Analysis of phenolic compounds in wines of Fogo Island – Cape Verde

Pereira, D., Esteves da Silva, J.C

Abstract

The phenolic compounds in wines are transferred from the grapes and others are formed during the fermentation process. In wines they serve as one of the quality parameters and one of the ways to verify the authenticity of a particular wine production. The analysis of these compounds in the wines from the Island of Fogo was done by HPLC-MS, employing the SPE and LLE extraction methods. Several compounds from anthocyanic and non-anthocyanic were detected with different concentrations in the analyzed wines.

Keywords: Wines, phenolic compounds, anthocianic and non-anthocyanic compounds, High Perfomance Liquid Chromatography -Mass Spectrometry

1. Introduction

Phenolic compounds are secondary plant metabolites which are found in the leaves, seeds, grapes and they are extracted from the wine during the vinification process. The type and concentration of these compounds depend on such factors as the type of grape and its ripening stage, climatic conditions, soil type and winemaking [1]. They are the major components of the wine with a percentage from 30% to 40% among macromolecular compounds present in wine [2]. They come from grapes and other results of chemical and biochemical processes in the production process, especially during fermentation and aging. During production, the must in contact with oxygen causes the oxidation of phenolic compounds causing wine browning. When the maturation is finished, the phenolic oxidation decreases and the concentration of phenolic compounds stabilizes [3]

These compounds have an important role in assessing the quality of the wine since they contribute in defining certain sensory characteristics such as color, flavor, hardness and astringency directly or by combination with other compounds [4].

The main phenolic compounds in wine and grapes are divided into two groups, the non-flavonoid and flavonoid. Flavonoids are composed of compounds of anthocyanins, flavonols and flavano-3-ols. In non-flavonoids phenolic compounds in wines are mainly hydroxycinnamic acids, hydroxybenzoic acids and volatile phenols such as stilbene (resveratrol) [4].

1.1. Anthocyanins

Anthocyanins are water-soluble pigments responsible for the red, blue and purple color of most flowers, grapes and young wine [5, 6]. Their molecular structures derived from glycosylated 3,5,7,3′-tetrahydroxyflavylium cation which is represented in figure 1 [5, 7]. The molecule of anthocyanin is constituted from an aglycone or anthocyanidin moiety which is glycosylated by one or more sugars in its natural state. The most prevalent sugars are D-glucose, L-rhamnose, D-galactose, D-xylose and arabinose and they usually link at carbons 3, 5, 7, 3’ and 5’. The difference between aglycone are the number of hydroxyl groups and the degree of methylation of those groups [5].

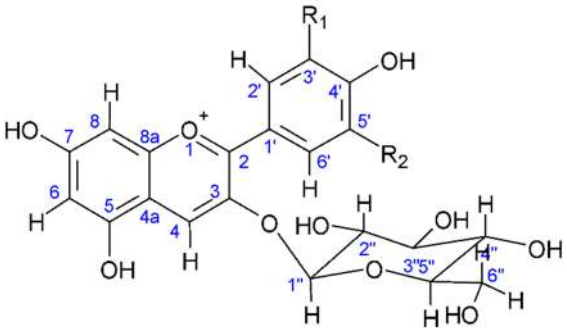


Figure 1 - Molecular structure of monoglucoside anthocyanin.

The glycosylated part can form esters with acetic, *p*-coumaric, caffeic, ferulic or sinapic acids and sometimes with *p*-hydroxybenzoic and malonic acids [5]. In the wines and grapes were identified five free anthocyanins of malvidine (MAL), cyanidine (CYA), delphinidine (DEL), petunidine (PET) and peonidine (PEO). Their formulas are represented in table 1 [7].

Table 1 - Substituents and the respective anthocyanins

Substituents		Aglycone
R_1	**R_2**	
OH	H	Cyanidine
OCH_3	H	Peonidine
OH	OH	Delphinidine
OH	OCH_3	Petunidine
OCH_3	OCH_3	Malvidine

In the wines and *Vitisvinifera* grapes species only monoglucoside anthocyanins (fig. 2) and acylated monoglucoside anthocyanins (fig. 3) were identified [7].

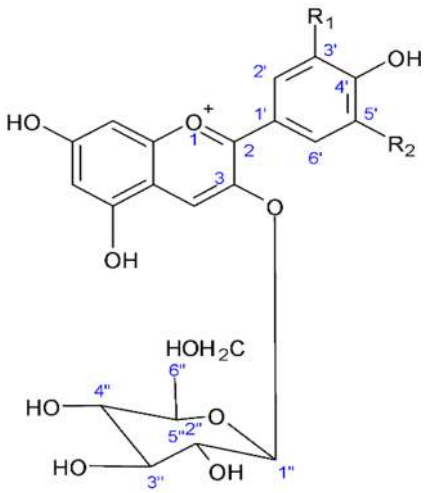


Figure 2 - Molecular structure of anthocyanin-3-monoglucoside.

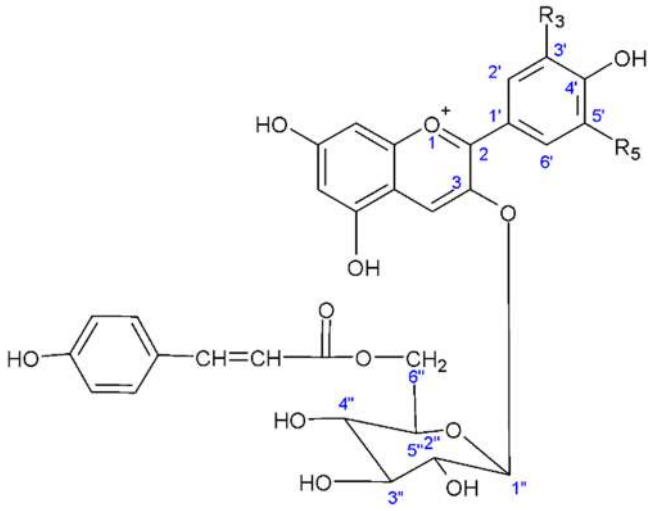


Figure 3 - Molecular structure of anthocynin-3-monoglucoside acylated by *p*-coumaric acid in carbon 5".

1.2. Flavonols

Flavonols are a subclass of flavonoids, the most common are quercetin, kaempferol, myricetin and isorhamnetin or quercetin-3-methylether [8, 9]. Their color vary from white to yellow and the molecular structure are presented in figures 4 to 7 [8].

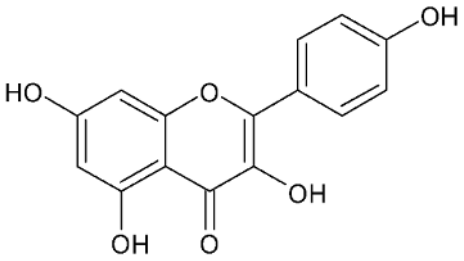


Figure 4 - Molecular structure of kaempferol.

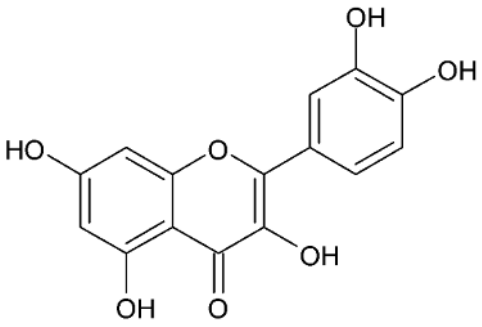


Figure 5 - Molecular structure of quercetin.

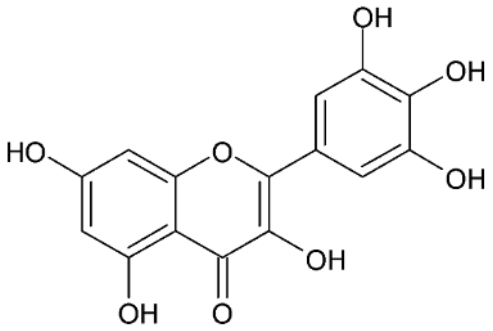


Figure 6 - Molecular structure of myricetin.

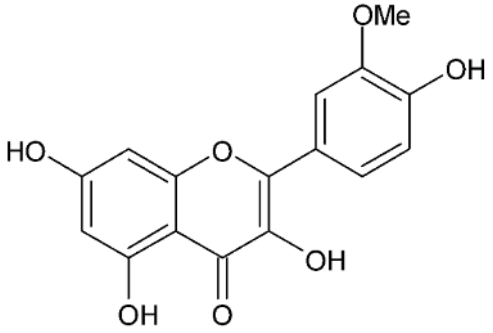


Figure 7 - Molecular structure of isohramnetin.

In grapes, the flavonols molecules are presented mainly in monoglycoside form in which molecules sugar are linked to hydroxyl group in the carbon 3 of the *O*-containing ring but the substitution can happen in other position. These flavonols glycosides of myricetin, quercetin and kaempferol form co-pigment with the anthocyanins in red wines and with oxidation products of tanins they are responsible for the color of white wines and grapes [8]. Currently there is much interest in the study of flavonols because of its antioxidant potential, anti-inflammatory, anti-allergic, hepatoprotective, anti-viral, anti-carcinogenic [9].

1.1. Flavan-3-ols

The flavan-3-ols are compounds that play an important role in defining the characteristics of wines. They are extracted from grapes skins and seeds during the winemaking process. During this process structural transformation takes place through oxidation and condensation reactions with influence on wine astringency and color [10]. They interact with anthocyanins to form co-pigment which help to stabilize the color of red wine and formation of new pigment during wine aging [10].
The basic unit of flavan-3-ols are catechin, epicatechin and their isomers present in the figures 9 and 10, and the nomenclature present in the tables 2 and 3. These molecules have two benzene cycle bonded by a saturated oxygenated heterocycle. The structure has two asymmetrical carbons (C2 and C3) that are the origin of the isomers [7].

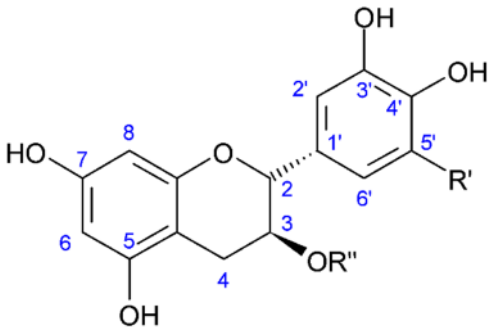


Figure 9 - Molecular structure of catechin series.

Table 2 - Nomenclature of catechin

R'	R''	Catechin
H	H	(+) – catechin (2R,3S)
H	H	(–) – catechin (2S,3R)
OH	H	Gallocatechin

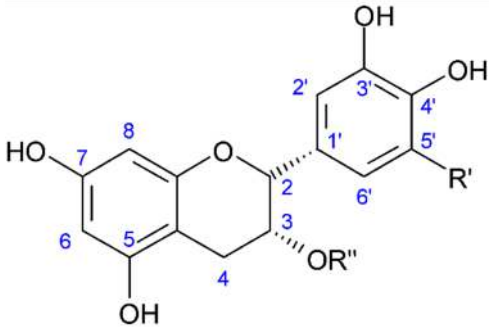


Figure 10 - Molecular structure of epicatechin series.

Table 3 - Nomenclature of epicatechin

R'	R''	Epicatechin
H	H	(+) – epicatechin (2S,3S)
H	H	(-) – epicatechin a (2R,3R)
OH	H	epigallocatechin

The flavano-3-ols can exist as monomers or polymers called proanthocyanidins or condensed tannins. These when heated in strongly acidic medium release anthocyanidins. The structure of proanthocyanidins varies with its sub-unit constituent, the degree of polymerization and the connection position. Figure 11 represents the general structure of a proanthocyanidin in which flavano-3-ols monomers are linked through carbon-carbon 4 and 8 or 4 and 6 [7, 11].

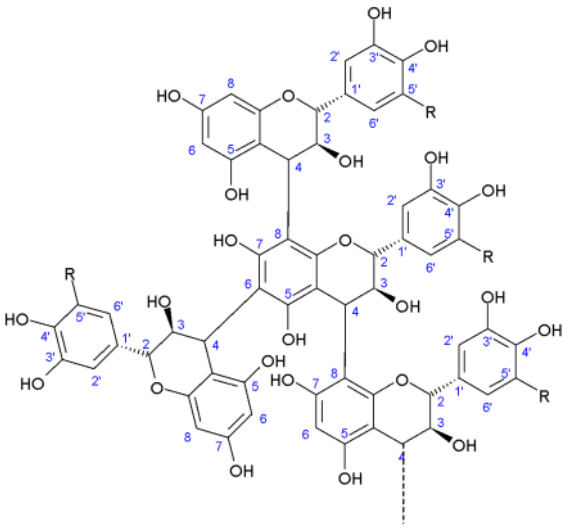


Figure 11 - General structure of proanthocyanidins

1.2. Benzoic, cinamic acids and derivates

The phenolic compounds no-flavonoids in wine are essentially derived from benzoic acid, cinnamic acid and volatile phenols including the stilbene (resveratrol). Their structures are elucidated in the figures 12 a) and b) and the derivatives are presented in table 4 [7].

a) b)

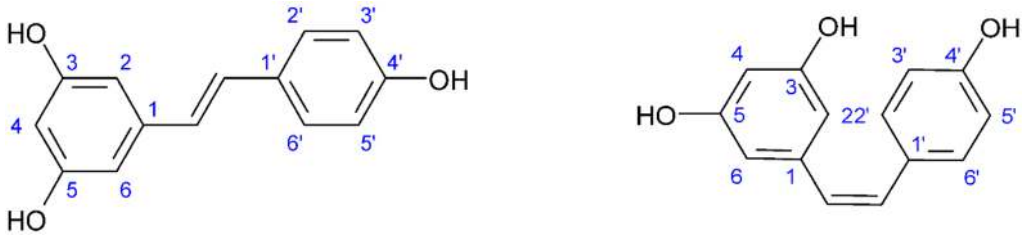
Figure 12 - Molecular structure of a) benzoic acid and b) cinammic acid.

Table 4 - Nomenclature of phenolic acids present in grapes and wines.

Benzoic acid	**R_1**	**R_2**	**R_3**	**R_4**	**Cinammic acid**
p-Hydroxybenzoic acid	H	H	OH	H	*p*-Coumaric acid
Protocatechuic acid	H	OH	OH	H	Caffeic acid
Vanilic acid	H	OCH_3	OH	H	Ferulic acid
Gallic acid	H	OH	OH	OH	
Syringic acid	H	OCH_3	OH	OCH_3	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentisic acid	OH	H	H	OH	

1.3. Stilbenes – Resveratrol

The stilbenes, particularly resveratrol, have been studied in recent years because of the benefits of those compounds may have on human health. They are biosynthesized in grapevines in defense of fungal diseases such as *Botrytis cinerea*, abiotic stress and UV irradiation [12]. Resveratrol can occur in two isomeric forms, *cis* and *trans*, as shown in figure 13 a) and b) [12].



a) b)

Figure 13 - Molecular structure of a) *cis* and b) *trans*-3,5,4'-trihidroxystilbene (resveratrol)

The objective of this work was to identify and quantify the different types of phenolic compounds found in wines from the Island of Fogo

2. Methosds, chemicals and materials

2.1. Methods

High performance liquid chromatography (HPLC) is the main analytical method and linked with mass spectrometry enables an identification of many phenolic compounds in wine. Because of the wine samples complexity and low concentration of phenolic compounds, it is needed an extraction process before injection on HPLC. The most common extraction methods for phenolic compounds in wine are solid phase extraction (SPE) and liquid liquid extraction (LLE) [13].

2.2. Chemicals and Materials

The sulfur standard studied were (CAS Number in bracket) *S*-ethylthioacetate (625-60-5), 2-mercaptoethanol (60-4-2), 2-(methylthio)-ethanol (5271-38-5), benzothiazole (95-16-9), dimethyl sulfone (67-71-0), 4-(methylthio)-1-butanol (20582-85-8), 3-(methylthio)-1-propanol (505-10-2), 3-mercapto-1-propanol (19721-22-3), ethyl-3-(methylthio)propionate (3047-32-3),2-methyltetrahydrothiophen-3-one (13679-85-1), 3-methylthio-1-propionic acid (646-05-01), 3-ethylthio-1-propanol (18721-61-4) and ethyl(methylthio)acetate (4455-13-4) (internal standard, IS) were purchased from Sigma-Aldrich and Lancaster. The *cis* and *trans*-2-methyltetrahydrothiophen-3-ol were prepared by reduction of 2-methyltetrahydrothiophen-3-one. The solvents used, dichloromethane, ethanol and water were all products with analytical grade.

2.3. Samples

The wine samples were Chã wine (white and red), Sodade wine (white, red and rosé), Montrond wine (white and red) and Sangue Vulcão wine (red). For each wine, six different samples were analysed. The samples analysed were from different producers but all from the same Island.

2.4. Preparation of standard solutions

Fifty milliliter of stock solution of each standard was prepared in ethanol at $1g.L^{-1}$ of concentration. One hundred ml of mix work solution was prepared in ethanol at $1mg.L^{-1}$ by dilution of stock solution . The internal standard solution, ethyl(methylthio)acetate, was prepared in 50 mL of hydroalcoholic solution of water/ethanol 12% (v/v) at 10 $mg.L^{-1}$. The calibration solutions were made with 12% hydroalcoholic standard solution, $3.5g.L^{-1}$ of tartaric acid and pH 3.5 adjusted with NaOH 0.1 M.

2.5. Procedure

2.5.1. Anthocyanins extraction by Solid Phase Extraction Procedure

The anthocyanins extraction from wine by SPE was done with Supelclean LC-18 6 mL cartridge according to the method proposed by Marquez et. al. [13]. A volume of 3 mL of wine was passed through a cartridge that was previously activated with 5 mL of methanol and washed with 7 mL aqueous 0.01% (v/v) HCl solution. The cartridge was successively washed with 10 mL of HCl 0.01% (v/v) and 5 mL ethyl acetate and the anthocyanins were recovered with 2.5 mL of methanol acidified to pH 2 with HCl. The anthocyanins samples were concentrated to 500 µL with nitrogen steam.

2.5.2. Non-anthocyanic compounds extraction by Liquid Liquid Extraction

The extraction of non-anthocyanics compounds was done according to the method proposed by Porgali & Büyüktuncel [14]. A volume of 5 mL was placed in Corning tube and 5 mL of ethyl acetate was added. The mixture was agitated for 5 minutes and the two phases, aqueous and organic phase, were separated by MIKRO centrifugater for 1 minute at 3000 rpm. Then 4,5 ml of organic phase was removed and the ethyl acetate was evaporated by nitrogen steam. The volume was adjusted to 500 µL with methanol solution.

2.6. Liquid chromatography mass spectrometry diode array detector conditions

The phenolic compounds were analysed in LC-MS-DAD. A Hypersil Gold C18 (250 x 4.6 mm, 5 µm) column was used and the eluents were A (99% H_2O: 1% HCO_2H) and B (80% CH_3CN: 19% H_2O: 1% HCO_2H). The gradient elution was 0-14 min, 8% B, 30 min, 8-20 %B, 16 min, 20-30% B, 20 min, 30-40% B, 10 min, 40-50% B and 10 min, 50-80% B. The detector is Thermo Fischer Scientific LTQ Orbitrap with an electrospray ion source and a high resolution fourier transform mass spectrometer (HR-FT-MS). The voltage on the electrospray needle was 3 kV and the capillary temperature 190 ºC. Full scan spectra were recorded over the range m/z 100-1000 in positive mode to anthocyanins and negative mode to other compounds. The data were processed by X-calibur software.

6. Presentation of results for phenolic compounds

6.1. Calibration curves of standard solutions

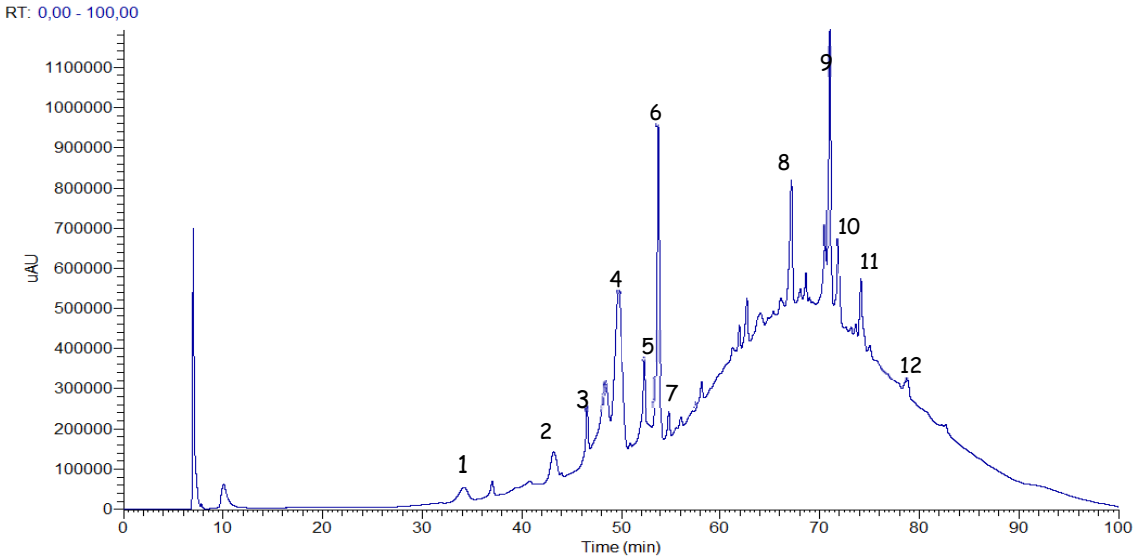
The results for chromatogram of a standard mix solution are presented in the table 5 with the retention time, RT, and their wavelength absorption, λ.
To each phenolic compound standard, calibration curve was determined by linear regression and the limit of detection (LOD) was estimated with the method proposed by ICH [15]. The LOD was expressed by *3.3*SD/S, S*, is the slope of the calibration curve and *SD* is the standard deviation of the response estimated by standard deviation of y-intercept of regression line. The table 5 presents the parameters of calibration curve of standard solutions. The second value of wavelength presented in the table correspond the maximum absorption spectrum.

Table 5 - Retention times, wavelength, concentration range, limit of detection, slope (m) and intercept (b) of the linear regression curves for the standard phenolic compounds.

Phenolic Compounds	RT /min	Wavelenght λ /nm	Concentration /mg.L^{-1}	LOD /mg.L^{-1}	R^2	Linear equation	
						Slope (m)	Intercept (b)
Anthocyanins							
Malvidin-3-*O*-glucoside	49.7	277 (526)	0.5 – 50	2.8	0.992	73908	-34990
Non-anthocyanic							
Gallic acid monohydrate	10.4	271	1 – 20	1.8	0.992	2333662	-2444644
(+)-catequin	33.3	280	1 – 20	1.5	0.987	8001006	-697892
Vanillic acid	38.1	260 (292)	1 – 30	1.2	0.998	1542217	-964593
Caffeic acid	39.7	269 (323)	0.5 – 30	2.2	0.992	7709937	-62599
Syringic acid	41.7	274	0.5 – 30	1.9	0.993	2340995	975930
p-Coumaric acid	53.0	310	0.5 – 30	2.4	0.989	5828715	-926006
Quercetin	61.4	352	1 – 20	2.0	0.993	8544324	-1298797
Kaempferol-3-*O*-glucoside	66.6	266 (346)	1 – 20	2.3	0.991	2297963	93301

6.2. Anthocyanins analysis in red wine

The figure 14 is one of the chromatograms obtained from Chã red wine extract, extracted by SPE.



1:**Dp-3-glc** (delphinidin-3-O-glucoside), 2:**Pt-3-glc** (petunidin-3-O-glucoside), 3:**Pn-3-glc** (peonidin-3-O-glucoside); 4:**Mv-3-glc** (malvidin-3-O-glucoside); 5:**Pn-3-glc-pyruvat** (peonidin-3-O-glucoside-pyruvic acid) 6:**VitisinA (malvidin-3-O-glucoside-pyruvic acid)**; 7:**Vitisin B (malvidin-3-O-glucoside vinyl adduct)**; 8: **Mv-3-p-coumglc-pyruvat** (malvidin-3-O-(6-*p*-coumaroyl)-glucoside pyruvic acid); 9: **Mv-3-glc-4-vinylcatechol** (malvidin-3-O-glucoside-4-vinylcatechol); 10:**Mv-3- p-coumglc** (malvidin-3-O-(6-p-coumaroyl)-glucoside); 11: **Mv-3-glc-4-vinylphenol** (malvidin-3-glucoside-4-vinylphenol); 12: **Mv-3-p-coumglc-4-vinylcatechol** (malvidin-3-(p-coumaroyl)glucoside-4-vinylcatechol)

Figure 14 – Chromatogram of *Chã* red wine extract for anthocyanins at 520 nm.

The chromatogram shows a deficient base line from 50 minutes which may indicate that all compounds had not been completely separated. However, the chromatogram baseline for anthocyanins analysis is always affected by the aging wine [16].With m/z, peak wavelengths and retention time values was possible to identify many anthocyanins present in the wines [17, 20]. In the table 6 are the anthocyanins identified in the chromatograms and theirs concentration, mg.L^{-1}, in Montrond, Chã, Sodade, Sangue de Vulcão red wines and Sodade rosé wine. In the same table are present the absorption wavelength, mass spectral (MS), mean concentration and standard deviation of each compound in the wine's samples

Six samples of each wine were analysed in triplicate and the quantification are expressed as malvidin-3-glucose equivalents. For each compound, Tukey test were applied at 5% of significance level, to verify the significant difference among the samples. Values not sharing the same superscript letter are different according to Tukey test.

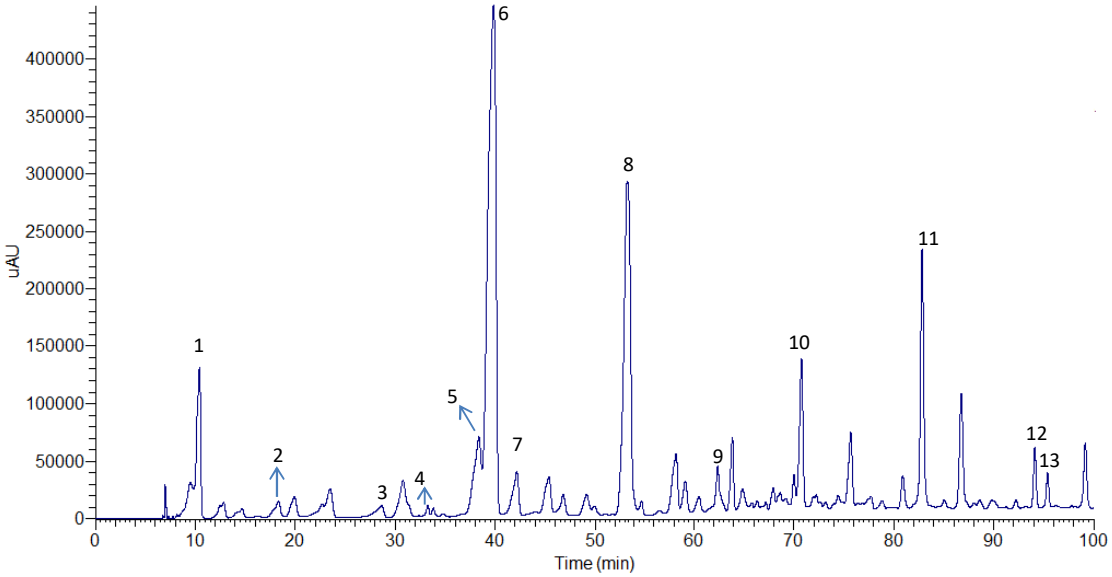
Table 6 – Absorption peak wavelengths, m/z of fragment and mean concentration with standard deviation (SD), $mg.L^{-1}$, of anthocyanins in red and rosé wines from Fogo Island.

Compounds	**λ**	**$[MS]^+$**	**Montrond**	**Chã**	**Sodade**	**Sangue Vulcão**	**Sodade rosé**
/mg.L^{-1}	nm	(m/z)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Dp-3-glc	520	465(303)	ND	8.90 ± 0.70	ND	ND	ND
Pt-3-glc	526	479(317)	ND	8.00$^{(a)}$ ± 8.00	4.85$^{(a)}$ ± 0.65	14.2$^{(a)}$ ± 0,7	ND
Pn-3-glc	520	463(301)	ND	10.1$^{(a)}$ ± 3.90	49.0$^{(b)}$ ± 1.4	24.6$^{(c)}$ ± 1,4	11,2$^{(a)}$ ± 1,1
Mv-3-glc	526	493(331)	19.6$^{(a)}$ ± 1.0	*74.2$^{(b)}$ ± 6.0	*61.4$^{(b,d)}$ ± 7.1	*116$^{(c)}$ ± 5	*51.9$^{(d)}$ ± 6.2
Pn-3-glc-pyruvat	504	531(369)	2.20$^{(a)}$ ± 0.10	7.95$^{(a.b)}$ ± 5.35	3.10$^{(a)}$ ± 0.60	13.7$^{(b)}$ ± 1.5	3.85$^{(a)}$ ± 0.05
Vitisin A	508	561(399)	10.2$^{(a)}$ ± 1.0	18.3$^{(a.b)}$ ± 10.2	9.10$^{(a)}$ ± 0.40	58.7$^{(b)}$ ± 38.3	8.95$^{(a)}$ ± 0.55
Vitisin B	490	517(355)	ND	<LOD	ND	ND	ND
Mv-3-*p*-coum-glc-pyruvic	512	707(399)	5.10$^{(a)}$ ± 0.90	9.45$^{(a)}$ ± 5.75	2.95$^{(a.b)}$ ± 0.65	25.7$^{(b)}$ ± 10.4	2.35$^{(a)}$ ± 0.15
Mv-3-glc-4-vinylcatechol	511	625(463)	5.65$^{(a)}$ ± 0.55	16.4$^{(b)}$ ± 0.7	13.1$^{(a)}$ ± 0.8	20.8$^{(b)}$ ± 6.5	33.1$^{(c)}$ ± 4.5
Mv-3- *p*-coum-glc	514	639(331)	4.85$^{(a)}$ ± 1.35	11.9$^{(a)}$ ± 0.9	ND	24.5$^{(b)}$ ± 14.2	10.8$^{(a)}$ ± 0.6
Mv-3-glc-4-vinylphenol	505	609(447)	3.65$^{(a.c)}$ ± 1.15	3.35$^{(a)}$ ± 0.65	3.10$^{(a)}$ ± 0.70	15.5$^{(b)}$ ± 4.4	9.05$^{(c)}$ ± 0.75
Mv-3-*p*-coum-glc-4-vinylcatechol	531	771(463)	ND	2.00 ± 0.50	ND	ND	ND

The concentration are expressed as malvidin-3-glucoside equivalents in $mg.L^{-1}$; *determined by dilution of sample with hydroalcoholic solution 12%. Values not sharing the same superscript letter (a-c) within the horizontal line are different according to the Tukey test; LOD – limit of detection; ND – not detected; SD – standard deviation from three determinations

6.3. Non-anthocyanic phenolic compounds analysis in wines

The identification of compounds was done with the values of retention time, wavelength of absorption and m/z for the compounds without standard solutions [21, 22]. The figure 14 represent one of chromatogram obtained from wine analysed.



1-gallic acid, 2- protocatechuic acid, 3- *cis*-caftaric acid, 4-(+)-catechin, 5-vanilic acid, 6-caffeic acid, 7 – syringic acid, 8-*p*-coumaric acid, 9- isorhamnetin-3-*O*-glucoside, 10-myricetin, 11-quercetin, 12-kaempferol, 13-isohramnetin.

Figure 14 – Chromatogram from non-anthocyanic compounds in wine

The values of concentrations for the compounds identified in red, white and rosé wines Chã, Sodade, Montrond and Sangue Vulcão are presented in the table 7 and 8.
It was needed to make a dilution of samples, to analyse some compounds like gallic acid and vanilic acid for some wine samples. The protocatechuic acid are expressed as gallic acid equivalent and *cis*-caftaric acid as caffeic acid equivalent. Myricetin, isohramnetin and isohramnetin-3-*O*-glucoside are expressed as quercetin equivalent.

Table 7 - Mean concentration with standard deviation (SD), mg.L^{-1}, of non-anthocyanic phenolic compounds determined in red and rosé wines from Fogo Island.

Compounds	**Montrond**	**Chã**	**Sodade**	**Sangue Vulcão**	**Sodade rosé**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Gallic acid	*24.5$^{(a)}$ ± 1.7	14.0$^{(b)}$ ± 0.1	*25.5$^{(a.d)}$ ± 1.0	*22.2$^{(a.e)}$ ± 0.6	2.35$^{(c)}$ ± 0,05
Protocatechuic acid [A]	6.25$^{(a)}$ ± 0.85	2.83$^{(b)}$ ± 0.47	ND	6.25$^{(a)}$ ± 0.85	2.35$^{(b)}$ ± 0.15
cis-Caftaric acid [B]	<LOD	<LOD	ND	<LOD	<LOD
(+)-Catechin	7.25$^{(a)}$ ± 0.35	3.85$^{(b)}$ ± 0.05	10.0$^{(c)}$ ± 2.1	6.40$^{(a.b)}$ ± 0.20	2.15 $^{(d.b)}$ ± 0.05
Vanilic acid	27.2$^{(a)}$ ± 3.8	19.9$^{(a)}$ ± 1.1	26.8$^{(a)}$ ± 5.4	*30.8$^{(a)}$ ± 7.4	7.00$^{(b)}$ ± 0.20
Caffeic acid	3.50$^{(a)}$ ± 0.70	15.7$^{(b)}$ ± 2.5	6.55$^{(a.c)}$ ± 0.05	6.35$^{(a.c)}$ ± 0.75	2.45$^{(a.e)}$ ± 0.05
Syringic acid	12.5$^{(a)}$ ± 1.3	6.00$^{(b)}$ ± 0.20	10.8$^{(a)}$ ± 0.1	13.7$^{(a)}$ 1.5	3.00$^{(c\ )}$ ± 0.20
p-Coumaric acid	7.40$^{(a)}$ ± 0.20	19.1$^{(b)}$ ± 0.9	9.25$^{(c)}$ ± 0.75	7.80$^{(a.c)}$ ± 0.20	<LOD
Myricetin [C]	4.50$^{(a)}$ ± 0.40	3.25$^{(b)}$ ± 0.15	3.35$^{(b)}$ ± 0.15	2.75$^{(b)}$ ± 0.05	2.05$^{(c)}$ ± 0.25
Quercetin	4.50$^{(a)}$ ± 0.40	4.25$^{(a)}$ ± 0.15	4.35$^{(a)}$ ± 0.15	3.45$^{(b)}$ ± 0.05	<LOD
Kaempferol [D]	<LOD	<LOD	<LOD	<LOD	<LOD
Isohramnetin [C]	<LOD	<LOD	<LOD	<LOD	<LOD
Isohramnetin-3-*O*-glucoside [C]	<LOD	<LOD	<LOD	<LOD	<LOD

Values expressed as: A-Gallic acid equivalents, B-Caffeic acid equivalents, C-Quercetin equivalents and D-Isohramnetin-*O*-glucoside equivalent in mg.l^{-1}. * - determined by dilution of sample with hydroalcoholic solution 12%. Values not sharing the same superscript letter (a-d) within the horizontal line are different according to the Tukey test. LOD – limit of detection; ND – not detected; SD – standard deviation from three determinations.

Table 8 - Mean concentration with standard deviation (SD), mg.L^{-1}, of non-anthocyanic phenolic compounds determined in white wines from Fogo Island.

Concentration	**MONTROND**	**CHÃ**	**SODADE**
	Mean ± SD	Mean ± SD	Mean ± SD
Gallic acid	2.45$^{(a)}$ ± 0.15	2.55$^{(a)}$ ± 0.15	2.35$^{(a)}$ ± 0.05
Protocatechuic acid [A]	<LOD	<LOD	<LOD
cis-Caftaric acid [B]	<LOD	<LOD	<LOD
(+)-Catechin	ND	<LOD	ND
Vanilic acid	<LOD	ND	<LOD
Caffeic acid	<LOD	<LOD	<LOD
Syringic acid	<LOD	<LOD	<LOD
p-Coumaric acid	<LOD	<LOD	<LOD
Myricetin [C]	ND	ND	<LOD
Quercetin	<LOD	<LOD	<LOD
Kaempferol [D]	<LOD	<LOD	<LOD
Isohramnetin [C]	<LOD	<LOD	<LOD
Isohramnetin-3-*O*-glucoside [C]	<LOD	<LOD	<LOD

Values expressed as: A-Gallic acid equivalents, B-Caffeic acid equivalents, C-Quercetin equivalents and D-Isohramnetin-3-O-glucoside equivalents in mg.L^{-1}. Values not sharing the same superscript letter (a-c) within the horizontal line are different according to the Tukey test; LOD – limit of detection; ND – not detected; SD – standard deviation.

7. Discussion of results

The analysis of anthocyanins in red wines revealed all monomeric anthocyanins in red wine Chã. The delphinidin-3-*O*-glucoside was detected only in Chã red wine with mean value of 8,90 ± 0,70 mg.L^{-1} of malvidin-3-*O*-glucoside equivalent. The figure 15 shows a graphic comparison of anthocyanins determined in all samples of red and rosé wines from Fogo Island.

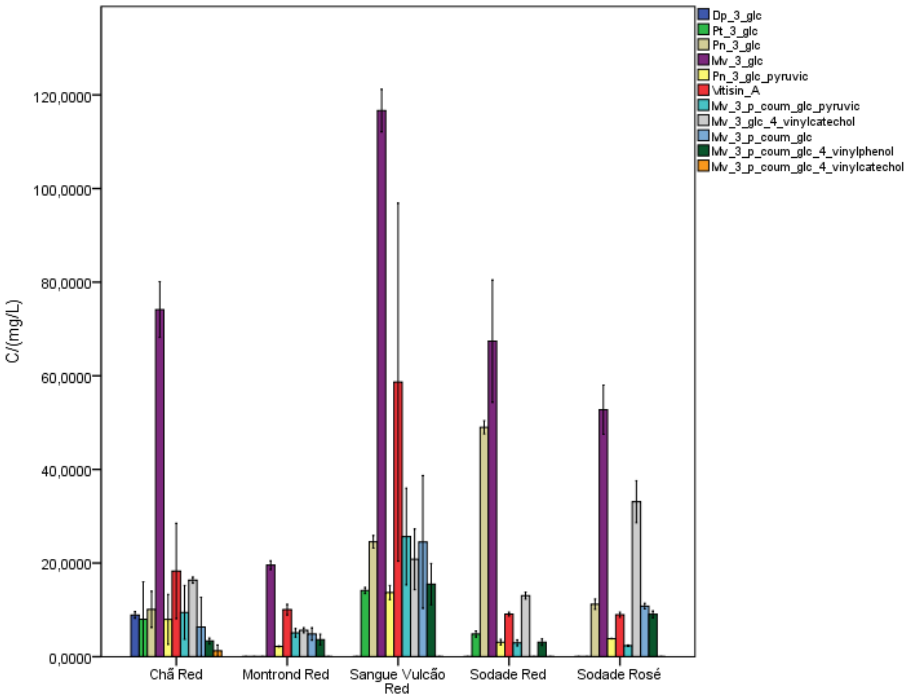


Figure 15 - Graphical comparison of anthocyanins mean concentration determined in red and rosé wines of Fogo Island.

The anthocyanin, petunidin-3-*O*-glucoside was detected in Chã, Sangue Vulcão and Sodade red wines with 8.00 ± 8.00 and 14.2 ± 0.7 mg.L^{-1} of malvidin-3-*O*-glucoside equivalent of concentration but 4,85 ± 0,65 mg.L^{-1} for Sodade red wine. The two values for Chã and Sangue de Vulcão red wines are similar according to Tukey test.

Peonidin-3-*O*-glucose was detected in all red wines samples except for Montrond red wine and maximum values determined in Sodade red wine with 49.0 ± 1.4 mg.L^{-1} of concentration. According to Tukey test, samples of Chã red and Sodade rosé wines with 10.1 ± 3.9 and 11.2 ± 1.1 mg.L^{-1} of malvidin-3-*O*-glucoside equivalent of concentration, do not have significant difference.

The malvidin-3-*O*-glucoside is the anthocyanin with higher concentration mainly in the Sangue Vulcão wine with 116 ± 5 mg.L^{-1} of concentration. The Montrond wine has the lower concentration of anthocyanin and significant difference in comparison with all the others wine samples. The Sodade rosé wine has 61.4 ± 7.1 mg.L^{-1}, and there are no significant difference with Chã red and Sodade rosé wines. These concentration of malvidin-3-O-glucoside are similar with some wines of other countries [23, 24].

Peonidin-3-*O*-glucose-pyruvic acid was detected in all wines samples. This compound, like malvidin-3-O-glucoside pyruvic acid, is formed by the reaction between peonidin-3-glucose with pyruvic acid released by yeast during alcoholic fermentation or by lactic bacteria during malolactic fermentation [25].In addition, with monomeric anthocyanins were detected other compounds derived from malvidin and peonidin, the pyroanthocyanins. These compounds are vitisin A, malvidin-3-O-(6-*p*-coumaroyl)-glucoside, malvidin-3-O-(6-*p*-coumaroyl)-glucoside-pyruvic acid (*p*-coumaroylvitisin

A), malvidin -3-O-glucoside-4-vinylcatechol, malvidin-3-O-glucoside-4-vinylphenol, malvidin-3-O-(6-p-coumaroyl)-glucoside-4-vinylcatechol. They are formed by the reaction between anthocyanins with phenolic acid derivate, pyruvic acid and acetaldehyde. The main compounds detected are derived from caffeic acid (vinylcatechol compounds) and *p*-coumaric acid (vinylphenol compounds) present in wine samples [26].

All pyranoanthocyanins detected are mainly malvidin derived with other compounds. It occurs because of the high concentration of the malvidin in relation to other anthocyanins.

The compound vitisin A was detected in all wines. Sangue Vulcão among the wines has the highest concentration with 58.7 ± 38.3 $mg.L^{-1}$ of Mv-3-gl equivalent.

Vitisin B was detected only in Chã red wine but its signal or peak on chromatogram was very low.

The malvidin-3-*O*-(6-p-coumaroyl)-glucoside pyruvic acid was determined in all wines samples. Except for Sodade red wine with the maximum value, all wines samples concentration have no significant difference. Malvidin-3-*O*-glucoside-4-vinylcatechol was also detected in all wines and Sodade rosé wine has the highest concentration and there are significant difference when compared with other analysed samples. Sangue Vulcão and Chã red wines have no significant difference according to Tukey test, and they have the highest concentration among red wines.

Malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside was not detected in Sodade red wine. Sangue Vulcão sample wine has the highest concentration with significant difference among other wines. The concentration of this compound in Montrond red wine, Chã red wine and Sodade Rosé wine has no significant difference.

Malvidin-3-*O*-glucose-4-vinylphenol was detected in all samples. Sangue Vulcão presented the highest concentration and according to Tukey test, this result has significant difference.

The last pyroanthocyanin analysed, malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside-4-vinylcatechol was detected only in Chã red wine. In the non-anthocyanic compounds, the red wines of Fogo Island have the major concentration of these compounds than white wines. The white wines have a concentration below of the limit of detection for most of these compounds, except for gallic acid. Some compounds were not detected in some white wines samples as show the table 8.

In the red and rosé wines, the gallic acid was detected in all samples and together with vanilic acid they presented the major concentration of the phenolic acid. The concentration of gallic acid determined in wines from Fogo Island is common in other wines [24].

The caffeic acid, *p*-coumaric acid and syringic acid were detected in all samples but in red wines they had a concentration lower than vanilic and gallic acid.

Caffeic acid was identified in all wines samples and the maximum values of concentration was determined in Chã red wine with 15.7 ± 2.5 $mg.L^{-1}$. This concentration is relatively high compared with some wines [23, 24].

The concentration of vanilic acid determined in wines from Fogo Island are very higher compared with Turkey wines [4]. The same happens with syringic acid for wines producedin Turkey but compared with Australian red wines, their concentration are similar [23].

Flavan-ols compounds, (+)-catechin, was the only detected in the wine samples but it was not detected in the Sodade white wine. The Sodade red wine had the highest concentration of this compound with 10.0 ± 2.1 $mg.L^{-1}$. This concentration is very low compared to Macedonian and Turkey red wines [4, 24]. Flavonols compounds, quercetin, myricetin, kaempferol, isohramnetin and isohramnetin-3-*O*-glucoside were detected in all wine samples.

In the red wines, quercetin was determined in all samples. The concentration of this compound determined in Montrond, Chã and Sodade wines samples have no significant difference according to Tukey test. The values of concentration determined in red wines are common comparing with other countries [23]. In Sodade rosé wine the concentration determined are below of LOD.

Myricetin were determined in all wines samples and the maximum concentration was obtained in Montrond red wine, 4.50 ± 0.40 as $mg.L^{-1}$ of quercetin equivalent.

The concentration of kaempferol, isohramnetin and isohramnetin-3-*O*-glucoside according to the calibration curve were below of LOD.
The figure 16 is a graphic representation of mean values of concentration for phenolic compounds non-anthocyanic in red wines samples.

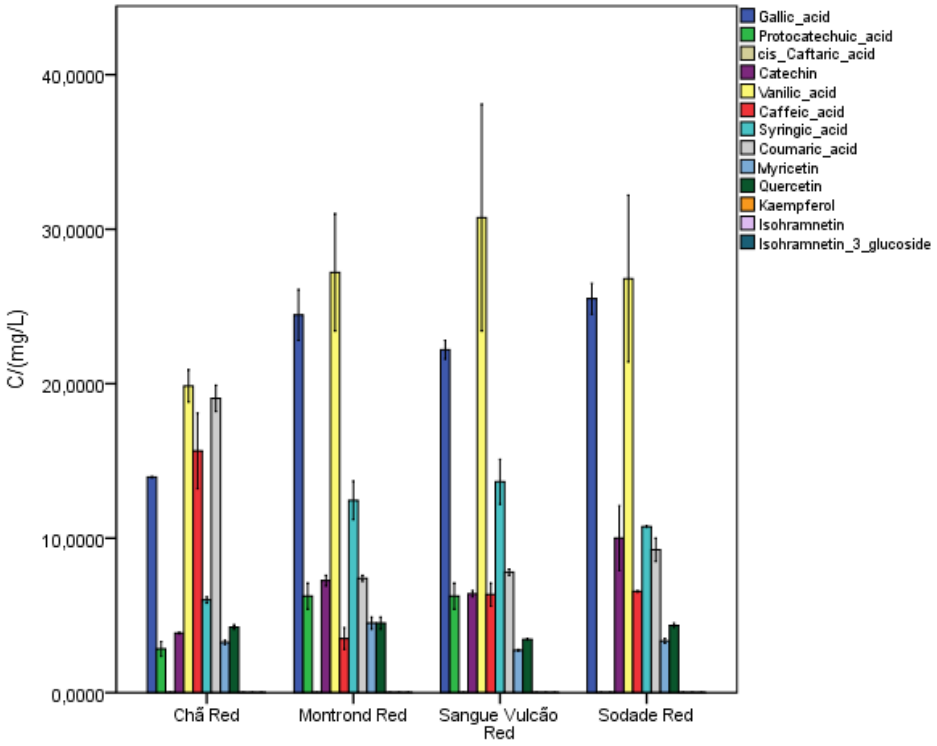


Figure 16 - Graphic comparison of mean concentration of non-anthocyanic compounds determined in red wines of Fogo Island.

8. Conclusion

The wines from Cape Verde presented several phenolic compounds and the red wines had more concentration of these compounds than white wines. Within anthocyanins, the malvidin derivates were the main compounds detected including the pyroanthocyanins. Malvidin-3-*O*-glucoside and vitisin A, were more concentrated among anthocyanins in analysed wines. The phenolic acids, syringic and gallic, were more concentrated than other acids in red wines. Quercetin, myricetin, kaempferol and (+)-catechin were de main flavonols and flavan-3-ols detected in analysed wines. Among wines analysed, Sangue Vulcão wine show the highest concentration of anthocyanic compounds.

9. Reference

[1] G. L. La Torre, M. Saitta, F. Vilasi, T. Pellicanò, and G. Dugo, “Direct determination of phenolic compounds in Sicilian wines by liquid chromatography with PDA and MS detection,” *Food Chem.*, vol. 94, no. 4, pp. 640–650, 2006, doi: http://dx.doi.org/10.1016/j.foodchem.2005.02.007.

[2] F. J. Gonçalves, S. M. Rocha, and M. A. Coimbra, “Study of the retention capacity of anthocyanins by wine polymeric material,” *Food Chem.*, vol. 134, no. 2, pp. 957–963, 2012, doi: http://dx.doi.org/10.1016/j.foodchem.2012.02.214.

[3] A. Andreu-Navarro, P. Russo, M. P. Aguilar-Caballos, J. M. Fernández-Romero, and A. Gómez-Hens, “Usefulness of terbium-sensitised luminescence detection for the chemometric classification of wines by their content in phenolic compounds,” *Food Chem.*, vol. 124, no. 4, pp. 1753–1759, 2011, doi: http://dx.doi.org/10.1016/j.foodchem.2010.08.014.

[4] H. Kelebek, A. Canbas, M. Jourdes, and P.-L. Teissedre, “Characterization of colored and colorless phenolic compounds in Öküzgözü wines from Denizli and Elazig regions using HPLC-DAD–MS,” *Ind. Crops Prod.*, vol. 31, no. 3, pp. 499–508, 2010, doi: 10.1016/j.indcrop.2010.01.012.

[5] I. J. Košir, B. Lapornik, S. Andrenšek, A. G. Wondra, U. Vrhovšek, and J. Kidrič, “Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance,” *Anal. Chim. Acta*, vol. 513, no. 1, pp. 277–282, 2004, doi: http://dx.doi.org/10.1016/j.aca.2003.12.013.

[6] M. Monagas, C. Gómez-Cordovés, and B. Bartolomé, “Evaluation of different Saccharomyces cerevisiae strains for red winemaking. Influence on the anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic content and colour characteristics of wines,” *Food Chem.*, vol. 104, no. 2, pp. 814–823, 2007, doi: http://dx.doi.org/10.1016/j.foodchem.2006.12.043.

[7] P. Ribéreau-Gayon, Y. Glories, A. Maujean, and D. Dubourdieu, “The Chemistry of Wine: Stabilization and Treatments.,” in *Handbook of Enology*, 2nd ed., vol. 2, J. W. and S. Ltd, Ed. 2006, pp. 141–203.

[8] D. P. Makris, S. Kallithraka, and P. Kefalas, “Flavonols in grapes, grape products and wines: Burden, profile and influential parameters,” *Journal of Food Composition and Analysis*, vol. 19, pp. 396–404, 2006.

[9] C. L. Silva, J. L. Gonçalves, and J. S. Câmara, “A sensitive microextraction by packed sorbent-based methodology combined with ultra-high pressure liquid chromatography as a powerful technique for analysis of biologically active flavonols in wines,” *Anal. Chim. Acta*, vol. 739, no. 0, pp. 89–98, 2012, doi: http://dx.doi.org/10.1016/j.aca.2012.06.020.

[10] S. González-Manzano, J. C. Rivas-Gonzalo, and C. Santos-Buelga, “Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration,” *Anal. Chim. Acta*, vol. 513, no. 1, pp. 283–289, 2004, doi: http://dx.doi.org/10.1016/j.aca.2003.10.019.

[11] B. Lorrain, I. Ky, L. Pechamat, and P. L. Teissedre, “Evolution of Analysis of Polyhenols from Grapes, Wines, and Extracts,” *Molecules*, vol. 18, no. 1, pp. 1076–1100, 2013, [Online]. Available: http://www.mdpi.com/1420-3049/18/1/1076.

[12] S. Kostadinović *et al.*, “Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: Effect of variety and enological practices,” *Food Chem.*, vol. 135, no. 4, pp. 3003–3009, 2012, doi: http://dx.doi.org/10.1016/j.foodchem.2012.06.118.

[13] A. Marquez, M. P. Serratosa, A. Lopez-Toledano, and J. Merida, “Colour and phenolic compounds in sweet red wines from Merlot and Tempranillo grapes chamber-dried under controlled conditions,” *Food Chem.*, vol. 130, no. 1, pp. 111–120, 2012, doi: http://dx.doi.org/10.1016/j.foodchem.2011.07.010.

[14] E. Porgalı and E. Büyüktuncel, “Determination of phenolic composition and antioxidant capacity of native red wines by high performance liquid chromatography and spectrophotometric methods,” *Food Res. Int.*, vol. 45, no. 1, pp. 145–154, Jan. 2012, doi: 10.1016/j.foodres.2011.10.025.

[15] ICH, “ICH Topic Q2 (R1) Validation of Analytical Procedures : Text and Methodology,” *Int. Conf. Harmon.*, vol. 1994, no. November 1996, p. 17, 2005, doi: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.

[16] D. Blanco-Vega, S. Gómez-Alonso, and I. Hermosín-Gutiérrez, “Identification, content and distribution of anthocyanins and low molecular weight anthocyanin-derived pigments in Spanish commercial red wines,” *Food Chem.*, vol. 158, no. 0, pp. 449–458, 2014, doi: http://dx.doi.org/10.1016/j.foodchem.2014.02.154.

[17] C. Alcalde-Eon, M. T. Escribano-Bailón, C. Santos-Buelga, and J. C. Rivas-Gonzalo, “Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study,” *Anal. Chim. Acta*, vol. 563, no. 1–2, pp. 238–254, 2006, doi: http://dx.doi.org/10.1016/j.aca.2005.11.028.

[18] C. Alcalde-Eon, M. T. Escribano-Bailón, C. Santos-Buelga, and J. C. Rivas-Gonzalo, “Separation of pyranoanthocyanins from red wine by column chromatography,” *Anal. Chim. Acta*, vol. 513, no. 1, pp. 305–318, 2004, doi: http://dx.doi.org/10.1016/j.aca.2003.10.076.

[19] E. Boido, C. Alcalde-Eon, F. Carrau, E. Dellacassa, and J. C. Rivas-Gonzalo, “Aging Effect on the Pigment Composition and Color of Vitis vinifera L. Cv. Tannat Wines. Contribution of the Main Pigment Families to Wine Color,” *J. Agric. Food Chem.*, vol. 54, no. 18, pp. 6692–6704, 2006, doi: 10.1021/jf061240m.

[20] F. He *et al.*, “Anthocyanins and Their Variation in Red Wines II. Anthocyanin Derived Pigments and Their Color Evolution,” *Molecules*, vol. 17, no. 2, pp. 1483–1519, 2012, [Online]. Available: http://www.mdpi.com/1420-3049/17/2/1483.

[21] H.-J. Chen, B. S. Inbaraj, and B.-H. Chen, “Determination of Phenolic Acids and Flavonoids in Taraxacum formosanum Kitam by Liquid Chromatography-Tandem Mass Spectrometry Coupled with a Post-Column Derivatization Technique,” *Int. J. Mol. Sci.*, vol. 13, no. 1, pp. 260–285, 2011, [Online]. Available: http://www.mdpi.com/1422-0067/13/1/260.

[22] M. Figueiredo-González, J. Rigueiro, B. Cancho-Grande, and J. Simal-Gándara, “Garnacha Tintorera-based sweet wines: Detailed phenolic composition by HPLC/DAD–ESI/MS analysis,” *Food Chem.*, vol. 143, pp. 282–292, 2014.

[23] I. Ginjom, B. D’Arcy, N. Caffin, and M. Gidley, “Phenolic compound profiles in selected Queensland red wines at all stages of the wine-making process,” *Food Chem.*, vol. 125, no. 3, pp. 823–834, 2011, doi: http://dx.doi.org/10.1016/j.foodchem.2010.08.062.

[24] V. Ivanova-Petropulos *et al.*, “Phenolic compounds and antioxidant activity of Macedonian red wines,” *J. Food Compos. Anal.*, vol. 41, pp. 1–14, 2015, doi: http://dx.doi.org/10.1016/j.jfca.2015.01.002.

[25] A. Morata, C. González, and J. A. Suárez-Lepe, “Formation of vinylphenolic pyranoanthocyanins by selected yeasts fermenting red grape musts supplemented with hydroxycinnamic acids,” *Int. J. Food Microbiol.*, vol. 116, no. 1, pp. 144–152, 2007.

[26] S. Benito, A. Morata, F. Palomero, M. C. González, and J. A. Suárez-Lepe, “Formation of vinylphenolic pyranoanthocyanins by Saccharomyces cerevisiae and Pichia guillermondii in red wines produced following different fermentation strategies,” *Food Chem.*, vol. 124, no. 1, pp. 15–23, 2011.