisted of water/formic acid/acetonitrile (87:10:3, *v/v*) (eluent A) and water/formic acid/acetonitrile (40:10:50, v/v) (eluent B) using the following program gradient: from 6 to 20% B (20 min), from 20 to 40% B (15 min), from 40 to 60% B (5 min), from 60 to 90% B (5 min), isocratic 90 B (5 min), from 90 to 6% B (0.5 min), isocratic 6% B (22.5 min). Total run time was 73 min, at a constant flow rate of 500 μL/min. Chromatograms were recorded at 280, 330 and 520 nm. Five microliter (μL) were injected. The phenolic compounds in the Verdelho wines were tentatively identified by comparison with retention times of individual authentic standard compounds and their ultraviolet–visible spectroscopy (UV–Vis) spectra; the quantification was performed on the basis of calibration curves obtained with the corresponding standards, namely gallic acid (≥ 99%), protocatechuic acid (≥97%), caftaric acid (≥97%), coutaric acid (≥97%), caffeic acid (≥98%), p-coumaric acid (≥98%), ferulic acid (99%), sinapic acid (≥ 98%), procyanidin B1

(≥90%), catechin (≥97%), procyanidin B2 (≥90%), epigallocatechin gallate (≥ 98%), epicatechin (≥ 98%), gallocatechin gallate (≥98%), epicatechin gallate (≥ 98%), catechin gallate (≥ 98%), quercetin-3-O-galactoside (≥97%), quercetin-3-O-glucoside (≥ 90%), kaempferol-3-O-rutinoside (≥ 98%), myricetin (≥ 96%), quercetin (≥ 95%),. For each standard, calibration curves at six different concentration levels were obtained, limit of detection (LOD) and limit of quantification (LOQ) established. Details concerning the method validation can be consulted in Bordiga et al. [15].

2.5. Determination of biogenic amines and amino acids

Analyses were carried out on a Shimadzu Class VP HPLC system, equipped with a temperature controller (Column Oven CTO-10AS) and a UV–Vis detector SPD-10A. Tyramine, histamine, 2-phenylethylamine, tryptamine and their precursors amino acids were determined without derivatization using an already-optimized ion-pair HPLC method [16], with a C_{18} reverse phase Spherisorb S5 ODS2 column (250 mm × 4.6 mm I.D., particle size 5 μm), with a pre-column (10 mm × 4.6 mm I.D.) packed with the same phase. Eluent A prepared by dissolving heptane sulphonate (8.3 mM) and KH_2PO_4 (9.0 mM) in ultra-pure water and adjusting the pH to 3.5 with phosphoric acid. Then, 20 μL/L of octylamine were added as second ion-pair reagent. Eluent B: Methanol. Gradient: 100% pump A for 1 min; pump B from 0 to 26% in 5.25 min; pump B from 26 to 35% in 9 min; pump B from 35 to 42% in 1.5 min; pump B at 42% for 24 min; pump A at 100% for 9.40 min. Flow rate: 1.0 mL/min, UV-detection: 215 nm. The column was kept at 27 °C during analyses. Medium samples were directly injected after 0.22 μm filtration (Millipore, HA type), volume injected five μL. The biogenic amines and amino acids in the Verdelho wines were tentatively identified by comparison with retention times of individual authentic standard compounds and their ultraviolet–visible spectroscopy (UV–Vis) spectra at 215 nm; the quantification was performed on the basis of calibration curves obtained with the corresponding standards, namely tyrosine (≥99%), histidine (98%), phenylalanine (≥98%), tryptophan (≥98%), tyramine (≥98%), histamine (≥97%), phenylethylamine (≥99%) and tryptamine (≥98%). For each standard, calibration curves at six different concentration levels were obtained, limit of detection (LOD) and limit of quantification (LOQ) established. Details concerning the method validation can be consulted in Bordiga et al. [16].

2.6. Statistical analysis

The obtained data was analyzed with Metaboanalyst 4.0 [17], which included a data pre-processing to remove compounds with missing values (MV) and normalization (data transformation by cubic root and data scaling by mean-center). The normalized data was further subjected to one-way ANOVA followed by Tukey's test for post-hoc multiple comparisons of means and multivariate statistical analysis namely, principal component analysis (PCA) to provide insights into the discrimination of Verdelho wines by vintage and to detect compounds that may indicate differences among the samples sets. Finally, Pearson's correlation was used to build the heat map of the Verdelho wines by age using the compounds identified with the aim of identify clustering patterns.

3. Results and discussion

As stated above, 18 monovarietal Verdelho wines from different vintages were selected for the current study. The pH, total phenolic content (TPC), antioxidant capacity (AC), total tannins (TT), polyphenols, biogenic amines and their precursor's amino acids were determined one year after wine production.

Table 1
Total polyphenols content (TPC), antioxidant activity (Ac) and total tannins (TT) determined in Verdelho wines from different vintages.

Verdelho wines	pH	TPC (mg (GAE)/L)	AC (% inhibition)	TT (mg/L)
2010	3.29 ± 1E-2[a]	221 ± 3	33.0 ± 8E-1	73.4 ± 2[e]
2011	3.25 ± 1E-2	178 ± 6	26.5 ± 2	166 ± 3E-1
2012	3.20 ± 6E-3	191 ± 3	22.3 ± 4E-1	108 ± 5E-1
2013	3.29 ± 4E-3[a]	242 ± 1[b]	43.0 ± 3E-1[c]	50.9 ± 2
2014	3.41 ± 5E-3	263 ± 8	41.0 ± 7E-1[c,d]	75.6 ± 8E-1[e]
2015	3.37 ± 2E-3	241 ± 2[b]	38.6 ± 2[d]	57.2 ± 7E-1

Means ± standard deviation. Column values with different lowercase letters in superscript are not significantly different at $p < 0.05$ by LSD in ANOVA.

3.1. Physical-chemical characterization of Verdelho wines

The average values of pH, TPC, AC and TT for Verdelho wines from six successive vintages (2010–2015) are listed in Table 1. The pH value is an important parameter, since the production of biogenic amines is suggested as a microbial approach to survive to acidic environments or to supply alternative metabolic energy [18]. In Verdelho wines the pH ranged from 3.20 to 3.41, which is in accordance with other white wines (3.1–3.4) [19].

Among all the vintages analyzed, Verdelho of the vintage 2014 showed the highest TPC (263 mg/L), followed by the 2013, 2015, 2010, 2012 and 2011 vintages. No significant differences of TPC were observed between the vintages of 2013 and 2015. The differences observed on TPC levels can be supported by the effect of terroir, since the Verdelho grapes came from the same vineyard to produce Verdelho wines using the same technological process [20]. The TPC found in Verdelho wines are in agreement with data from the literature for white wines [12,21–23]. Moreover, the differences in the TPC level observed between different vintages may also explain the differences observed in AC of Verdelho wines.

All Verdelho wines analyzed showed a decreasing trend in AC in the followed order 2013, 2014, 2015, 2010, 2011 and 2012 vintages. On average, the Verdelho wines from 2010 to 2012 (27.3% inhibition) showed an AC two times lower when compared to Verdelho wines from 2013 to 2015 vintages (40.9% inhibition). No statistical significant differences were observed among Verdelho wines from 2013 to 2015 vintages (Table 1).

On average, the TT levels of Verdelho wines from 2010 to 2012 vintages (115.8 mg/L) are about two times higher when compared to vintages from 2013 to 2015 (61.2 mg/L). Nevertheless, no statistical significant differences were observed in TT levels of Verdelho wines of the 2010 and 2014 vintages.

3.2. Polyphenols profile of Verdelho wines

The results related to the analyses of polyphenols in Verdelho wines are reported in Table 2. On average, the total phenolic acids content was two times lower in vintages from 2010 to 2012 (42.2 mg/L) compared to the vintages from 2013 to 2015 (87.9 mg/L), with the highest concentration (98.5 mg/L) determined in 2013 vintage. Caftaric and coutaric acid were the most abundant hydroxycinnamic acids in all vintages, while ferulic acid and sinapic acid were found in trace amounts, 0.42 and 0.09 mg/L, respectively. These results are in agreement with reported in previous works [24,25], and support the suggestion that caftaric and coutaric acids could be 'marker compounds' for the differentiation of young wines.

The 2011 vintage exhibited the highest flavan-3-ols concentration (93.7 mg/L) followed by the 2010, 2012, 2014, 2015 and 2013 vintages, 63.6, 54.1, 40.1, 39.8 and 37.8 mg/L, respectively. Insignificant changes were observed between Verdelho wines from 2015 and 2014 vintages, since the difference in total concentration was only 0.37 mg/

Table 2
Concentration (mg/L) of polyphenols identified in Verdelho wines from different vintages using RP-HPLC.

Polyphenol groups	λ (nm)	CODE	Concentration (mg/L) ± standard deviation					
			2010	2011	2012	2013	2014	2015
Phenolic acids								
Gallic acid	280	Gal	0.81 ± 2E-2	0.14 ± 3E-3	0.40 ± 7E-3	27.8 ± 5E-2	14.2 ± 5E-2	11.9 ± 4E-2
Protocatechuic acid	280	Prot	6.02 ± 1E-2	9.33 ± 1E-2	7.00 ± 9E-3	5.31 ± 2E-2	7.10 ± 3E-2	3.88 ± 2E-2
Caftaric acid	280	Caft	25.4 ± 3E-2	23.9 ± 4E-2	13.8 ± 3E-2	45.5 ± 4E-2	35.4 ± 5E-2	54.8 ± 2E-2
Coutaric acid	280	Cout	12.6 ± 2E-2	7.03 ± 3E-2	4.88 ± 2E-2	16.5 ± 3E-2	7.30 ± 4E-2	13.3 ± 5E-3
Caffeic acid	330	Caf	2.58 ± 1E-2	2.92 ± 9E-3	2.77 ± 1E-2	2.21 ± 6E-3	6.70 ± 3E-2	4.00 ± 2E-2
p-Coumaric acid	330	Coum	2.01 ± 1E-2	0.46 ± 3E-2	2.82 ± 5E-2	0.66 ± 2E-2	4.61 ± 3E-2	1.23 ± 2E-3
Ferulic acid	330	Fer	0.53 ± 1E-2^{a}	0.52 ± 1E-2^{a}	0.31 ± 5E-3	0.48 ± 4E-3	0.41 ± 1E-2	0.27 ± 1E-3
Sinapic acid	330	Sin	0.18 ± 1E-3	0.04 ± 2E-3^{b}	0.03 ± 1E-3^{b}	0.08 ± 1E-3	0.04 ± 1E-3^{b}	0.15 ± 2E-3
Flavan-3-ols								
Procyanidin B1	280	ProB1	1.25 ± 5E-3^{c}	1.23 ± 2E-3^{c}	1.63 ± 4E-2	1.32 ± 1E-2^{d}	1.31 ± 1E-2^{d}	2.04 ± 1E-2
Catechin	280	Cat	7.04 ± 6E-2	3.92 ± 7E-2	6.31 ± 6E-2	8.95 ± 9E-2	12.1 ± 9E-2	8.69 ± 9E-2
Procyanidin B2	280	ProB2	3.39 ± 7E-2	4.01 ± 5E-2	4.93 ± 7E-2	3.70 ± 6E-2	5.14 ± 1E-1	3.02 ± 4E-2
Epigallocatechin gallate	280	EGCgal	2.78 ± 8E-2	1.25 ± 1E-1^{e}	1.22 ± 8E-3^{e}	1.59 ± 1E-2	1.17 ± 1E-2	2.04 ± 3E-2
Epicatechin	280	Epi	30.9 ± 1E-1	61.6 ± 1E-1	20.4 ± 3E-1	4.09 ± 2E-1	6.40 ± 3E-1	3.06 ± 4E-2
Gallocatechin gallate	280	GCgal	11.4 ± 4E-2	12.7 ± 6E-2	12.2 ± 2E-1	9.59 ± 2E-1	4.11 ± 1E-1	10.2 ± 1E-1
Epicatechin gallate	280	Epigal	3.70 ± 1E-1^{f}	2.91 ± 2E-2	3.53 ± 2E-1^{f}	5.07 ± 1E-1	6.11 ± 7E-2	7.30 ± 7E-2
Catechin gallate	280	Catgal	1.30 ± 1E-2	1.37 ± 3E-2	2.08 ± 4E-3	1.94 ± 9E-3	1.85 ± 3E-2	2.24 ± 1E-2
Procyanidin A2	280	ProA2	1.84 ± 4E-2 g	4.66 ± 1E-1	1.79 ± 1E-2 g	1.54 ± 2E-1	1.94 ± 2E-2	1.17 ± 1E-2
Flavonols								
Quercetin-3-O-galactoside	330	QueGal	0.53 ± 8E-3	0.64 ± 1E-2	0.80 ± 1E-2	0.77 ± 1E-2	0.46 ± 9E-3	1.27 ± 9E-3
Quercetin-3-O-glucoside	330	QueGlu	0.87 ± 9E-3	0.62 ± 8E-3^{h}	0.53 ± 1E-2	0.58 ± 4E-3	0.63 ± 8E-3^{h}	0.40 ± 1E-2
Kaempferol-3-O-rutinoside	330	KaeRut	3.68 ± 2E-2	4.13 ± 5E-2	1.99 ± 3E-2	4.42 ± 3E-2	2.14 ± 5E-2	0.36 ± 4E-3
Myricetin	330	Myr	1.58 ± 2E-2	1.67 ± 1E-2	1.48 ± 1E-2	1.53 ± 1E-2	1.45 ± 1E-2^{i}	1.45 ± 6E-3^{i}
Quercetin	330	Que	–	–	1.64 ± 9E-3	–	–	1.56 ± 5E-3
Stilbenes								
Trans-piceid	305	t-pic	–	–	0.001 ± 1E-4	0.145 ± 5E-3	0.101 ± 1E-3	0.006 ± 2E-3
Cis-piceid	285	c-pic	–	–	–	0.030 ± 1E-4	0.095 ± 2E-3	0.059 ± 3E-3
Total			120.39	145.05	92.54	143.81	120.77	134.40

Room values with same lowercase letters in superscript are not significantly different at $p < 0.05$ by LSD in ANOVA -: not detected.

L. This result is mainly explained by the increase of the concentration of epicatechin and gallocatechin gallate that is highest in vintages from 2010 to 2012. On average, epicatechin and gallocatechin gallate was 8.32 and 1.52 fold higher in vintages from 2010 to 2012 (37.6 and 12.1 mg/L) when compared to vintages from 2013 to 2015 (4.52 and 7.97 mg/L), respectively. The highest concentration of epicatechin and gallocatechin gallate was detected in 2011 vintage, 61.6 and 12.7 mg/L, respectively. According to the literature, white wines contain, on average, 10 mg/L of catechin and epicatechin [23,26]. The concentration of epicatechin in Verdelho wines ranged from 3.06 to 61.6 mg/L, whereas catechin ranged from 3.92 to 12.1 mg/L. From healthy point of view, flavan-3-ols act as antioxidant, free radical scavengers and anti-carcinogenic; they have cardio-preventive, anti-microbial and anti-viral properties and may also play an important role in maintaining neurological health [27,28]. The total flavonols concentration ranged from 4.68 (2014) to 7.30 (2013) mg/L. From the flavonols, the quercetin was only detected in Verdelho wines of 2012 and 2015 vintages, whereas kaempferol-3-O-rutinoside and myricetin were the most abundant in all vintages. Regarding to myricetin, no statistical differences were observed between 2012 and 2014 vintages, as well as between 2014 and 2015 vintages.

Stilbenes are an essential group of polyphenols in wine, specially from the point of view of their health benefits, since it has been shown to have cancer chemopreventive activity and to protect lipoproteins from oxidative damage [29]. As can be seen in Table 2, only *trans*-piceid and *cis*-piceid were detected in Verdelho wines from 2013 to 2015 vintages. *Trans*-piceid level in Verdelho wines ranged from 0.001 (2012) to 0.15 (2013) mg/L, with a mean value of 0.06 mg/L, whereas *cis*-piceid level in Verdelho wines ranged from 0.03 (2013) to 0.10 (2015) mg/L, with a mean value of 0.06 mg/L. In addition, in Verdelho wines independently of vintage no *cis*- or *trans*-resveratrol was detected. The stilbenes content depends on several parameters, namely grape variety, climate conditions, among others [30]. The results obtained are in agreement with the literature in which the levels of *cis*-piceid were lower than their *trans*-forms in most wines analyzed, except in Verdelho wine 2015 [29,30]. The ratio of *trans*-/*cis*-piceid was 4.83 and 1.06, for Verdelho 2013 and 2014, respectively.

3.3. Biogenic amines and its amino acids precursors in Verdelho wines

Nowadays, the Office International de la Vigne et du Vin (OIV) did not establish the maximum content of biogenic amines in wine. However, in several countries establish For histamine, a different maximum limit in wines were established in several countries, namely 2 mg/L in Germany, 5–6 mg/L in Belgium, 10 mg/L in Switzerland and Austria, 8 mg/L in France and 3 mg/L in Holland [31,32]. Nevertheless, previous studies recommended that the concentration of biogenic amines in wines should be lower than 8 mg/L [31–34]. The concentrations (mg/L) of biogenic amines and their respective amino acids in Verdelho wines from different vintages is reported in Table 3. In the current study, total biogenic amine concentration was considered as the sum of tyramine, histamine, phenylethylamine and tryptamine, as well as the total of amino acids concentration is the sum of tyrosine, histidine, phenylalanine and tryptophan, precursors of the corresponding biogenic amines.

Overall, on average, phenylethylamine present the highest level (1.56 mg/L), followed by histamine (1.02 mg/L), tyramine (0.57 mg/L) and tryptamine (0.11 mg/L). Phenylethylamine, histamine and tyramine, reported in the literature as biogenic amines suspected to have toxicological effects, are not a concern in Verdelho wines, since their concentrations (Table 3) did not exceed 8 mg/L [31]. Regarding to vintage, the total concentration of biogenic amines in 2015 (7.27 mg/L) was nine times higher than in 2011 (0.84 mg/L), five times than in 2012 (1.47 mg/L), three times than in 2010 (2.36 mg/L), two times than in 2013 (3.29 mg/L) and 2014 (2.69 mg/L). These significant differences among vintages of Verdelho wines was explained by the higher concentration of histamine (3.00 mg/L) and phenylethylamine (3.82 mg/L) determined in 2015. According to the reported in the

Table 3
Concentration (mg/L) of biogenic amines and its amino acids precursors in Verdelho wines from different vintages.

Vintages	Amino acids (mg/L)				Biogenic amines (mg/L)			
	Tyrosine TYR	Histidine HIST	Phenylalanine PHEN	Tryptophan TRYP	Tyramine TYRA	Histamine HISTA	Phenylethylamine PHENA	Tryptamine TRYPA
2010	2.63 ± 3E-2	1.39 ± 3E-2[b]	18.8 ± 2E-2	1.09 ± 6E-3	1.24 ± 3E-2	0.87 ± 5E-3	0.12 ± 5E-3	0.13 ± 2E-3
2011	2.43 ± 3E-2[a]	1.33 ± 2E-2[b]	6.41 ± 3E-2	0.84 ± 5E-3	0.53 ± 2E-2	0.22 ± 1E-2[c]	–	0.09 ± 1E-3
2012	5.72 ± 5E-2	1.65 ± 3E-2	17.9 ± 5E-2	1.41 ± 4E-3	1.18 ± 2E-2	0.20 ± 1E-2[c]	0.01 ± 6E-3	0.08 ± 1E-3[d]
2013	5.89 ± 3E-2	2.77 ± 1E-2	25.2 ± 4E-2	1.34 ± 5E-3	–	0.45 ± 2E-2	2.73 ± 1E-1	0.11 ± 1E-3
2014	9.45 ± 7E-2	6.48 ± 6E-2	33.2 ± 9E-2	2.84 ± 4E-3	0.08 ± 3E-3	1.35 ± 7E-2	1.12 ± 1E-2	0.14 ± 2E-3
2015	2.31 ± 6E-2[a]	15.0 ± 1E-1	27.6 ± 5E-2	0.96 ± 2E-3	0.37 ± 7E-3	3.00 ± 4E-2	3.82 ± 1E-1	0.08 ± 1E-3[d]

Column values with same lowercase letters in superscript are not significantly different at $p < 0.05$ by LSD in ANOV: not detected.

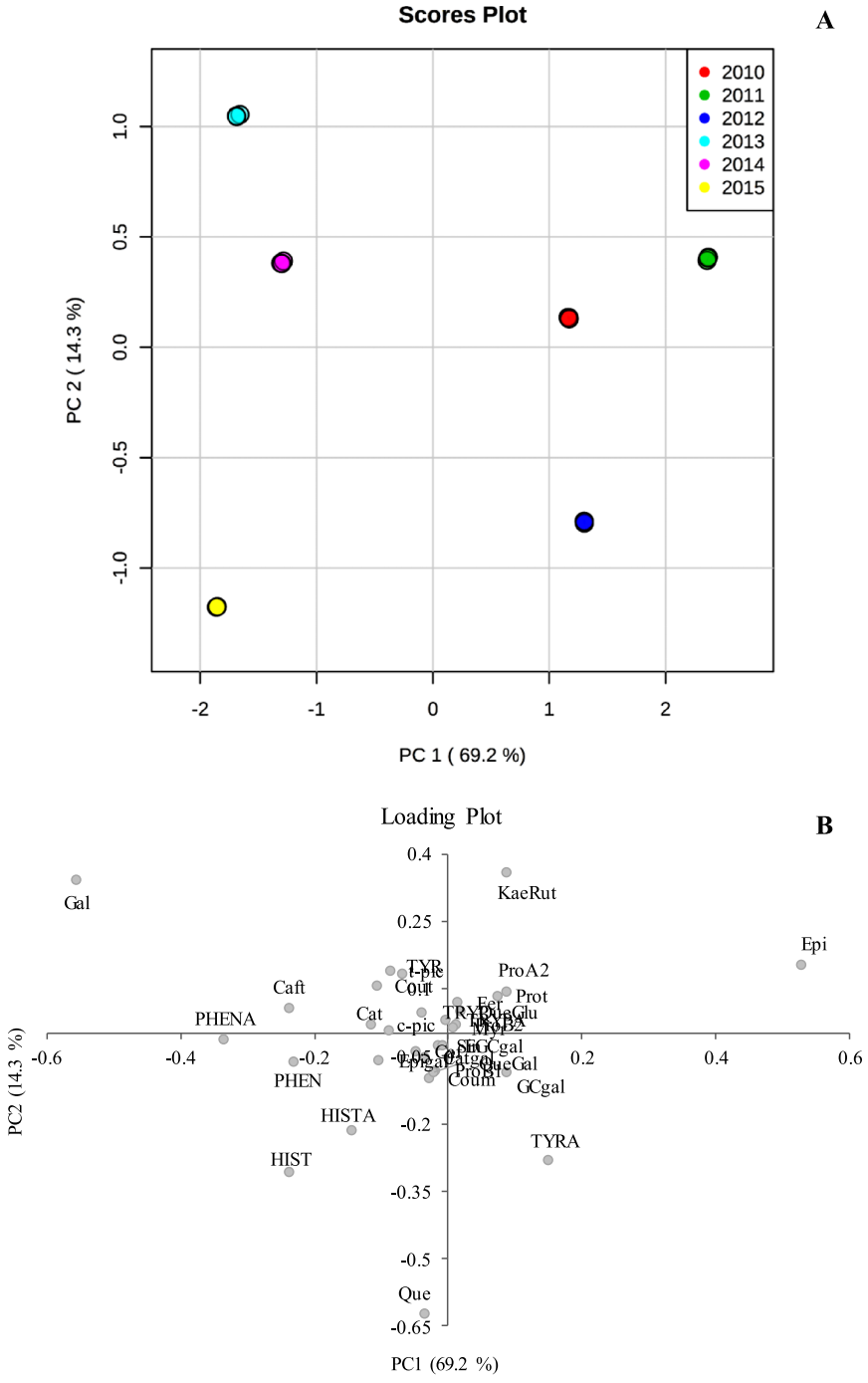

Fig. 1. PCA of the polyphenols, biogenic amines and amino acids of Verdelho wines according to vintage (n = 3 for each data point). A) PC1 × PC2 score scatter plot and B) loading weight plot (attribution of the abbreviations is shown in Tables 2 and 3).

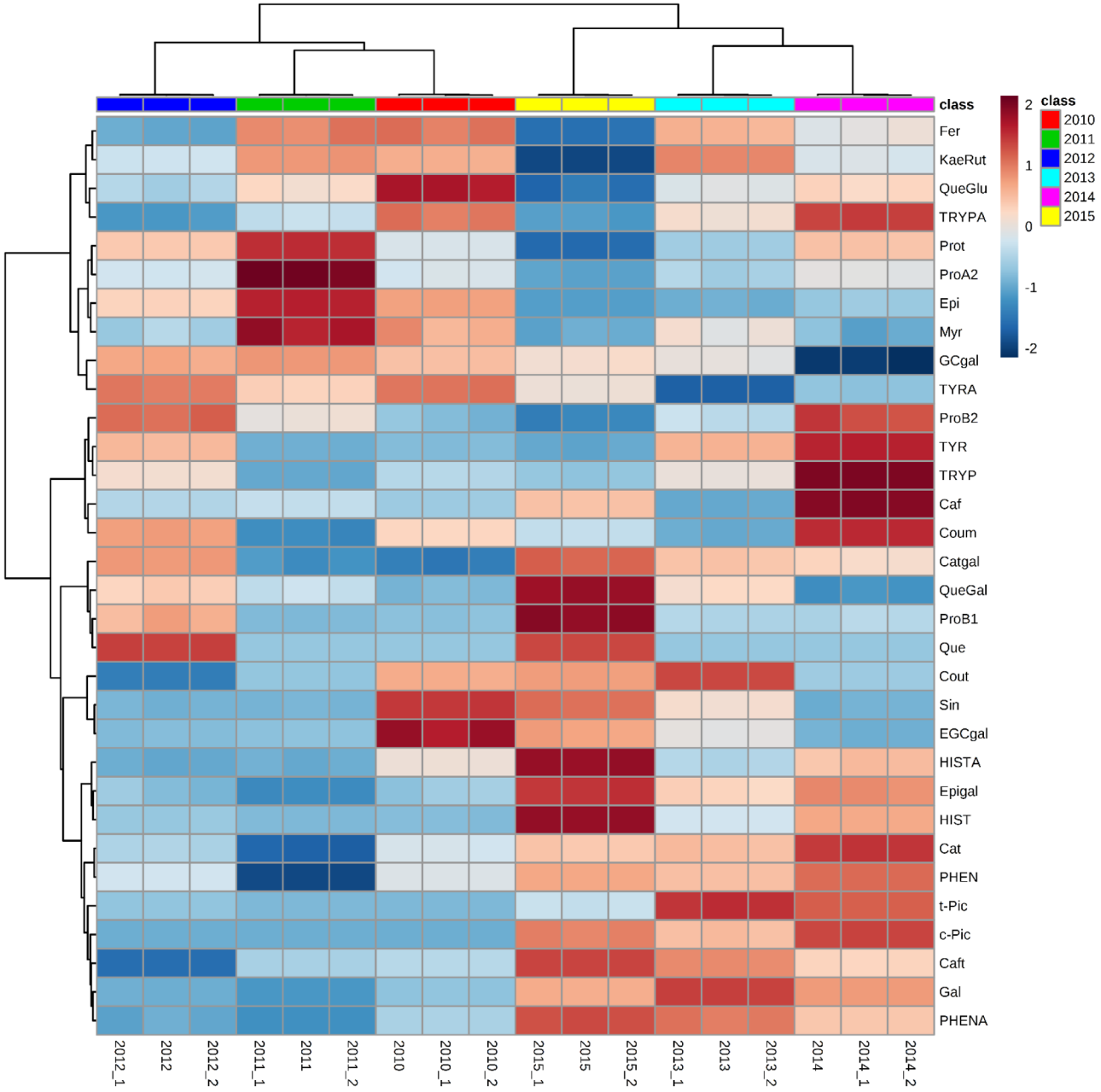

Fig. 2. Hierarchical cluster analysis (HCA). The heat maps of the polyphenols, biogenic amines and their precursor amino acids identified in all Verdelho samples were generated by average algorithm and Pearson distance analysis (attribution of the abbreviations is shown in Tables 2 and 3).

literature, higher concentrations of histamine was expected in less acid wines (higher pH). This trend can be related with the growth of microorganisms with decarboxylase activity, which is improved at higher pH, and thus, histidine decarboxylation is enhanced [34]. The pH of Verdelho wines is higher in 2014 and 2015, being the histidine concentration higher in these two vintages, whereas the Verdelho wines with lowest pH also has the lowest histidine concentration, (Verdelho wines from 2012), indicating the potential influence of decarboxylase at low pH medium.

3.4. Multivariate statistical data analysis

The concentration of the 34 analytical variables (26 polyphenols, four biogenic amines, four amino acids) of the 18 Verdelho wines from six successive vintages (2010–2015) were submitted to a PCA analysis. The aim of this analysis was to visualize the similarity/difference among Verdelho wines and establish a possible relationship amongst polyphenols, biogenic amines, amino acids and vintage. Fig. 1 shows a PCA biplot of the two first principal components (PC1 vs PC2), which explains 83.5% of the total variability of the data set, allowing us to organize the Verdelho wine by vintage (as a function of PC1 axis). According to vintage, the Verdelho wines from 2013 to 2015 projected in PC1 negative, are mainly characterized by gallic acid, caftaric acid, phenylethylamine, phenylalanine, histamine and histamine, while Verdelho wines from 2010 to 2012 placed in PC1 positive by epicatechin, gallocatechin gallate, tyramine and kaempferol-3-O-rutinoside.

Moreover, Fig. 2 shows the resulting dendrogram associated with heat map constructed using Pearson's correlation, providing intuitive visualization of the dataset, and it is often applied to identify samples or features that are unusually high or low. An analogous color tone to the heat-map indicates the area, a group of samples, taking into account the concentration of the analyzed compounds is similar. However, apart

from vintages, other factors such as grape variety, vineyard location, vine cultivation practices, ripeness, and winemaking process [4,8] could also influence the polyphenols content.

4. Conclusions

In the current study Verdelho wines from six successive vintages, 2010 to 2015, were characterized according to their content of total phenolic content, antioxidant capacity, total tannins, in addition to their individual polyphenols, biogenic amines and amino acids. The dataset obtained was submitted to multivariate statistical analysis (e.g., PCA) to distinguish the Verdelho wines by vintages. The results indicated that polyphenols, biogenic amines and amino acids might provide a suitable tool for classification of Verdelho wines by vintages.On average, the Verdelho wines from 2013 to 2015 showed higher content of phenolic acids, amino acids and biogenic amines when compared to Verdelho wines from 2010 to 2012. Regarding flavan-3-ols the higher concentration was obtained for Verdelho wines from 2010 to 2012. The results demonstrated that Verdelho wines might exert a positive effect on human health, taking into consideration the estimation values for antioxidant capacity and polyphenols concentration, in addition to low levels of biogenic amines, lower than the legal limits set by some European Union countries.

Declaration of Competing Interest

The authors declare there are no conflict of interest

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (Project PEst-OE/QUI/UI0674/2019, CQM, Portuguese Government funds), and through Madeira 14–20 Program, project PROEQUIPRAM - Reforço do Investimento em Equipamentos e Infraestruturas Científicas na RAM (M1420-01–0145-FEDER-000008) and by ARDITI - Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação, through the project M1420-01–0145-FEDER-000005 - Centro de Química da Madeira - CQM^{+} (Madeira 14–20 Program).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2019.104383.

References

[1] R. Perestrelo, C. Silva, J.S. Câmara, A useful approach for the differentiation of wines according to geographical origin based on global volatile patterns, J. Sep. Sci. 37 (2014) 1974–1981.
[2] A.A. Artero, A.A. Artero, J.J. Tarín, A. Cano, The impact of moderate wine consumption on health, Maturitas 80 (2015) 3–13, https://doi.org/10.1016/J.MATURITAS.2014.09.007.
[3] N. Paixão, V. Pereira, J.C. Marques, J.S. Câmara, Quantification of polyphenols with potential antioxidant properties in wines using reverse phase HPLC, J. Sep. Sci. 31 (2008) 2189–2198.
[4] V. Pereira, F. Albuquerque, J. Cacho, J. Marques, Polyphenols, antioxidant potential and color of fortified wines during accelerated ageing: the Madeira wine case study, Molecules 18 (2013) 2997–3017.
[5] A. Tassoni, N. Tango, M. Ferri, Comparison of biogenic amine and polyphenol profiles of grape berries and wines obtained following conventional, organic and biodynamic agricultural and oenological practices, Food Chem. 139 (2013) 405–413.
[6] C.I.G. Tuberoso, G. Serreli, F. Congiu, P. Montoro, M.A Fenu, Characterization, phenolic profile, nitrogen compounds and antioxidant activity of Carignano wines, J. Food Compos. Anal. 58 (2017) 60–68.
[7] R.-R. Xing, D. Liu, Z. Li, Y. Tian, X.-X. Zhang, J.-M. Li, Q.-H. Pan, Impact of different types of stoppers on sensorial and phenolic characteristics evolution during a bottle storage time of a white wine from Chardonnay grape variety, J Food Sci Technol 53 (2016) 4043–4055.
[8] I. Budić-Leto, G. Zdunić, J. Gajdoš-Kljusurić, A. Mucalo, U. Vrhovšek, Differentiation between Croatian dessert wine Prošek and dry wines based on phenolic composition, J. Food Compos. Anal. 62 (2017) 211–216.
[9] S. Kallithraka, M.I. Salacha, I. Tzourou, Changes in phenolic composition and antioxidant activity of white wine during bottle storage: accelerated browning test versus bottle storage, Food Chem. 113 (2009) 500–505.
[10] M. Papageorgiou, D. Lambropoulou, C. Morrison, J. Namieśnik, J. Płotka-Wasylka, Direct solid phase microextraction combined with gas chromatography–Mass spectrometry for the determination of biogenic amines in wine, Talanta 183 (2018) 276–282.
[11] A.C. Manetta, L. Di Giuseppe, R. Tofalo, M. Martuscelli, M. Schirone, M. Giammarco, G. Suzzi, Evaluation of biogenic amines in wine: determination by an improved HPLC-PDA method, Food Control. 62 (2016) 351–356.
[12] N. Paixão, R. Perestrelo, J.C. Marques, J.S. Câmara, Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines, Food Chem 105 (2007) 204–214.
[13] E.C. Bate-Smith, Haemanalysis of tannins: the concept of relative astringency, Phytochemistry 12 (1973) 907–912, https://doi.org/10.1016/00319422(73)80701-0.
[14] M. Locatelli, R. Gindro, F. Travaglia, J.D. Coïsson, M. Rinaldi, M. Arlorio, Study of the DPPH-scavenging activity: development of a free software for the correct interpretation of data, Food Chem. 114 (2009) 889–897.
[15] M. Bordiga, F. Travaglia, M. Locatelli, M. Arlorio, J.D. Coïsson, Spent grape pomace as a still potential by-product, Int. J. Food Sci. Technol. 50 (2015) 2022–2031.
[16] M. Bordiga, R. Guzzon, R. Larcher, F. Travaglia, M. Arlorio, J.D. Coïsson, Variation in content of tyramine, histamine, 2-phenylethylamine, tryptamine and their precursor amino acids in a Chardonnay wine by using different commercial active dry yeasts and nitrogen sources, Int. J. Food Sci. Technol. (2019), https://doi.org/10.1111/ijfs.14307.
[17] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, M. 4, towards more transparent and integrative metabolomics analysis, Nucleic. Acids. Res. 46 (2018) W486–W494.
[18] M. Martuscelli, G. Arfelli, A.C. Manetta, G. Suzzi, Biogenic amines content as a measure of the quality of wines of Abruzzo (Italy), Food Chem. 140 (2013) 590–597.
[19] R.S. Jackson, Wine science , principles, practice, Perception, Academic Press, San Diego, 2000.
[20] I. Balga, A. Leskó, M. Ladányi, M. Kállay, Influence of ageing on changes in polyphenolic compounds in red wines, 2014.
[21] S.-V. Oscar, O.-C.L. Fernando, C.-M.M. del Pilar, Total polyphenols content in white wines on a microfluidic flow injection analyzer with embedded optical fibers, Food Chem. 221 (2017) 1062–1068.
[22] P. Simonetti, A. Piergiorgio Pietta, G. Testolin, Polyphenol content and total antioxidant potential of selected italian wines, J. Agric. Food Chem. 45 (1997) 1152–1155.
[23] R.C. Minussi, M. Rossi, L. Bologna, L. Cordi, D. Rotilio, G.M. Pastore, N. Durán, Phenolic compounds and total antioxidant potential of commercial wines, Food Chem. 82 (2003) 409–416.
[24] A. Bimpilas, D. Tsimogiannis, K. Balta-Brouma, T. Lymperopoulou, V. Oreopoulou, Evolution of phenolic compounds and metal content of wine during alcoholic fermentation and storage, Food Chem. 178 (2015) 164–171.
[25] E. Puértolas, I. Álvarez, J. Raso, Changes in phenolic compounds of aragón red wines during alcoholic fermentation, Food Sci. Technol. Int. 17 (2011) 77–86.
[26] S. Carando, P.-L. Teissedre, A. laurence pascual-martinez, J.-C. cabanis, levels of flavan-3-ols in french wines, J. Agric. Food Chem. 47 (1999) 4161–4166.
[27] P.M. Aron, J.A. Kennedy, Flavan-3-ols: nature, occurrence and biological activity, Mol. Nutr. Food Res. 52 (2008) 79–104, https://doi.org/10.1002/mnfr.200700137.
[28] S.C. Forester, A.L. Waterhouse, Metabolites are key to understanding health effects of wine polyphenolics, J. Nutr. 139 (2009) 1824S–1831S.
[29] M.T. Ribeiro de Lima, P. Waffo-Téguo, P.L. Teissedre, A. Pujolas, J. Vercauteren, J.C. Cabanis, J.M. Mérillon, Determination of stilbenes (trans-astringin, cis- and trans-piceid, and cis- and trans-resveratrol) in Portuguese wines, J. Agric. Food Chem. 47 (1999) 2666–2670.
[30] A. Peçuli, F. Lorenzini, M. Angjellari, S. Schnee, K. Gindro, Á. Dienes-Nagy, Polyphenolic profile and stilbene content of Albanian “Kallmet” monovarietal wine, OENO One 52 (2018) 135–144.
[31] M.C. Leitão, A.P. Marques, M.V. San Romão, A survey of biogenic amines in commercial portuguese wines, Food Control 16 (2005) 199–204.
[32] O. Busto, J. Guasch, F. Borrull, Biogenic amines in wine : a review of analytical methods, J. Int. Des. Sci. de La Vigne et Du Vin 30 (1996) 85.
[33] V. Pereira, A.C. Pereira, J.P. Pérez Trujillo, J. Cacho, J.C. Marques, Amino acids and biogenic amines evolution during the estufagem of fortified wines, J. Chem. (2015) 1–9 2015.
[34] C. Proestos, P. Loukatos, M. Komaitis, Determination of biogenic amines in wines by HPLC with precolumn dansylation and fluorimetric detection, Food Chem. 106 (2008) 1218–1224.