ANALYSIS OF PHENOLIC, SULFUR AND VOLATILES AROMA COMPOUNDS IN WINES OF FOGO ISLAND – CAPE VERDE

À minha filha Karen Pereira

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Abstract

The Island of Fogo, one of the islands of the Cape Verde archipelago has invested in the production of wines. The wine trade is important to the economy of the island and has been an asset for the ecotourism mainly in the *Chã das Caldeiras* region. Wine is a complex alcoholic beverage resulting from the fermentation of the grapes must. This complexity is due to the presence of various substances which are transferred from grapes or form during fermentation or in another stage of production. Several of these compounds may contribute to the quality of the wine but there are other that harm the quality of this beverage depending on their concentrations and the limits of sensory perception. Several compounds were analysed in the wines of Fogo Island including phenolic compounds, sulfur compounds and volatile compounds responsible for the aroma of the wines. For the phenolic compounds were analysed anthocyanins and non-anthocyanic compounds. Among the non-anthocyanic, flavonols, flavanols and phenolic acids were analysed. These phenolic compounds were analysed by high perfomance liquid chromatography with diode array detector and mass spectrometry. Because of the presence of the sulfur in the *Chã das Caldeiras* and because majority of the sulfur compounds influence negatively the quality of the wine, the sulfur compounds were also analysed. Several low volatile sulfur compounds, like methionol, were analysed by gas chromatography with flame photometric detector. The volatile compounds that give fruity and floral aromas to the wines were also analysed. Many of these compounds were analysed, including various esters, alcohols such as 2-phenylethanol and 1-hexanol, terpenes, nor-isoprenoids, sesquiterpenes and some acids such as hexanoic and decanoic acids. The analysis of these compounds was carried out by solid phase microextraction gas chromatography coupled with mass spectrometry. Several samples of red, white and rosé wines of different producers of the Fogo Island were analysed and all results were submitted to the Tukey test to check significant differences between the values of the concentrations determined. The application of chemiometric analysis including principal component analysis, linear discriminant analysis and hierarchical cluster analysis in the wines, allowed to differentiate the wines of Cape Verde based on phenolic, heavy sulfur and volatiles aroma compounds. With chemometric analysis was possible to distinguish the four analysed red wines Chã, Sodade, Montrond and Sangue de Vulcão through the phenolic compounds. Sodade rosé wine through sulfur compounds presented a distinct classification of other wines with chemometric analysis while for volatile aromatic compounds, the white wine Chã stood out from other wines.

Keywords: Cape Verde – Fogo Island, Chã das Caldeira, wines, phenolic compounds, sulfur compounds, volatiles aroma compounds, liquid chromatography, gas chromatography, chemometric analysis.

Resumo

A ilha do Fogo, uma das ilhas do arquipélago de Cabo Verde, tem apostado fortemente na produção dos vinhos. O comércio do vinho influencia de forma importante na economia da ilha e tem sido uma mais-valia no ecoturismo principalmene na região de Chã das Caldeiras. O vinho é uma bebida alcoolica complexa resultante da fermentação do mosto das uvas. Esta complexidade deve-se a presença de diversas substâncias que são transferidas das uvas, que se formam durante a fermentação do mosto ou numa outra fase de produção. Vários desses compostos podem contribuir para a qualidade do vinho mas também existem outros que prejudicam a qualidade desta bebida dependendo das suas concentrações e dos limites de perceção sensorial. Nos vinhos da ilha do Fogo foram analisados vários compostos entre os quais compostos fenólicos, compostos sulfurados e compostos voláteis responsáveis pelo aroma dos vinhos. Nos compostos fenólicos foram analisados as antocianinas e os compostos não-antociânicos. Entre os não-antociânicos foram analisados os flavonóis, flavanóis e ácidos fenólicos. Esses compostos fenólicos foram analisados através da cromatografia líquida de elevada eficiência com detector por arranjo de diodos e espetrometria de massa. Por causa da presença do enxofre na região de Chã das Caldeiras e porque a maioria dos compostos de enxofre ou sulfurados influenciam de forma negativa a qualidade do vinho também foram analisados os compostos sulfurados. Vários compostos sulfurados pouco voláteis ou pesados, como o metionol, foram analisados através da cromatografia gasosa com detetor fotométrico de chama. Também foram analisados os compostos voláteis que conferem aromas frutados e florais aos vinhos. Varios destes compostos foram analisados, destacando-se diversos ésteres, os álcoois como o 2-feniletanol e o 1-hexanol, vários terpenos, norisoprenoides, sesquiterpenos e alguns ácidos como o ácido hexanóico e ácido decanóico. A análise destes compostos foi feita através da microextração em fase sólida com cromatografia gasosa acoplado com espetrometria de massa. Foram analisados amostras de vinhos tintos, brancos e rosé dos principais produtores da ilha do Fogo e todos os resultados foram submetidos ao teste de Tukey a fim de verificar se existem diferenças significativas entre os valores determinados. A aplicação de análise quimiométrica nomeadamente análise de componentes principais, análise discriminante linear e análise hierárquica de *cluster* permitiu diferenciar os vinhos de Cabo Verde com base nos compostos fenólicos, sulfurados e voláteis. Da análise quimiométrica, foi possível distinguir os quatro vinhos tintos analisados Chã, Sodade, Montrond e Sangue de Vulcão atraves dos compostos fenólicos. O vinho rosé Sodade com compostos sulfurados apresentou uma

classificação distinta dos outros vinhos através da análise quimiométrica enquanto que nos compostos voláteis aromáticos o vinho branco Chã destacou-se dos outros vinhos.

Palavras chaves: Ilha do Fogo – Cabo Verde, Chã das Caldeiras, vinhos, compostos fenólicos, compostos sulfurados, compostos voláteis, cromatografia líquida e cromatografia gasosa, análise quimiométrica.

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Abbreviations and symbols

$A_{analyte}$	Area of analyte
A_{IS}	Area of internal standard
CYA	Cyanidine
DEL	Delphinidine
Dp-3-glc	Delphinidin-3-O-glucoside
GC-FPD	Gas Chromatograph - Flame Photometric Detector
GC-MS	Gas Chromatograph – Mass Spectrometer
HCA	Hierarchical Cluster Analyis
HPLC	High Performance Liquid Chromatography
HR-FT-MS	High Resolution Fourier Transform Mass Spectrometer
HS	Headspace
IS	Internal Standard
LC-MS-DAD	Liquid Chromatography Mass Spectrometry Diode Array Detector
LDA	Linear Discriminant Analysis
LLE	Liquid Liquid Extraction
LOD	Limit of Detection
MAL	Malvidine
MS/IT	Mass Spectrometry/Ion Trap
Mv-3- p-coumglc	Malvidin-3-O-(6-p-coumaroyl)-glucoside
Mv-3-glc	Malvidin-3-O-glucoside
Mv-3-glc-4-vinylcatechol	Malvidin-3-O-glucoside-4-vinylcatechol
Mv-3-glc-4-vinylphenol	Malvidin-3-glucoside-4-vinylphenol
Mv-3-p-coumglc-4-vinylcatechol	Malvidin-3-(p-coumaroyl)glucoside-4-vinylcatechol
Mv-3-p-coumglc-pyruvat	Malvidin-3-O-(6-*p*-coumaroyl)-glucosidepyruvicacid
ND	Not Detected
PCA	Principal Components Analysis
PEO	Peonidine
PET	Petunidine
Pn-3-glc	Peonidin-3-O-glucoside
Pn-3-glc-piruvat	Peonidin-3-O-glucoside-pyruvic acid
Pt-3-glc	Petunidin-3-O-glucoside

RI	Retention Index
RT	Retention Time
SD	Standard Deviation
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
WTO	World Trade Organization

1. INTRODUCTION AND CHARACTERIZATION OF FOGO ISLAND CAPE VERDE

1.1. CHARACTERIZATION OF CAPE VERDE

The archipelago of Cape Verde is located on the West Coast of Africa at 400 km of westwards of Senegal in the Atlantic Ocean (Olehowski et. al., 2008). It consists of ten islands as shown in figure 1.1 discovered in 1460 during the trips of the Portuguese expansion. The colonization of the islands began in 1462 and until now only nine of the ten islands are inhabited. The total population in the country is around 500 000 inhabitants, with the island of Santiago, the largest, with 200 000 inhabitants. The predominant economic activities in the different islands is distributed in agriculture, livestock, fisheries and tourism.

Figure 1.1 - Map of Cape Verde in the Atlantic Ocean[1]

1.2. CHARACTERIZATION OF FOGO ISLAND

The island of Fogo, the fourth biggest island, is roughly circular, as shown in figure 1.2 with a surface area of 476 km^2 (Olehowski et. al., 2008). The island is of volcanic origin and it resembles a volcano. Because of the altitude of the volcano, the Peak of Fogo Island is the central cone of the volcano and is the highest point in the country with 2829 meters.

[1] Available in the web page < http://www.nationsonline.org/oneworld/map/cape-verde-map2.htm> last access 06/04/2017.

The climate as the other islands is dominated by the trade winds but does not supply moist air masses. Between August and October there are monsoon winds, hot and humid, although with irregular rainfall in these months.

The climate range from tropical to semi-arid and the average annual temperature is 25 °C but in Chã das Caldeiras in the months of December to January the climate can reach below 0 ºC. The humid region (> 600 mm/a) is located in the north-east, and arid region (~ 600 mm/a) are in the southeast of the island but above 1300 meters of altitude there is lower rainfall (Marques et. al., 2014; Mota Gomes, 2006).

Figure 1.2 - Aerial photograph of Fogo Island[2].

Agriculture is a dominant practice in this island. The soil consists essentially of volcanic material rich in nutrients which are generally covered by the layer of volcanic slag basaltic gravel of different sizes. The permeability of this material allows the storage of water that enables the development of flora in dry seasons (Leyens, 2002).

[2] Available in the web page < https://www.pinterest.pt/aagodinho/cabo-verde/> last access 06/04/2017.

1.3. THE VITIVINICULTURE IN FOGO ISLAND - CAPE VERDE

The culture of vines in Cape Verde was started by the first Portuguese settlers during 16th century in the islands of Santo Antão, São Nicolau, Santiago, Brava and Fogo. Because of the natural conditions of Fogo Island this culture had and still has more success. The first production of the wine at the time were for domestic consumption but in the 18th century, the wine began to be exported to Guinea and Brazil. But those exports were banned by order of the Marquês de Pombal as well as the cultivation of vines. The cultivation was restarted with some plants that remained on the island and today it continues despite some recent volcanic eruptions in 2015 (Figure 1.3).

The grapes grown are possibly originating from Touriga Nacional and Moscatel de Setubal and they have adapted to the dry climate of Cape Verde.

The regions of Chã das Caldeiras and Mosteiros are the main producers of the vineyard. Especially the region of Chã das Caldeiras located at 1600 meters above sea level, next to a volcano, offers optimal conditions for the cultivation of the vine because there is always fog formation which provides sufficient moisture for the practice of agriculture (Mota Gomes, 2006).

Figure 1.3 - Grapevine culture in Chã das Caldeiras (Centeio, 2015).

The fertile volcanic soil, rich in minerals, the fluctuation of temperatures between day and night, good sun exposure and brightness favor the development of grapes with good sugar content and aromatic compounds.

Because of the climatic characteristics of the local soil and harvest realised before the rainy season, culture is not subjected to chemical fertilizers and pesticides, unless the treatment against powdery mildew (the vineyard dust) that is made with sulfur collected in the region.

The first wines produced are handmade and fully biological called *Manecon*. Later with the support of the German Cooperation, Italian Cooperation and the Ministry of Agriculture were introduced modern technologies in the production of wines.

Now there are several producers of wine, Chã, Sodade, Montrond and others, all of the Fogo Island who sell red, white and rosé wines.

The marketing the Fogo Island wine is widespread in the whole of the Cape Verde archipelago and have the perspective of being exported. The trade of this product is one of the important sources of income specifically for Fogo Island farmers of Chã das Caldeiras region. Together with agriculture, tourism has been very important to the economy of this region.

Tourism also has gradually developed making it a good source of revenue. In the last decade it has contributed significantly to the development of the island's economy and some typical products of the island, such as coffee, wine and the presence of the volcano have been an asset for the development of rural tourism (López-Guzmán et. al., 2011).

Combining tourism with the commercialization of typical products of the region contributes to a typical trademark of the island of Fogo. To achieve this objective, it is necessary to accomplish certain quality requirements and even the socio-economic conditions that the country demands.

The ease of access to information, awareness of the population of their rights, tourism development and the adherence of Cape Verde to the World Trade Organization (WTO) in 2008, puts Cape Verde in a larger market with greater competition. These news national and international conditions require producers and Cape Verdean traders

highest demand in terms of quality of its products. So, it is always necessary to make an exhaustive study of any product commercialized in order to ascertain its quality or even to improve it for increased competition.

1.4. OBJECTIVE

This work has the purpose to identify and quantify, in the wines of Fogo Island, the following compounds:

- Phenolic compounds;
- Sulfur compounds;
- Volatiles aroma compounds.

Apply the chemometrics analysis to classify and distinguish the wines according to their concentrations of:

- Phenolic compounds;
- Sulfur compounds;
- Volatiles aroma compounds.

2. PHENOLIC COMPOUNDS IN WINE

2.1. INTRODUCTION TO PHENOLIC COMPOUNDS

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 (La Torre et. al., 2006). They are the major components of the wine with a percentage from 30% to 40% among macromolecular compounds present in wine (Gonçalves et. al., 2012).

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 (Andreu-Navarro et. al., 2011).

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 (Kelebek et. al., 2010).

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) (Kelebek et al., 2010).

2.1.1. Anthocyanins

Anthocyanins are water-soluble pigments responsible for the red, blue and purple color of most flowers, grapes and young wine (Košir et al., 2004; Monagas et. al., 2007). Their molecular structures derived from glycosylated 3,5,7,3′-tetrahydroxyflavylium cation which is represented in the figure 2.1 (Košir et al., 2004; Ribéreau-Gayon et.al., 2006).

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 (Košir et al., 2004).

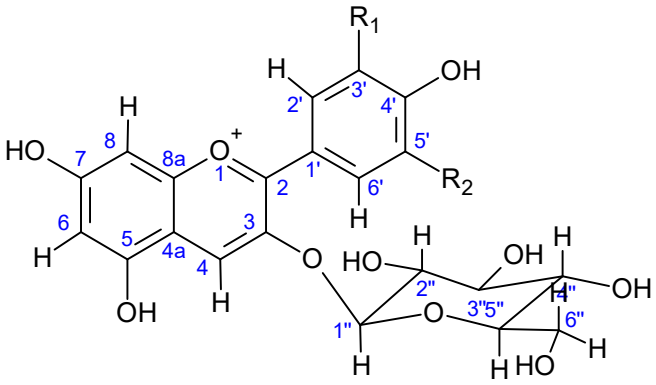


Figure 2.1 - Molecular structure of monoglucoside anthocyanin.

The glycosylated part can form esters with acetic, *p*-coumaric, caffeic, ferulic or sinapic acids and sometimes with *p*-hydroxybenzoic and malonic acids (Košir et. al., 2004). 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 2.1 (Ribéreau-Gayon et. al., 2006).

Table 2.1 - Substituents and the respective anthocyanins (Ribéreau-Gayon et. al., 2006)

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. 2.2) and acylated monoglucoside anthocyanins (fig. 2.3) were identified (Ribéreau-Gayon et. al., 2006).

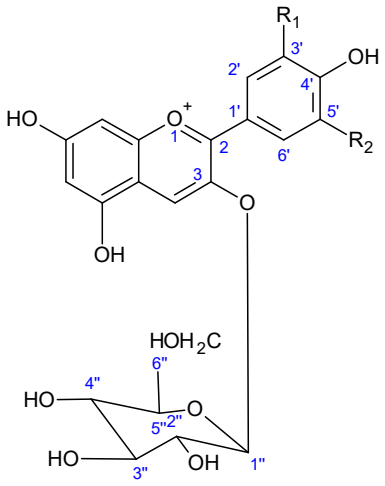


Figure 2.2 - Molecular structure of anthocyanin-3-monoglucoside.

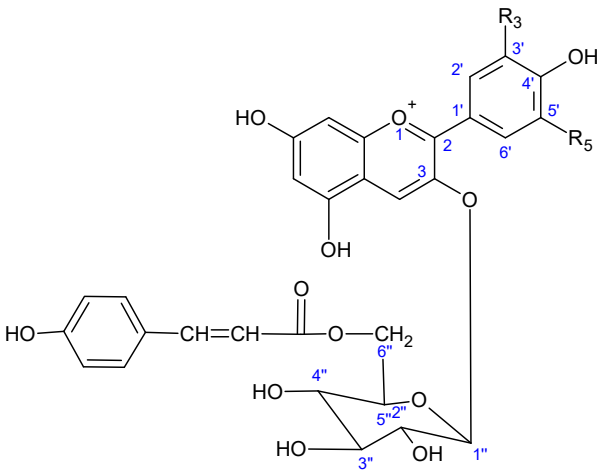


Figure 2.3 - Molecular structure of anthocynin-3-monoglucoside acylated by *p*-coumaric acid in carbon 5".

The structure of anthocyanins is pH dependent. Below pH 3.2 there are two indistinct interconvertible forms, the red flavilium cation and the blue quinoidal. At pH 1.5 ninety-six percent of anthocyanins are in the flavilium cation and to pH 2.5 sixty-seven percent in the flavilium cation. Above pH 2 there are several peak broadening because the slow interconversion between species (Košir et. al., 2004). This variation with pH is represented in figure 2.4 with species *Vitis vinifera* in wine.

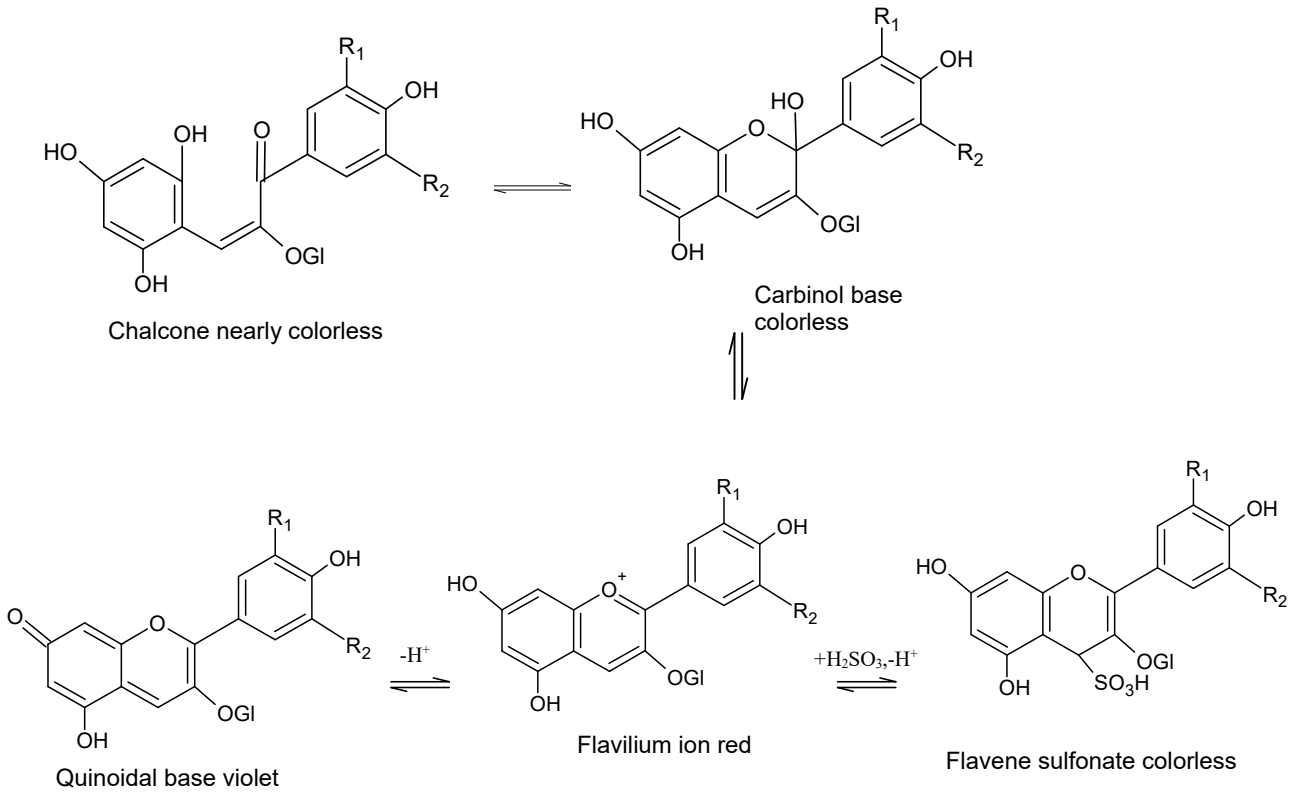


Figure 2.4 - Structure and equilibria of the anthocyanins present in *Vitis vinifera* species in wine with pH (Košir et. al., 2004).

2.1.2. Flavonols.

Flavonols are a subclass of flavonoids, the most common are quercetin, kaempferol, myricetin and isorhamnetin or quercetin-3-methylether (Makris et. al., 2006; Silva et. al., 2012). Their color vary from white to yellow and the molecular structure are presented in figures 2.5 to 2.8 (Makris et. al., 2006).

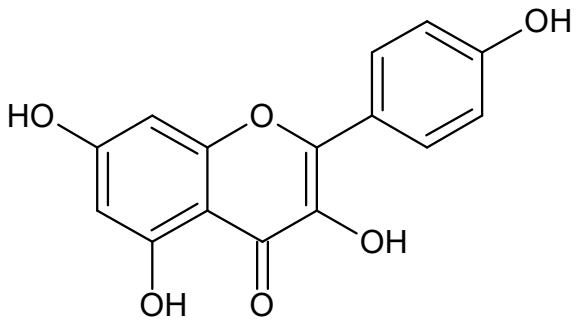


Figure 2.5 - Molecular structure of kaempferol.

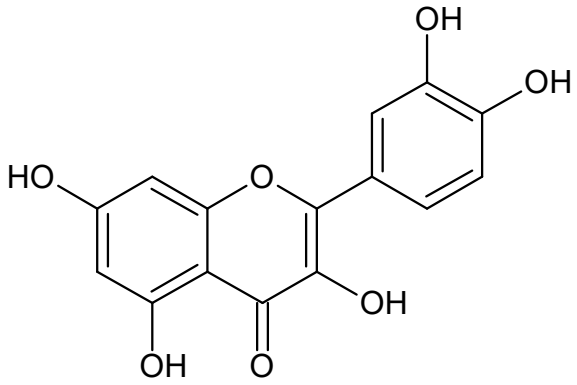


Figure 2.6 - Molecular structure of quercetin.

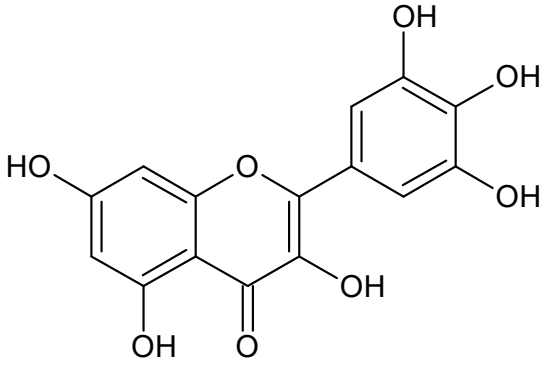
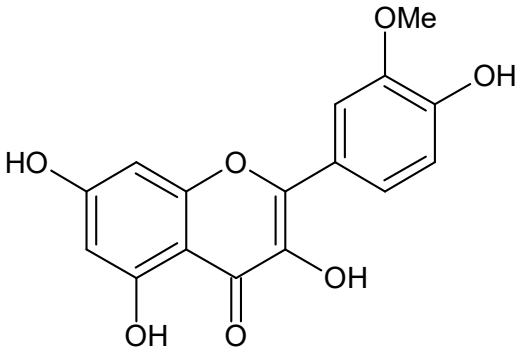


Figure 2.7 - Molecular structure of myricetin.



Me – methylethyl radical

Figure 2.8 - 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 (Makris et al., 2006).

The basic structure rings and with the convention labelling is presented in figure 2.9 (Makris et. al., 2006).

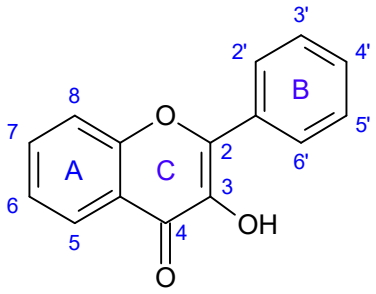


Figure 2.9 - Basic ring structure of flavonols and convention labelling.

Currently there is much interest in the study of flavonols because of its antioxidant potential, anti-inflammatory, anti-allergic, hepatoprotective, anti-viral, anti-carcinogenic (Silva et. al., 2012).

2.1.3. 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 (González-Manzano et. al., 2004). 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 (González-Manzano et al., 2004).

The basic unit of flavan-3-ols are catechin, epicatechin and their isomers present in the figures 2.10 and 2.11, and the nomenclature present in the tables 2.2 and 2.3. 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 (Ribéreau-Gayon et al., 2006).

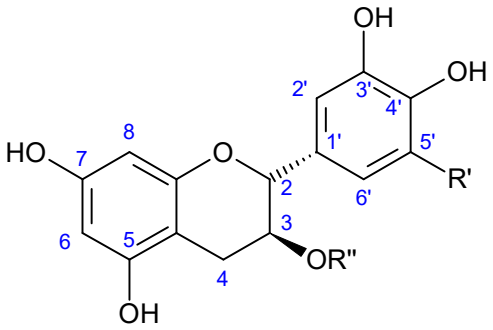


Figure 2.10 - Molecular structure of catechin series.

Table 2.2 - Nomenclature of catechin (Ribéreau-Gayon et. al., 2006).

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

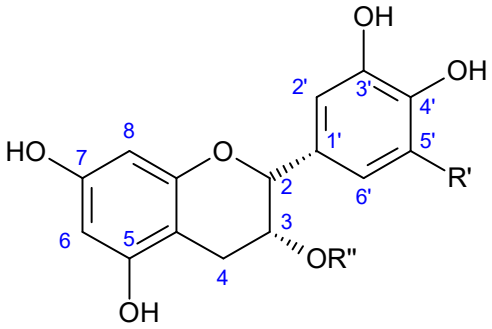


Figure 2.11 - Molecular structure of epicatechin series.

Table 2.3 - Nomenclature of epicatechin (Ribéreau-Gayon et. al., 2006).

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 2.12 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 (Lorrain et. al., 2013; Ribéreau-Gayon et. al., 2006).

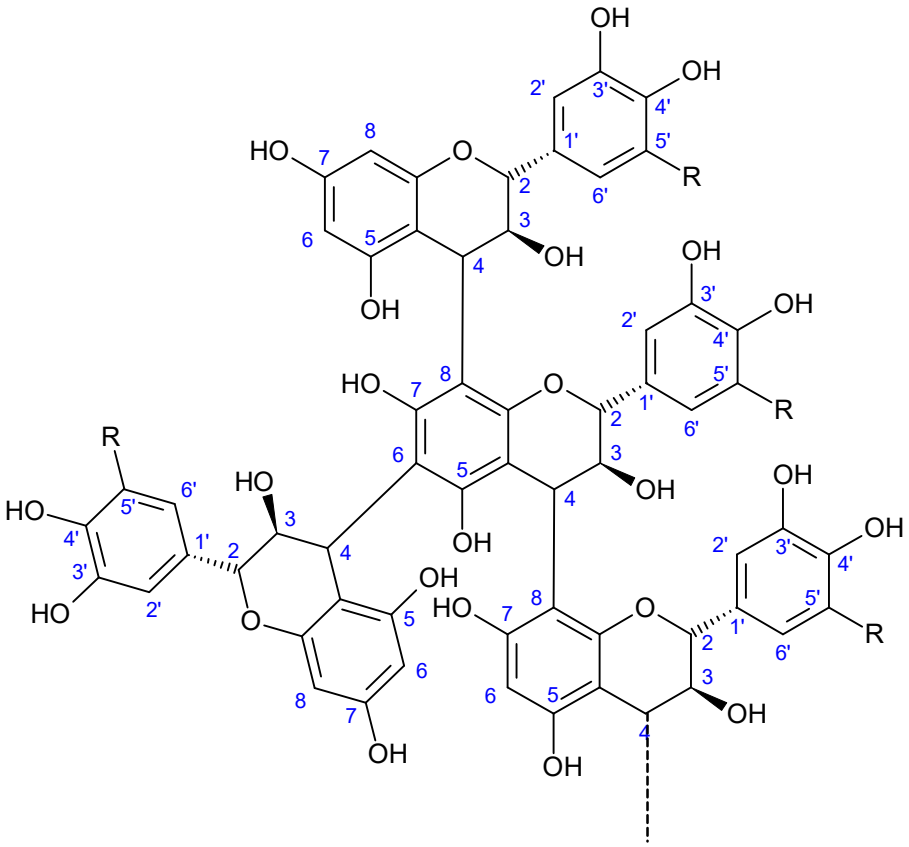


Figure 2.12 - General structure of proanthocyanidins (Lorrain et. al., 2013).

2.1.4. 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 2.13 a) and b) and the derivatives are presented in table 2.4 (Ribéreau-Gayon et. al., 2006).

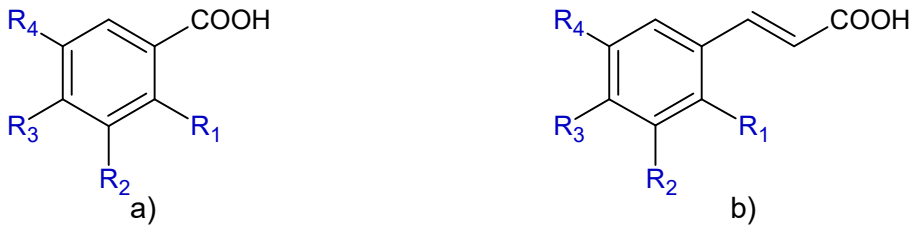


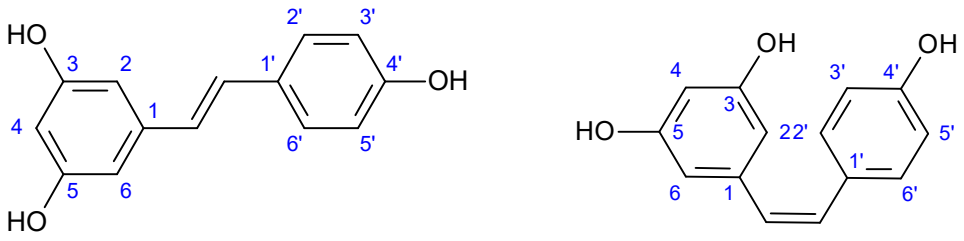
Figure 2.13 - Molecular structure of a) benzoic acid and b) cinammic acid.

Table 2.4 - 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	

2.1.5. 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 (Kostadinović et al., 2012). Resveratrol can occur in two isomeric forms, *cis* and *trans*, as shown in figure 2.14 a) and b).



a) b)

Figure 2.14 - Molecular structure of a) *cis* and b) *trans*-3,5,4'-trihidroxystilbene (resveratrol) (Wu, et. al., 2013).

3. SULFUR COMPOUNDS IN WINES

3.1. INTRODUCTION TO SULFUR COMPOUNDS IN WINES

The sulfur compounds are parts of a large group of compounds which affect the sensorial quality of the wines. The majority contributes to unpleasant characteristic in wines but there are other like 3-mercapto-1-hexanol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate, 3-mercapto-3-methyl-1-butanol which have a great impact (Capone et. al., 2011; Moreira et. al., 2010; Ribéreau-Gayon et al., 2006). The threshold values of these compounds are very low, producing strong olfactory impact in wines even if the concentration are low (Mestres et. al., 2000). There are many kinds of sulfur aroma in wine, pleasant like passion fruits, grapefruits and coffee; and unpleasant like rotten eggs, onion, garlic, which indicated bad storage conditions or a deficient production process (Capone et. al., 2011; Mestres et. al., 2000; Mora et. al., 1986).

The sulfur compounds can be classified by molecular structure in thiols, sulfides, polysulfides and heterocycle compounds. In wine, they are split in two groups according with their volatilities: the volatiles or light sulfur compounds, those with boiling point below 90 ºC and the less volatiles or high sulfur compounds with boiling point above 90ºC (Mestres et. al., 2000). The volatiles have low perception values but because their volatility can be eliminated by aeration, racking or by a copper treatment (Moreira et. al., 2004). Otherwise, the high sulfur cannot be eliminated by an easy process remaining in the products therefore affecting the wine quality.

The sulfur compound's formation mechanism it is not very understood, but some authors suggest the formation by two processes involving the enzymatic and non-enzymatic process. The enzymatic process results from sulfur-containing amino acids degradation like methionine and cysteine; metabolism of pesticides and formation of fermentation products. The non-enzymatic involves chemical, photochemical and thermal reaction during winemaking and storage (Berger & Media, 2007; Fedrizzi et. al., 2007; Landaud et. al., 2008; Mestres et. al., 2000). The figure 3.1 is an explanation of the sulfur compound's mechanism formation with methionine (Landaud et. al., 2008; Moreira et. al., 2008; Wang et. al., 2003).

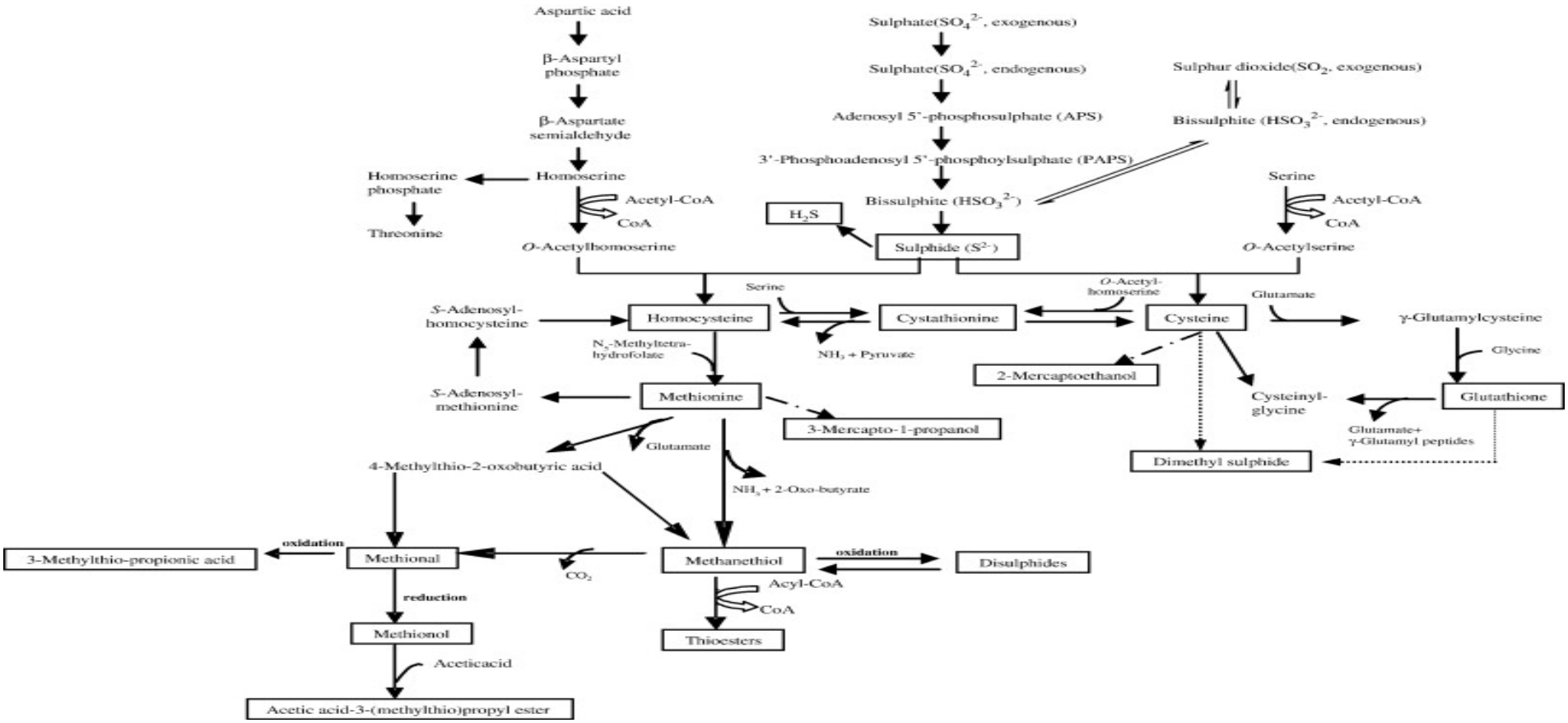


Figure 3.1 – Mechanism of formation of sulfur compounds in wines (Landaud et. al., 2008; Moreira et. al., 2008; Wang et. al., 2003).

There are many heavy sulfur compounds identified in wine with different odor, including 2-mercaptoethanol (boxer, farmyard, poultry smell), methylthioethanol (french bean aroma), 3-methylthio-1-propanol (methionol) (potato, soap, cauliflower, cooked cabbage aroma), 4-methylthio-1-butanol (onion, garlic, earthy aroma), 2-methyltetrahydrothiophen-3-one (metallic, natural gas aroma), *cis*-2-methyltetrahydrothiophen-3-ol (odorless), *trans*-2-methyltetrahydrothiophen-3-ol (onion, chive-garlic odor) benzothiazol (rubber odor), 3-methylthiopropionic acid (butter, rancid odor) and dimethylsulphone (odorless) (Mestres et. al., 2000; Landaud et. al., 2008; Moreira et. al., 2010).

On the other side, there are sulfur compounds with pleasant odor like 3-mercapto-1-hexanol (grapefruit flavor, passion fruit), 3-mercaptohexyl acetate (boxwood, passion fruit), 4-mercapto-4-methyl-1-butanol (citrus zest) identified in Sauvignon blanc wines (Capone et. al., 2011; Moreira et al., 2010; Ribéreau-Gayon et. al., 2006).

In the tables 3.1 and 3.2 are presented the molecular structure of heavy sulfur compounds, their odor, threshold values and concentration founds in wines.

Table 3.1 - Molecular structure of heavy sulfur compounds in wines (Mestres et. al.,2000)

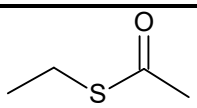
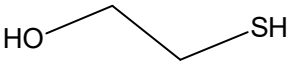
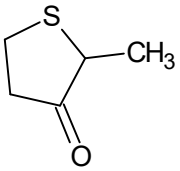
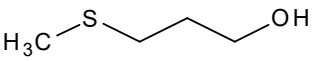
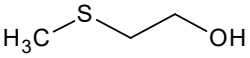
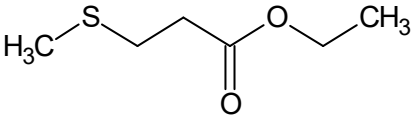
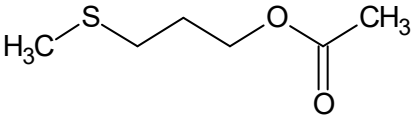
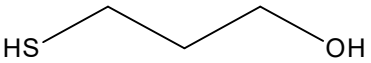
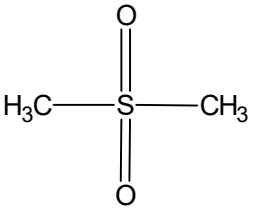
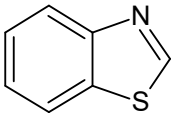
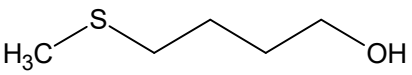
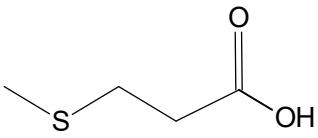
Sulfur compounds	Molecular structure
S-Ethylthioacetate	
2-Mercaptoethanol	
2-Methyltetrahydrothiophen-3-one	
3-Methylthio-1-propanol	
2-Methylthioethanol	
Ethyl-3-methylthiopropionate	
3-(Methylthio)propyl acetate	
3-Mercapto-1-propanol	
Dimethyl sulfone	
Benzothiazole	
4-Methylthio-1-butanol	
3-Methylthio-1-propionic acid	

Table 3.2 - Odor, threshold values and concentration of heavy sulfur compounds in wines (Mestres et. al., 2000; Moreira et. al., 2011; Ye et. al., 2016).

Sulfur compound	**Odor/flavour**	**Threshold values ($\mu g.L^{-1}$)**	**Wine concentration ($\mu g.L^{-1}$)**
2-Mercaptoethanol	Box tree, poultry, farmyard, burnt rubber	600 in red wine 450 in white wine	ND - 400
2-Methyltetrahydrothiophen-3-one	Metallic, natural gas, butane-like	250 in red wine 150 in white wine	14.8 - 237
3-Methylthio-1-propanol (Methionol)	Cooked potato, cauliflower, cabbage	3200 in red wine 4500 in white wine	224 - 5655
2-(Methylthio)ethanol	French been, cauliflower	640 in red wine 800 in white wine	25 - 98
Ethyl-3-methylthiopropionate	Sulfurous, metallic	300 - 1000 wine	0 - 10
3-(Methylthio)propyl acetate	Mushroom, onion, garlic	115 in red whine 100 in white wine	0 - 17
3-Mercapto-1-propanol	Sweat odor, roasted, potato, broth	60 in model solution	*
Dimethyl sulfone	Odorless	-	-
Benzothiazole	Rubber	-	0 - 6
4-Methylthiobutanol	Metallic-bitter, grassy, onion, chive-garlic	80 in wine 100–1000 in hydroalcoholic solution	ND - 181
3-Methylthio-1-propionic acid	Chocolate, roasted, butter, rancid	50 in model solution; 244 in red wine	1 - 140

ND – not detected;

4. VOLATILES AROMA COMPOUNDS IN WINES

4.1. INTRODUCTION TO VOLATILES AROMA COMPOUNDS IN WINES

Wine volatiles comprise several compounds of different chemical classes with a large range of concentration between $ng.L^{-1}$ to $mg.L^{-1}$. Several volatiles compounds such as alcohols, terpenes, hydrocarbons, esters, ketones, acids, aldehydes, ethers, sulfur, nitrogen and lactones were identified in wines (Barros et. al., 2012). They play a very important role in wine flavor which depends on the correlation between chemical composition and perception threshold since many volatiles compounds have a concentration below of their sensory threshold (Vilanova & Sieiro, 2006). Many of these compound confer a floral, fruity and citrus attributes to wines which is very important to wine qualities (Ugliano & Henschke, 2009).

4.1.1. Terpenes

Terpenes belong to important class of volatiles compounds in wine because of their high concentration and low aroma threshold (Vilanova & Sieiro, 2006). They are from grape, also formed during maturation and influenced by the characteristics of soil, climate and viticulture processes (Michlmayr et. al., 2012; Vilanova & Sieiro, 2006).
The most predominant monoterpenes in white wine Muscatel are linalool, geraniol, nerol, α-terpeniol, β-citronellol and hotrienol (Dziadas & Jeleń, 2010; Mateo & Jiménez, 2000; Rocha et. al., 2007; Takoi et. al., 2010). Typical floral aroma of these compounds are rose-like (geraniol, nerol), coriander (linalool), camphoraceous (linalool oxides) and green (nerol oxides) (Marais, 1983; Rocha et. al., 2007). Monoterpenes can occur in wine and grapes in free form or bound with sugar as glycosides and these glycosides form are the more abundant (Dziadas & Jeleń, 2010; Mateo & Jiménez, 2000).

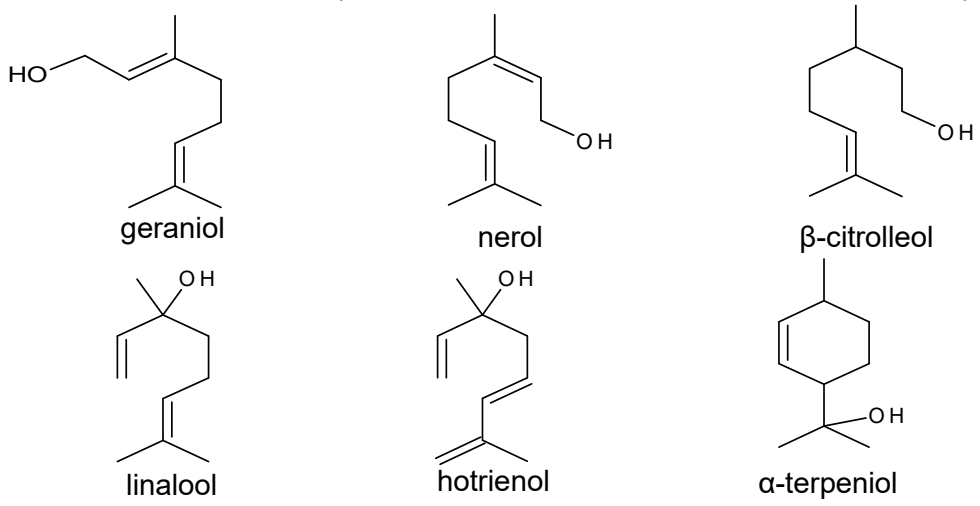


Figure 4.1 - Molecular structure of monoterpenes in wines Muscat (Takoi et al., 2010).

4.1.2. Nor-isoprenoids

Nor-isoprenoids are substances formed by degradation of carotenoid such as β-carotene, lutein, neoxanthin and violaxanthin, released by hydrolysis of glycosides molecules during winemaking or aging processes (Silva Ferreira & Guedes de Pinho, 2004; Vinholes et. al., 2009). The nor-isoprenoids compounds identified in wines were β-damascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and vitispirane (Fig. 4.2) (Silva Ferreira & Guedes de Pinho, 2004). Like so many others volatiles compounds, these compounds have an important role in sensorial wine aroma because of their low olfactory perception threshold and a pleasant odor descriptor related to tea, violet, exotic flowers, stewed apple, eucalyptus and camphor (Mendes-Pinto, 2009; Vinholes et. al., 2009).

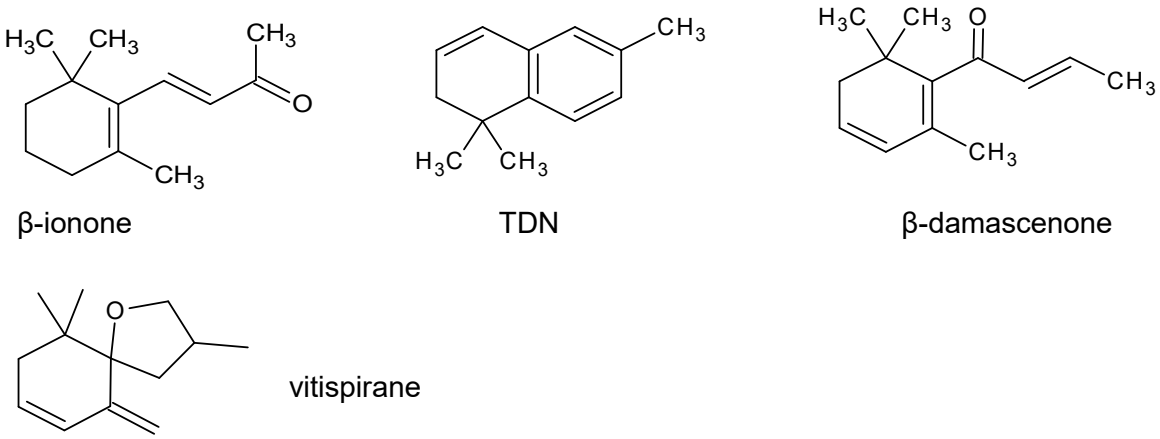


Figure 4.2 - Chemical structure of some nor-isoprenoids detected in wines.

4.1.2. Esters

Esters are very common in wines, they are formed in wine by two ways, enzymic esterification during fermentation and chemical esterification during aging steps (Ribéreau-Gayon et. al., 2006). Ethyl esters like acetates are formed enzymically during fermentation and are very important wine aroma (Roussis et. al., 2005). Because of their fruity fragrance, many esters are denominated “fruity esters” principally those who have low molecular weight. Others like ethyl esters of hexanoic, octanoic and decanoic acids, isoamyl acetate and isobutyl acetates are often considered to give wine much of its vinous fragrance (Roussis et. al., 2005).

In the table 4.1 are presented some esters and others volatile compounds with their odor characteristics (Vilanova et. al., 2010).

Table 4.1 - Characteristics of some volatile compounds in wine (Vilanova et. al., 2010)

Volatile compounds	Odor descriptor	Odor threshold/ $\mu g.L^{-1}$
Alcohols		
1-propanol	-	750000
2-methyl-1-propanol	Álcohol, banana, solvent	65000
1-butanol	Álcohol, fusel	150000
3-methyl-1-pentanol	-	-
2-phenylethanol	Rose, sweetish	10000
1-Hexanol	Vegetable, grass	800
Ethyl esters		
Ethyl butyrate	Papaya, sweetish, butter	20
Ethyl 2-methylbutyrate	Fruity	18
Ethyl 3-methylbutyrate	Fruity, apple	3
Ethyl hexanoate	Fruity, apple, sweetish	14
Ethyl lactate	Strawberry, raspberry	154700
Ethyl octanoate	Fruity, apple	5
Ethyl decanoate	Fruity, apple, solvent	200
Acetates		
3-Methylbutyl acetate	Banana, apple, estery	30
Hexyl acetate	Sweetish, perfumed	670
2-Phenylethyl acetate	Rode, honey, tobacco	250
Volatile fatty acids		
2 + 3-methylbutyrate	Cheese, oldhops, sweaty	34
Butyric acid	Rancid, cheese	173
Hexanoic acid	Geranium, vegetable	30
Octanoic acid	Sweat, cheese	500
Decanoic acid	Rancid, fat	1000
Dodecanoic acid	Soapy, waxy	6100
Monoterpenes		
Linalool	Flower, lavander	25
α-terpineol	Pine, lily of the valley	250
Citronelol	Green lemon	100
Nerol	Rose, lime	400

5. CHEMOMETRICS ANALYSIS

Chemometric analysis is an important tool when we have results with many samples and variables, from them extract significant and useful information. This allows to simplify the results and facilitate its analyses. The field of application is very wide, as example, signal processing, experimental design, optimization, data mining, multivariate calibration and classification (Moncayo et. al., 2015).

Rergading classification methods, the chemometric analysis comprises several statistic methods which can be grouped in unsupervised and supervised methods. In unsupervised methods there are no prior assumed classification model over the data in a matrix, while in supervised methods are defined by two data sets, objects (input) and classes (target) (Moncayo et. al., 2015). In unsupervised methods the sample gives the algorithm without information that belong to any class, but in supervised methods, data training samples and to perform the models and output of cases, are used training samples (Martelo-Vidal & Vázquez, 2014).

In unsupervised chemometric methods, the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are the most popular methods (Jiang et. al., 2015; Yi et. al., 2015; Zhao et. al., 2014). The Linear Discriminant Analysis (LDA) is one of supervised method of classification (Azcarate et. al., 2013; Zhao et. al., 2014).

These methods try to discovery a relationship between classes and objects, referred as a model, which represents a set of features that define the classification process. The membership of new objects (unknowns for the models) is predicted on the basis of their similiarity to a certain class in the model (Moncayo et. al., 2015).

In the wines, there are many studies using chemometrics methods like PCA, LDA, HCA to distinguish or to classify wines from diferent regions or grapes varieties (Versari et. al, 2014).

5.1. PRINCIPAL COMPONENTS ANALYSIS (PCA)

The PCA attempted to reduce the dimensions of an initial multivariate dataset to a smaller number of uncorrelated variables with the maximized variances, that permits the analysis of a dataset using the most important variables. Its not useful for discriminating classes as it just provides an overview of the overall data without taking into account the class information to build the model. Its usually coupled with other chemometrics

methods (LDA, SIMCA, etc) in order to achieve a classification model (De Andrade, Do Nascimento, Pereira, Hallwass & Paim, 2013).

PCA involves a transformation of the data represented in the follow equation:

$$\boldsymbol{X} = \boldsymbol{TP} + \boldsymbol{E}$$

Where $\boldsymbol{X}$ is the original data matrix of dimension *I x J*. *I* is objects and *J* variables. The individual variables (columns) of $\boldsymbol{X}$ are denoted by $\boldsymbol{x_j}$ and are all vector in the *I*-dimensional space (Moncayo et al., 2015).
A linear combination of those $\boldsymbol{x}$ variables can be written as $t = \omega_1 x_1 + \quad + \omega_j x_j$ where ω are the weight of variables. $\boldsymbol{T}$ is score matrix with dimension *I x A*, where A is the number of principal components (PC) considered. $\boldsymbol{P}$ is the loading matrix with *A x J* dimension, where each vector $\boldsymbol{p_A}$ contains the regression coefficient. $\boldsymbol{E}$ represent the matrix of residuals. The principal component is defined for the pair of eigenvector $\boldsymbol{t}$ and $\boldsymbol{p}$ (Moncayo et al., 2015).

5.2. LINEAR DISCRIMINANT ANALYSIS (LDA)

LDA classification model is created on the basis of the estimation of several discriminant functions, which are linear combinations of the original variables, minimizing the variances within-class S_w and maximizing the variance between classes S_b:

$$S_w = \sum_{i=1}^{n} \sum_{x \in c_i} (x \quad \mu_i)(x_j \quad \mu_i)^T$$

$$S_b = \sum_{i=1}^{n} n_k (\mu_i \quad \mu)(\mu_i \quad \mu)^T$$

Where n and n_k are the number of classes and the number of training objects for each classes, x is each class object, μ_i is the means for each class and μ is the total mean vector. The model takes into account different variances of each variable and also the correlation between variables. The prediction results for the validation set is obtained projecting each unknown object on the discriminant functions and these are always assigned to a single class according to the minimal distance to the centroid of each class (Moncayo et al., 2015).

5.3. HIERARCHICAL CLUSTER ANALYSIS (HCA)

HCA is a multivariate approach that aims to identify natural groups or clusters among objects in a dataset, through minimization of the within-cluster variance and maximization of the between cluster variance (Bayo & Lopez-Castellanos, 2016). This method characterizes similarities among samples by examining interpoint distances representing all possible samples pairs in high dimensional space. The sample similarities are represented on two dimensional diagrams call dendograms (Lima et. al., 2010).

The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. In the single linkage method, the distance or similarities between two clusters A and B is defined as minimum distance between a point A and B (Patras et. al., 2011).

$$D(A,B) = min\{d(y_i, y_j), \text{ for } y_i \text{ in A and } y_j \text{ in B}\}$$

Where $d(y_i, y_j)$ is the Euclidean distance in the equation.

6. ANALYSIS OF COMPOUNDS IN WINES OF FOGO ISLAND

6.1. ANALYSIS OF PHENOLIC COMPOUNDS IN WINES OF FOGO ISLAND

6.1.1. Methods of analysis

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) (Marquez et. al., 2012).

6.1.2. Chemicals and materials

The compounds used in the study were (CAS number in brackets) malvidin-3-*O*-glucoside chloride (7228-78-6), (±)-catechin trihydrate (7295-85-4), *t*-ferulic acid (537-98-4, Aldrich), *p*-coumaric acid (501-98-4, Sigma), gallic acid monohydrate (5995-86-8, Sigma-Aldrich), trihydrate caffeic acid (331-39-5), syringic acid (530-57-4), vanilic acid (121-34-6) and quercetin (117-39-5), all purchased from Sigma-Aldrich and Janssen Chimica. The solvent used were acetonitrile, ethyl acetate, deionized water, formic acid and methanol. All standard and solvent used were analytical grade. The SPE Supelclean cartridge LC-18 6 mL was purchased from Sigma-Aldridge.

6.1.3. Preparation of standard solutions

The standard solution was prepared dissolving individuals weighted standard in methanol at 1000 $mg.L^{-1}$ of concentration. The standard solution was protected from light and maintained at -10ºC. The works solutions were prepared in 12% hydroalcoholic standard solution with 3.5 $g.L^{-1}$ of tartaric acid and pH 3.5 adjusted with NaOH 0.1 M.

6.1.4. Samples

The wines samples were Chã (white and red), Montrond (white and red), Sodade (white, red and rose) and Sangue de Vulcão. All wines were from Fogo Island and each one was randomly chosen three samples. The samples analysed were from different producers but all from the same Island.

6.1.5. Procedure

6.1.5.1. Anthocyanins extraction by Solid Phase Extraction

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. (2012). 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.

6.1.5.2. Non-anthocyanic compounds extraction by Liquid Liquid Extraction

The extraction of non-anthocyanics compounds was done according to the method proposed by Porgali & Büyüktuncel (2012). 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.

6.1.6. 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.

6.2. ANALYSIS OF HEAVY SULFUR COMPOUNDS IN WINE

6.2.1. Method of analysis

The analysis of sulfur compounds in wine was carried out by gas chromatography with flame photometric detector (GC-FPD). The method applied was proposed by Moreira et. al. (2004) with liquid liquid extraction and analysis by GC-FPD. This detector has a particularity and an advantage of detecting only sulfur compounds in the samples.

6.2.2. 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.

6.2.3. 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). The samples analysed were from different producers but all from the same Island.

6.2.4. 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.

6.2.5. Liquid liquid extraction

The internal standard was added to 50 mL of wine sample or standard solution at 30 $\mu g.L^{-1}$. Four grams of anhydrous sodium sulphate was added to the samples and extracted twice with 5 mL of dichloromethane for 5 min. The organic phases were mixed and 2 mL of extract was concentrated to 1/10 under nitrogen flow. Two microliters of concentrated extract were injected into the chromatograph.

6.2.6. GC-FPD conditions

Analyses were carried out on a Hewlett-Packard (HP) 5890 gas chromatograph, equipped with a ame photometric detector (FPD), and the HP Chemstation software was used. The FPD used an interference lter set at 394 nm. The extract was injected in the splitless mode for 0.3 min, into a CP-WAX 58(FFAP)-CB column (Chrompack) of 30 m × 0.32 mm and 0.2 µm phase thickness. The oven temperature programme start at 50 ºC to 220 ºC (40 min) at 2 $ºC.min^{-1}$. The injector and detector temperatures were 250 ºC. The carrier gas used was hydrogen at 1–2 $mL.min^{-1}$. The FPD used hydrogen at 90 $mL.min^{-1}$, air at 100 $mL.min^{-1}$ and make up gas (nitrogen) at 20 $mL.min^{-1}$.

6.2.7. Calibration curve and limit of detection

The FPD response is a power function between peak area and concentration. Since the response for all sulfur compounds was nearly quadratic, the Hubaux-Vous limit detection was applied (Catalan et. al., 2006). The graph was plotted by square root of ratio between peak area of analyte with internal standard, $(A_{analyte}/A_{IS})^{1/2}$, versus concentration and the determination coefficient were good for all compounds. The limit of detection (LOD) was expressed as $3.3SD/S$, 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 (ICH, 2005). The calibration curve was evaluated by coefficient of determination R^2.

6.3. ANALYSIS OF VOLATILES COMPOUNDS IN WINE

6.3.1. Method of analysis

The analysis of all volatile compounds in wine was made by HS-SPME-GC-MS/IT, headspace solid phase microextraction and gas chromatography with mass spectrometry/ ion trap, a method optimized by Barros et. al. (2012). All calibration curve parameters were set up by Barros et. al. (2012).

6.3.2. Materials and chemicals

The volatile compounds studied were (CAS number in brackets): limonene (5989-54-8, Fluka), *cis*-linalool oxide (5989-33-3, Fluka), terpinolene (586-62-9, Aldrich), β-linalool (78-70-6, Sigma), β-terpineol (138-87-4, Sigma), α-terpineol (98-55-5, Sigma), nerol (106-25-2, Aldrich), geraniol (106-24-1, Sigma), α-ionone (6901-97-9, Aldrich), neryl acetate (141-12-8, Aldrich), β-ionone (6901-97-9, Aldrich), nerolidol (7212-44-4, Aldrich), ethyl butanoate (105-54-4, Merck), ethyl hexanoate (123-66-0,Sigma), hexyl acetate (142-92-7, Merck), diethyl succinate (123-25-1, Merck), ethyl octanoate (106-32-2, Merck), phenylethyl acetate (103-45-7, Merck) and phenylethyl alcohol (60-12-8, Sigma). A hydrocarbon mixture C_6–C_{20} was obtained from Fluka. NaCl and NaOH were purchased from Merck. The SPME fiber used was divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm (DVB/CAR/PDMS) purchased from Supelco.

6.3.3. Samples wine

The samples wine from Cape Verde were Chã (red and white), Sodade (red, white and rose), Montrond (red and white) and Sangue de Vulcão (red).

6.3.4. Chromatographic conditions

GC-IT/MS analysis were performed on a Varian CP-3800 gas chromatograph (USA) equipped with a Varian Saturn 4000 ion trap mass detector (USA), a Saturn GC-IT/MS workstation software version 6.8, a Combi-PAL autosampler (Varian Pal Autosampler, Switzerland) and the Cycle Composer software (CTC Analytics System Software, Switzerland). Chromatographic separation was achieved using a capillary column VF-5 ms (30 m x 0.25 mm x 0.25 μm) from Varian and a high purity helium C-60 (Gasin, Portugal) as carrier gas at a constant flow of 1.0 mL.min ^{1}, in splitless injection mode.

An initial oven temperature of 40 °C was held for 1 min, and then increased 5 °C.min ^{1} to 250 °C (5 min) followed to increase 5 °C.min ^{1} to 300 °C (10 min). The ion trap detector was set as follow: the transfer line, manifold, and trap temperatures were 280 °C, 50 °C and 180 °C, respectively. All mass spectra were acquired in the electron impact (EI). The mass range was 35–600 m/z, with a scan rate of 6 scan.s ^{1}. The emission current was 50 μA, and the electron multiplier was set in relative mode to auto-tune procedure. The analysis was performed in full scan mode (Barros et. al., 2012).

6.3.5. Procedure

Before the analysis the fiber was conditioned according to the manufacturer recommendation. Five millimeter of wine sample or standard was put in a vial of 20 ml with 0.5 g of NaCl. The wine sample was stirring at 250 rpm for 5 min at 45 ºC. Then the fiber was exposed to the headspace at 45 ºC for 20 min, under continuous stirring (250 rpm). The desorption time into GC injector was 2 min at 230 ºC.

6.4. STATISTICAL ANALYSIS

For all result, Tukey test was carried out with PAST software to verify statistically significant differences among mean values. The level of significance in the Tukey test was $\alpha = 0.05$. All chemometrics analyses and graphics presented were carried out with SPSS version 20 software.

7. PRESENTATION AND DISCUSSION OF RESULTS

7.1. PRESENTATION OF RESULTS FOR PHENOLIC COMPOUNDS

7.1.1. Calibration curves of standard solutions

The results for chromatogram of a standard mix solution are presented in the table 7.1 with the retention time, RT, and their wavelength absorption.

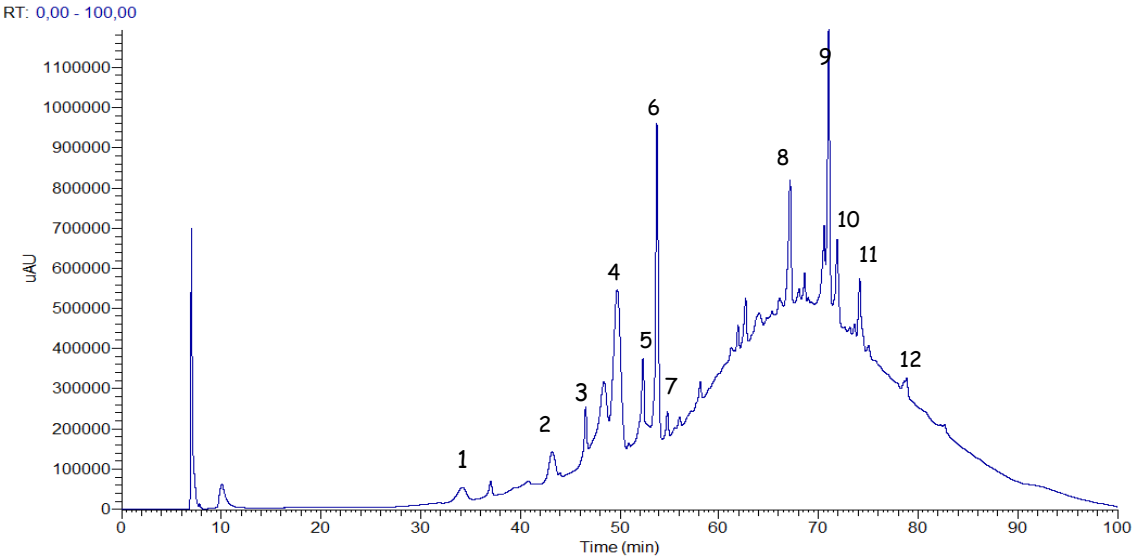
To each phenolic compound standard, calibration curve was determined by linear regression and the limit of detection (LOD) was estimated with the method proposed by ICH (2005). The LOD was expressed by *3.3*SD/S, S*, is the slope of the calibration curve and *SD* is the standard deviation of the response estimated by standard deviation of y-intercept of regression line. The table 7.1 presents the parameters of calibration curve of standard solutions. The second value of wavelength presented in the table correspond the maximum absorption spectrum.

Table 7.1 - Retention times, wavelength, concentration range, limit of detection, slope and intercept 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

7.1.2. Anthocyanins analysis in red wine

The figure 7.1 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 7.1 – 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 (Blanco-Vega et. al., 2014).With m/z, peak wavelengths and retention time values was possible to identify many anthocyanins present in the wines (Alcalde-Eon et. al., 2004; Alcalde-Eon et. al., 2006; Boido et. al., 2006; He et al., 2012).

In the table 7.2 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.

Three samples of each wine were analysed and the quantification are expressed as malvidin-3-glucose equivalents.

For each compounds, 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.

In the table 7.2 are present the absorption wavelength, mass spectral (MS), mean concentration and standard deviation of each compound in the wines samples.

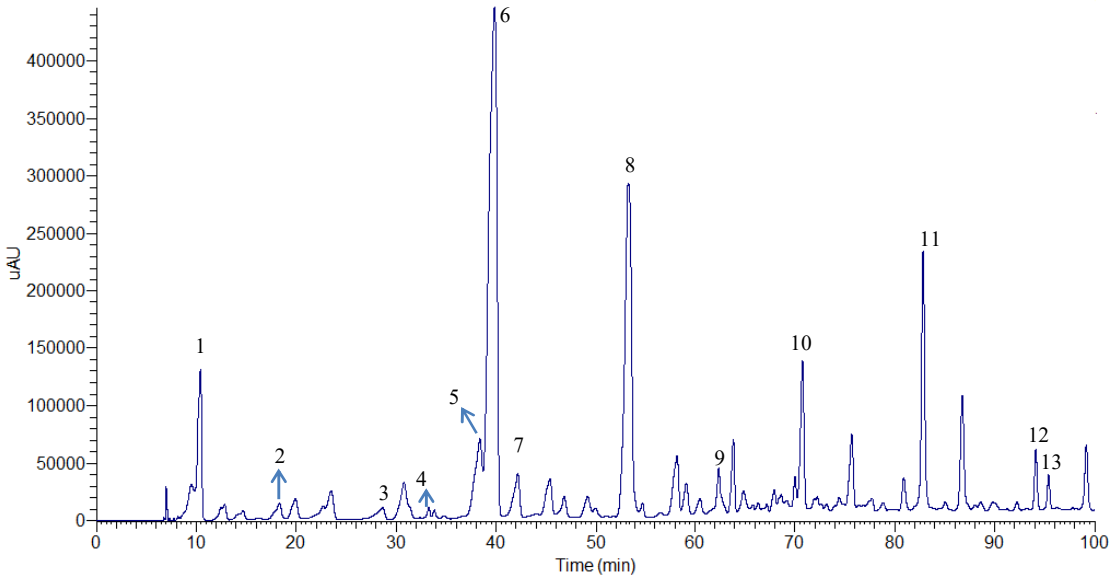
Table 7.2 – Absorption peak wavelengths, m/z of fragment and mean concentration with standard deviation (SD), mg.L^{-1}, of anthocyanins in red and rosé wines from Fogo Island.

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

7.1.3. 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 (Chen et. al., 2011; Figueiredo-González et. al., 2014).



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 7.2 - Chromatogram of Chã red wine extract for non-anthocyanic compounds at total scan.

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 6.3 and 6.4.

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 7.3 - 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 7.4 - 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.

7.1.4. Discussion of phenolic compounds 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 7.3 shows a graphic comparison of anthocyanins determined in all samples of red and rosé wines from Fogo Island.

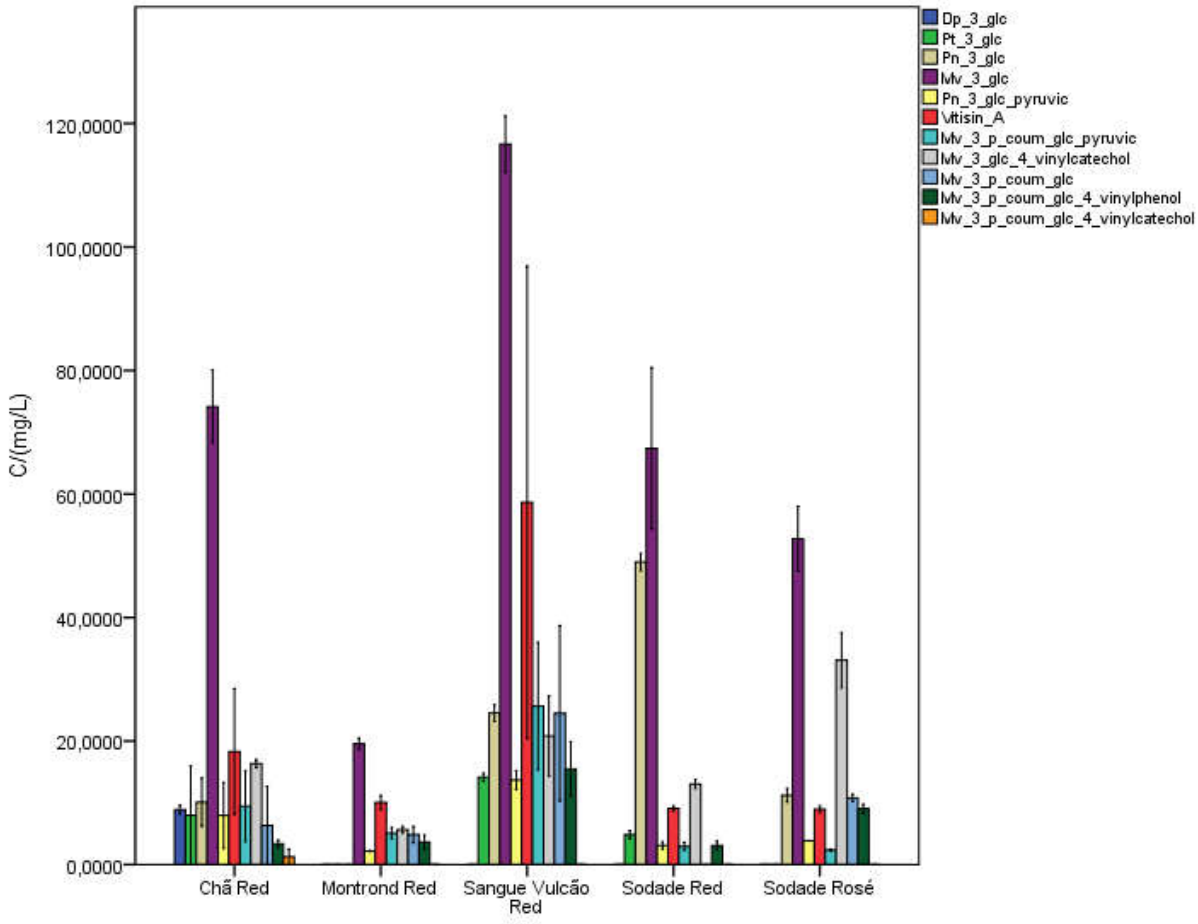


Figure 7.3 - Graphical comparison of anthocyanins mean concentration determined in red and rosé wines of Fogo Island.

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 (Ginjom et. al., 2011; Ivanova-Petropulos et. al., 2015).

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 (Morata et. al., 2007). The maximum and minimum values were founded in Sangue Vulcão and Montrond red wine with 13.7 ± 1.5 and 2.20 ± 0.10 mg/L of concentration. This compound determined in Montrond, Chã, Sodade red wines and Sodade rosé wine have no significant difference according to Tukey test.

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 (Morata et. al., 2007; Benito et. al., 2011). The main compounds detected are derived from caffeic acid (vinylcatechol compounds) and *p*-coumaric acid (vinylphenol compounds) present in wine samples (Benito et. al., 2011). 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 these compound in Montrond red wine, Chã red wine and Sodade rosé wine have 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 the majority of these compounds, except for gallic acid. Some compounds were not detected in some white wines samples as show the table 7.4.

White wines have always lower concentrations of phenolic compounds than red wines. This is because of the processing of the red wines, which are made with the skin of the grapes, which does not occur with the white wines.

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 (Ivanova-Petropulos et. al., 2015). 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 (Ginjom et. al., 2011; Ivanova-Petropulos et. al., 2015).

The concentration of vanilic acid determined in wines from Fogo Island are very higher compared with Turkey wines (Kelebek et. al., 2010). The same happens with syringic acid for wines produced in Turkey but compared with Australian red wines, their concentration are similar (Ginjom et. al., 2011).

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 (Ivanova-Petropulos et. al., 2015; Kelebek et. al., 2010). 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 (Ginjom et. al., 2011). 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 7.4 is a graphic representation of mean values of concentration for phenolic compounds non-anthocyanic in red wines samples.

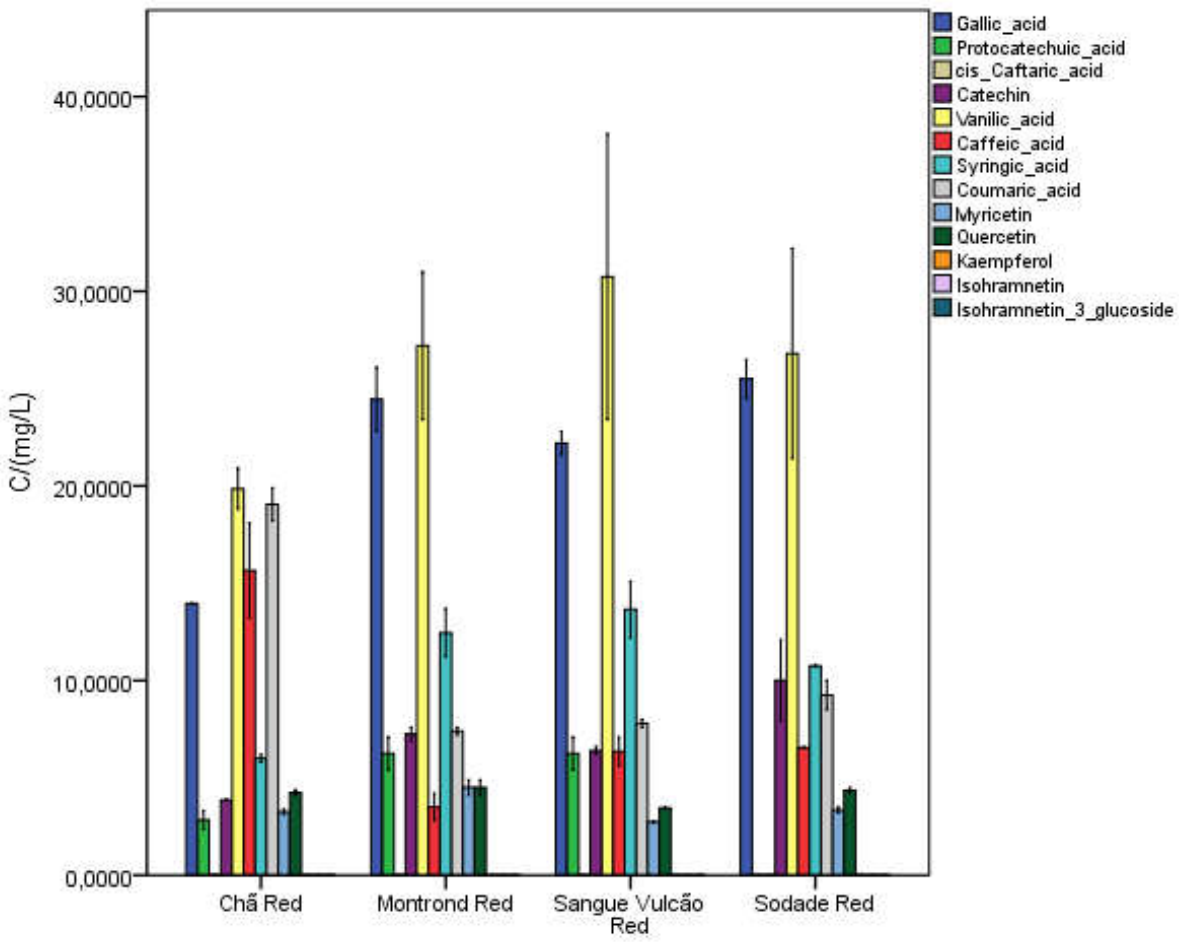


Figure 7.4 - Graphic comparison of mean concentration of non-anthocyanic compounds determined in red wines of Fogo Island.

7.1.5. Chemometric analysis for phenolic compounds in the wines

The PCA was not possible for phenolic compounds because the data were not enough. For phenolic compounds was made linear discriminant analysis and hierarchical cluster analysis.

The discriminant analysis is represented by the figure 7.5 which represent the plot of discriminant function for the wines.

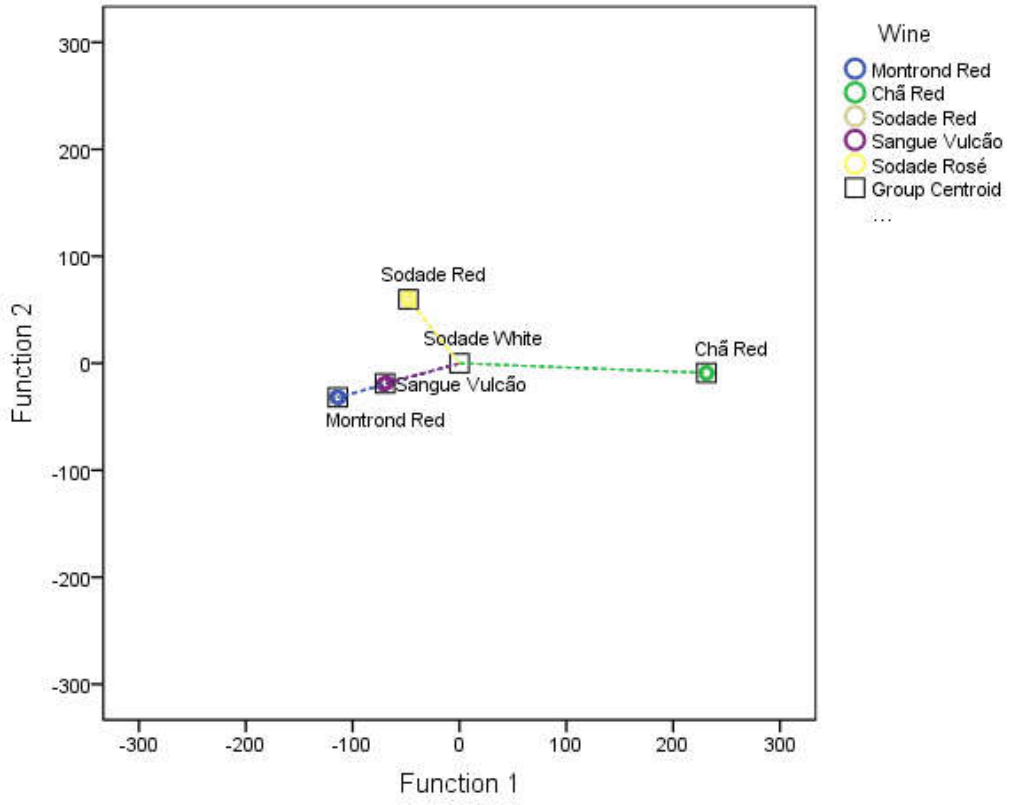


Figure 7.5 - 2D scatter plot of discriminant functions to four wine classification functions with phenolic compounds.

The figure 7.5 is a scatter plot of the two discriminant functions and it show a good separation of the four red wines of Cape Verde mainly the Chã and Sodade red wines. The Montrond and Sangue Vulcão red wines are almost similar according to the graphical representation.

For the phenolic compounds according to the results present in the table 7.5, the variables which most contributed to the discriminant model were, Dp-3-glc, Pt-3-glc, Pn-3-glc and Vitisin A.
These results are present in the tables 7.5 and 7.6 with the values of coefficients for each variables discriminating.

Table 7.5 - Classification function coefficients for phenolic compounds

	Wine			
	Montrond Red	Chã Red	Sodade Red	Sangue Vulcão
Dp-3-glc	-394.540	12101.005	1945.608	1184.492
Pt-3-glc	3.127E-010	865.152	261.302	133.964
Pn-3-glc	-8.685	407.601	225.553	62.198
Vitisin A	30.397	-302.772	-14.820	4.402E-010
(Constant)	-154.131	-56599.483	-6093.661	-949.185

Fisher's linear discriminant functions

Three discriminating functions were gerated but the first two functions have 100% of variance and the majority of eigenvalue.

Table 7.6 - Standardized canonical discriminant function coefficients for phenolic compounds

	Function		
	1	2	3
Dp-3-glc	12.695	-0.190	-0.750
Pt-3-glc	9.758	4.228	6.141
Pn-3-glc	2.257	3.669	-0.127
Vitisin A	-5.081	0.898	6.672

The classification matrix presented on the table 7.7 shows that 100% of total samples were correctly classified.

Table 7.7 - Classification matrix for phenolic compounds.

		Wine	Predicted Group Membership				Total
			Montrond Red	Chã Red	Sodade Red	Sangue Vulcão	
Original	Count	Montrond Red	3	0	0	0	3
		Chã Red	0	3	0	0	3
		Sodade Red	0	0	3	0	3
		Sangue Vulcão	0	0	0	3	3
	%	Montrond Red	100	0	0	0	100
		Chã Red	0	100	0	0	100
		Sodade Red	0	0	100	0	100
		Sangue Vulcão	0	0	0	100	100

The cluster analysis of wines with centroid clustering method and squared Euclidean distance is represented by dendogram in the figure 7.6.

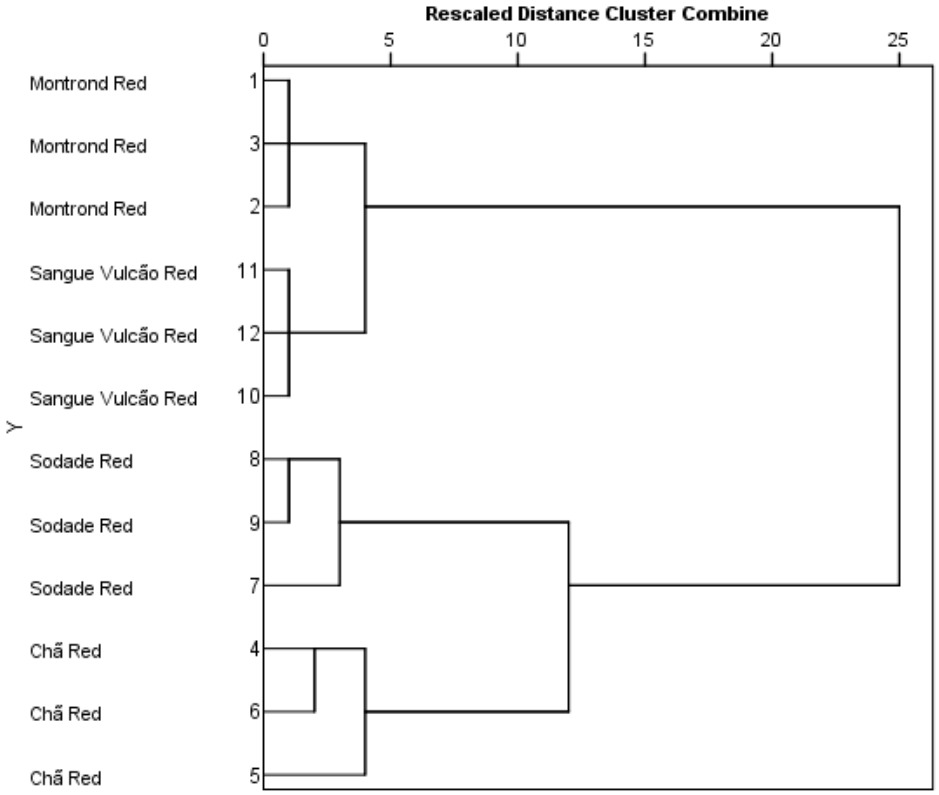


Figure 7.6 – Dendogram of cluster analysis obtained with phenolic compounds in the wines.

The analysis of dendogram it is possible to distinguish four different wine classes, Chã red, Sodade red, Sangue Vulcão red and Montrond red wines, as had been previously determined by discriminant analysis.

The Montrond and Sangue Vulcão red wines belong to classes with some similarity as shown in the dendogram while Sodade and Chã red wines have very different classes of others.

The white wines did not enter this classification. This probably is due to low concentration of phenolic compounds in relation to red wines that perhaps prevents this analysis.

7.2. PRESENTATION OF RESULTS FOR SULFUR COMPOUNDS

In table 7.8 are presented the retention times, LOD, and R^2 for all standard solution used for calibration curve. The R^2 was around 0.99 for all compounds except 3-mercapto-1-propanol.

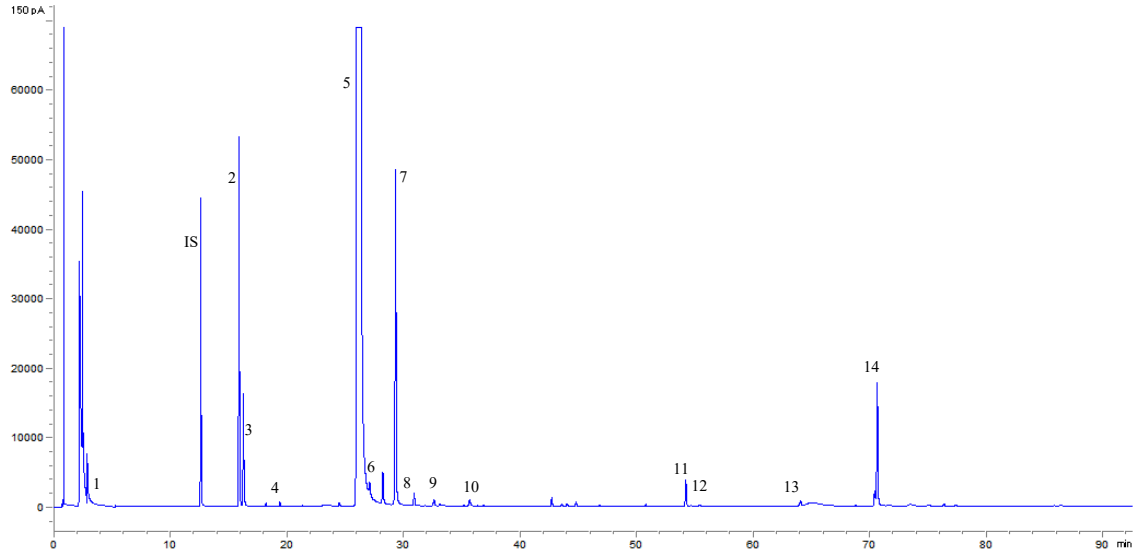
Table 7.8 - Parameter of calibration curve for sulfur standard solution analysed.

Sulfur standard	Abbreviation	RT /mn	C /µg.L^{-1}	/R^2	LOD /µg.L^{-1}	Linear equation	
						Slope (m)	Intercept (b)
S-Ethylthioacetate	ETA	2.6	5 – 100	0.993	7.2	3.52E-02	-5.82E-02
2-Methyltetrahydrothiophen-3-one	MHT	15.8	5 – 100	0.996	4.6	3.49E-02	-1.59E-01
2-(Methylthio)-ethanol	MTE	16.2	10 – 100	0.992	7.4	1.13E-02	-7.25E-02
Ethyl-3-methylthiopropionate	EMTP	18.2	5 – 100	0.997	3.9	3.44E-02	-1.75E-01
3-Mercapto-1-propanol	MCP	22.8	20 – 200	0.984	28.7	6.70E-03	-1.69E-01
3-(Methylthio)-1-propanol	MTP	26.1	5 – 100	0.993	7.5	1.35E-02	-7.55E-02
4-(Methylthio)-1-butanol	MTB	32.6	5 – 150	0.997	0.002	2.11E-02	-1.17E-01
Dimethylsulfone	DTS	35.7	10 – 200	0.994	11.5	5.00E-03	-6.20E-03
Benzothiazole	BZT	37	10 – 100	0.991	5.3	3.56E-02	-1.81E-01

The chromatogram presented in figure 7.7 belongs to Sodade red wine extract chromatogram, an example of one of chromatograms of samples wines of Fogo Island.

Because of FPD detector specificity only sulfur compounds in the samples are detected by this device.

The peaks in chromatograms who were not possible to identify the respective compound, they were mentioned as Unidentified.



1: *S*-Ethylthioatate; *IS*-internal standard; 2: methyltetrahydrothiophen-3-one; 3: 2-(methylthio)ethanol; 4: ethyl-3-(methylthio)propionate; 5: methionol; 6: *cis*-2-methyltetrahydrothiophen-3-ol; 7: 3-(ethylthio)-1-propanol; 8: *trans* - 2-methyltetrahydrothiophen-3-ol; 9: 4-methylthio-1-butanol; 10: dimethylsulfone; 11: benzothiazole; 12: 3-(methylthio)propionic acid; 12: Unidentified ; 13: Unidentified; 14: Unidentified.

Figure 7.7 - Chromatogram of Sodade red wine extract for heavy sulfur compounds by GC-FPD.

The identification of compounds in chromatogram was based on the retention time of the standard solution available. The concentrations of *c*-methyltetrahydrothiophen-3-ol, *t*-methyltetrahydrothiophen-3-ol, 3-ethylthio-1-propanol, 3-methylthio propionic acid and the four unidentified compounds whose standard were available were expressed by peak area x 10^3/peak area IS (Moreira et. al., 2010). Because of high intensity of methionol peak area, to determine the concentration of this compound it was necessary to make dilution of the sample by 2/50 factor.

The concentration of all compounds analysed in the wines samples are presented in the table 7.9 for white wines and 7.10 for rosé and red wines. The values presented are the mean values and standard deviation of three determinations.

Table 7.9 - Mean value and standard deviation, $\mu g.L^{-1}$, of sulfur compounds determined in white wines of Fogo Island.

Compounds	MONTROND	CHÃ	SODADE
	Mean ± SD	Mean ± SD	Mean ± SD
S-Ethylthioacetate	<LOD	<LOD	<LOD
2-Mercaptoethanol	ND	ND	ND
2-Methyltetrahydrothiophen-3-one	11.4$^{(a)}$ ± 4.1	13.7$^{(a)}$ ± 2.2	13.9$^{(a)}$ ± 3,2
2-Methylthioethanol	*116$^{(a)}$ ± 25	90.0$^{(a)}$ ± 6.7	79.3$^{(a)}$ ± 2,3
Ethyl-3-(methylthio)propianate	37.8$^{(a)}$ ± 9.4	ND	20.4$^{(a)}$ ± 7.9
3-Methylthio-1-propanol	*452$^{(a)}$ ± 6	*1611$^{(b)}$ ± 90	*844$^{(c)}$ ± 89
cis-2-Methyltetrahydrothiophen-3-ol**	229$^{(a)}$ ± 159	93.8$^{(a)}$ ± 19.0	50.9$^{(a)}$ ± 7.0
3-Ethylthio-1-propanol**	*862$^{(a)}$ ± 192	*1157$^{(a)}$ ± 503	*641$^{(a)}$ ± 129
trans-2-Methyltetrahydrothiophen-3-ol**	201$^{(a)}$ ± 122	142$^{(a)}$ ± 22	53.1$^{(a)}$ ± 4,2
4-Methylthiobuthanol	31.1$^{(a)}$ ± 12.1	21.7$^{(a)}$ ± 1.8	ND
Dimethyl sulphone	*283$^{(a)}$ ± 245	31.9$^{(a)}$ ± 6.3	28.2$^{(a)}$ ± 10,5
Benzothiazole	<LOD	ND	ND
3-(Methylthio)propionic acid**	309$^{(a)}$ ± 247	76.8$^{(a)}$ ± 21.8	172$^{(a)}$ ± 28
Unidentified 1**	279$^{(a)}$ ± 211	425$^{(a)}$ ± 360	43.4$^{(a)}$ ± 8,0
Unidentified 2**	1.36E03$^{(a)}$ ± 7.2E01	880$^{(a)}$ ± 214	318$^{(a)}$ 102
Unidentified 3**	2.28E03$^{(a)}$ ± 1.82E03	ND	90.2$^{(a)}$ ± 60.2
Unidentified 4**	ND	ND	146 ± 8

**Peak área x 10^3/ Peak área IS; ND: not detect. *Determined by dilution of samples in hydroalcoholic solution 12%.SD: standard deviations from three determinations. Values not sharing the same superscript letter (a–c) within the horizontal line are different according to the Tukey test;

Table 7.10 - Mean value and standard deviation, $\mu g.L^{-1}$, of sulfur compounds determined in red and rosé wines of Fogo Island.

RT	Compounds	Montrond	Chã	Sodade	Sangue Vulcão	Sodade Rosé
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
2,6	*S*-Ethylthioacetate	<LOD	<LOD	13.5$^{(a)}$ ± 4.5	6.00$^{(b)}$ ± 2.00	11.0$^{(a)}$ ± 1.0
15	2-Mercaptoethanol	ND	ND	ND	ND	ND
15,8	2-Methyltetrahydrothiophen-3-one	6.50$^{(a)}$ ± 1.50	12.5$^{(a.c)}$ ± 6.5	33.0$^{(b)}$ ± 3.0	11.5$^{(a.c)}$ ± 3.5	16.5$^{(c)}$ ± 1,5
16,2	2-(Methylthio)-ethanol	53.5$^{(a)}$ ± 5.5	25.5$^{(a.b)}$ ± 10.5	74.5$^{(a.c)}$ ± 10.5	63.0$^{(a)}$ ± 14.0	73.0$^{(a.c)}$ ± 3,0
18,2	Ethyl-3-(methylthio)propianate	17.0$^{(a)}$ ± 5.0	14.0$^{(a)}$ ± 1.0	20.5$^{(a)}$ ± 10.5	21.0$^{(a)}$ ± 7.0	12.0$^{(a)}$ ± 1,0
26,1	3-Methylthio-1-propanol	*626$^{(a)}$ ± 112	*1.59E03$^{(b)}$ ± 9.9E01	*2.03E03$^{(b)}$ ± 8.0E01	*1.57E03$^{(b)}$ ± 4.41E01	*6.03E03$^{(c)}$ ± 3,8E01
28,3	*cis*-2-Methyltetrahydrothiophen-3-ol**	14.5$^{(a)}$ ± 2.5	ND	265$^{(b)}$ ± 14	47.5$^{(a)}$ ± 5.5	342$^{(c)}$ ± 44
29,6	3-Ethylthio-1-propanol**	169$^{(a)}$ ± 99	1.17E03$^{(b)}$ ± 5.0E02	2.28E03$^{(c)}$ ± 5.1E02	212$^{(a)}$ ± 111	1.20E03$^{(b)}$ ± 3,7E01
31	*trans*-2-Methyltetrahydrothiophen-3-ol**	ND	43$^{(a)}$ ± 36	103$^{(a)}$ ± 27	76.5$^{(a)}$ ± 25.5	347$^{(b)}$ ± 46
32,6	4-Methylthiobuthanol	9.50$^{(a)}$ ± 1.50	12.0$^{(a)}$ ± 4.0	17.0$^{(b.a)}$ ± 1.0	10.5$^{(a.b)}$ ± 3.5	22.5$^{(b)}$ ± 2,5
35,7	Dimethyl sulphone	139$^{(a)}$ ± 42	63.5$^{(b)}$ ± 18.5	40.5$^{(b)}$ ± 15.5	49.5$^{(b)}$ ± 17.5	0.00 ± 0,00
37	Benzothiazole	<LOD	<LOD	<LOD	ND	ND
54,2	3-(Methylthio)propionic acid**	44.5$^{(a)}$ ± 2.5	135$^{(a)}$ ± 120	214$^{(a)}$ ± 70	227$^{(a)}$ ± 102	723$^{(b)}$ ± 69
55	Unidentified 1**	44.0$^{(a)}$ ± 19.0	60.0$^{(a.b)}$ ± 20.0	39.0$^{(a)}$ ± 14.0	ND	92.5$^{(b)}$ ± 3.5
64	Unidentified 2**	150$^{(a)}$ ± 21	4.47E03$^{(a)}$ ± 4.29E03	757$^{(a)}$ 705	4.23E03$^{(a)}$ ± 2.64E03	514$^{(a)}$ ± 62
70,5	Unidentified 3**	969$^{(a)}$ ± 179	1.24E03$^{(a.c)}$ ± 3.2E02	886$^{(a)}$ ± 191	2.33E03$^{(b)}$ ± 6.4E02	321$^{(a.d)}$ ± 23
70,7	Unidentified 4**	323$^{(a)}$ ± 338	282$^{(a)}$ ± 198	ND	478$^{(a)}$ ± 20.5	353$^{(a)}$ ± 33

**Peak area x 10^3/Peak area IS. ND – not detect, *determined by dilution of samples in hydroalcoholic solution 12%. SD: standard deviations from three determinations. Values not sharing the same superscript letter (a-c) within the horizontal line are different according to the Tukey test.

7.2.1. Discussion of results for heavy sulfur compound

The formation of sulfur compound during wine production mainly after and during fermentation is related with yeast strain and their nutrition, temperature of fermentation within others (Moreira et. al., 2008; Specht, 2010). The sunlight exposition also activates synthesis of some sulfur compounds in wines during aging (Jackson, 2008).

The formation of *S*-ethylthioacetate is also influenced during fermentation step. There is a relation between biological formation of H_2S and *S*-ethylthioacetate during fermentation (Kinzurik et. al., 2016). In the white wines this compound was found below of limit of detection as in the red wines such as Montrond and Chã cultivars.

The concentration of *S*-ethylthioacetate was determined in the Sodade and Sangue Vulcão red wines and Sodade rosé wine. The highest concentration was detected in Sodade red wine at 13.5 ± 4.5 $\mu g.L^{-1}$, which is not significantly different compared with Sodade rosé wine.

The figures 7.8, 7.9 and 7.10 represent a comparison of some sulfur compounds determined in white, red and rosé wines of Fogo Island.

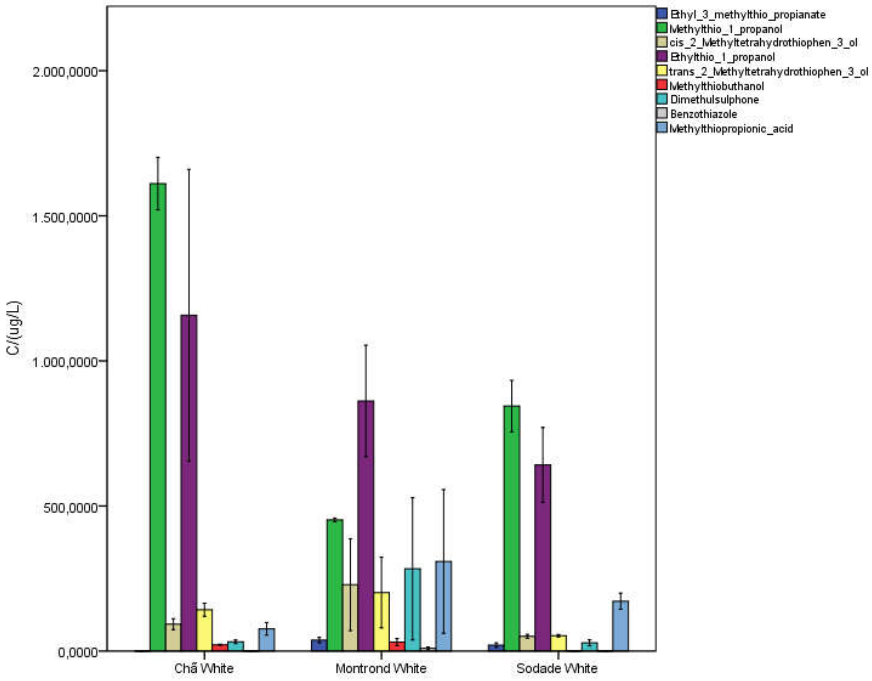


Figure 7.8 - Graphical comparison of sulfur compounds determined in white wines of Fogo Island.

2-Mercaptoethanol was not detected in any samples of wines. This value is not usual in young wines but it is common in old wines like old tawny port wine and aging Cabernet Sauvignon wines, and one reason for that is the presence O_2 causes the reduction of this sulfur compound (Moreira & Guedes de Pinho, 2011; Ye et. al., 2016).

2-Methyltetrahydrothiophen-3-one was detected in all wines samples. According to literature values the concentration of this compound vary 3.3 to 478 $\mu g.L^{-1}$ (Mestres et. al., 2000). In white wines, Montrond, Sodade and Chã varieties, presented a similar content in 2-methyltetrahydrothiophen-3-one, 11.4 ± 4.1 to 13.9 ± 3.2 $\mu g.L^{-1}$. Those values determined in the white wines are below of threshold values which is 150 $\mu g.L^{-1}$ therefore not affecting the quality of wines (Moreira et. al., 2010). The concentration values of 2-methyltetrahydrothiophen-3-one determined in white wines of Cape Verde are similar those determined by Ye et. al. (2016) in Sauvignon Blanc wines.

In the red wines and rosé wine, the concentration of 2-methyltetrahydrothiophen-3-one are below of threshold value for red wine, 250 $\mu g.L^{-1}$. The highest concentration determined was in the Sodade red wine, 33.0 ± 3.0 $\mu g.L^{-1}$. This concentration is normal in wines not affecting the quality of wines. The maximum concentration found in wines was 478 $\mu g.L^{-1}$ (Mestres et. al., 2000).

The 2-(methylthio)-ethanol was detected in all wine samples, red, white and rosé. This compound above threshold value in wines, 250 $\mu g.L^{-1}$, contribute to unpleasant odor of french bean (Mestres et. al., 2000). The concentration determined of this compound in the wines of Cape Verde are in the range of values found in the literature which are 5 to 139 $\mu g.L^{-1}$ (Mestres et. al., 2000; Moreira & Guedes de Pinho, 2011; Ye et. al., 2016). Among white wines, Montrond white wine has the highest concentration, 116 ± 25 $\mu g.L^{-1}$ but it is not significantly different from the other white wines samples. The concentration in white wines are higher than in red wines.

Among red wines, Sodade has the higher concentration of 2-(methylthio)-ethanol, 74.5 ± 10.5 $\mu g.L^{-1}$ while Sodade rosé wine has 73.0 ± 3.0 $\mu g.L^{-1}$ of concentration. Chã red wine has the lowest concentration, 25.5 ± 10.5 $\mu g.L^{-1}$, among all wines, although it is not significantly different from Sangue Vulcão and Montrond red wines and they are in the range of concentration determined by Moreira & Guedes de Pinho (2004) and Ye et. Al. (2016) in the wines. Despite all analysed wines samples have this substance, their concentrations are below of the perception threshold (Mestres et. al., 2000; Moreira et. al., 2011).

The ethyl-3-methylthiopropianate, in the white wines was detected in the Montrond and Sodade wines. This sulfur compounds above its threshold value, 300 - 1000 $\mu g.L^{-1}$, gives to the wines an unpleasant metallic and sulfurous odor (Mestres et. al., 2000; Ye et. al., 2016). Its concentration in the wines vary 0 to 14.3 $\mu g.L^{-1}$ (Mestres et. al., 2000). The concentration of ethyl-3-methylthiopropianate determined in the two white wines, Montrond and Sodade white wines, 37.8 ± 9.4 and 20.4 ± 7.9 $\mu g.L^{-1}$ are relativaley very high when compared with those reported in the literature but they are below of threshold values in wines (Mestres et. al., 2000; Moreira & Guedes de Pinho, 2011; Ye et. al., 2016).

For the red wines, ethyl-3-methylthiopropianate was determined in all red wines samples and also to Sodade rosé wine. The concentration determined in those wines are high when compared with others wines in the literature (Mestres et. al., 2000; Moreira & Guedes de Pinho, 2011; Ye et. al., 2016).

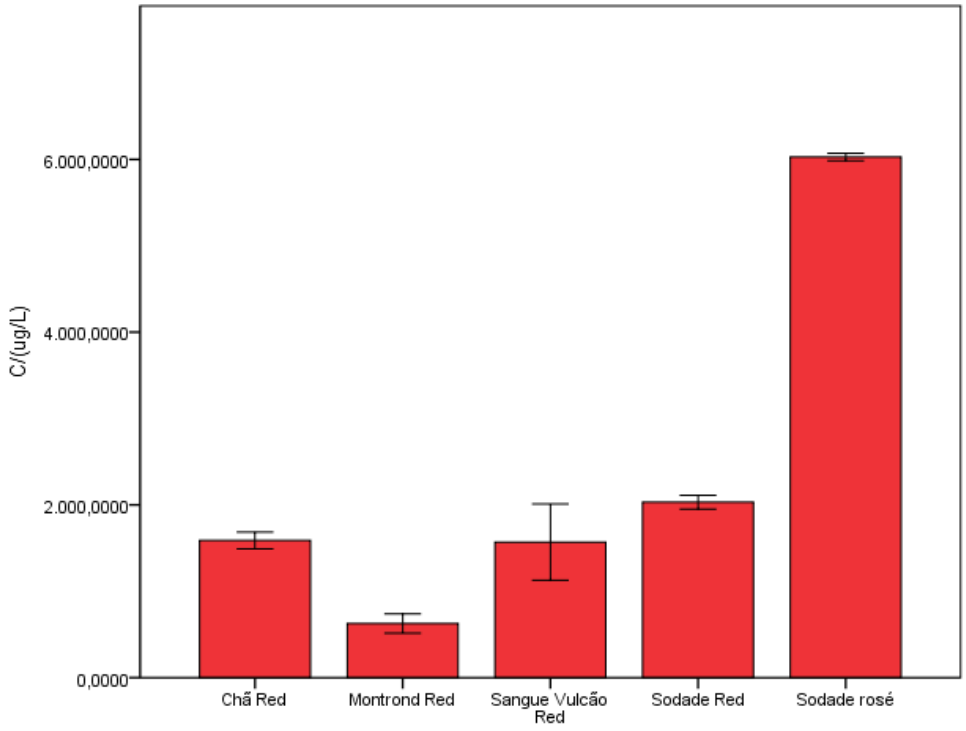


Figure 7.9 - Graphic comparison of 3-methylthio-1-propanol determined in red and rosé wines.

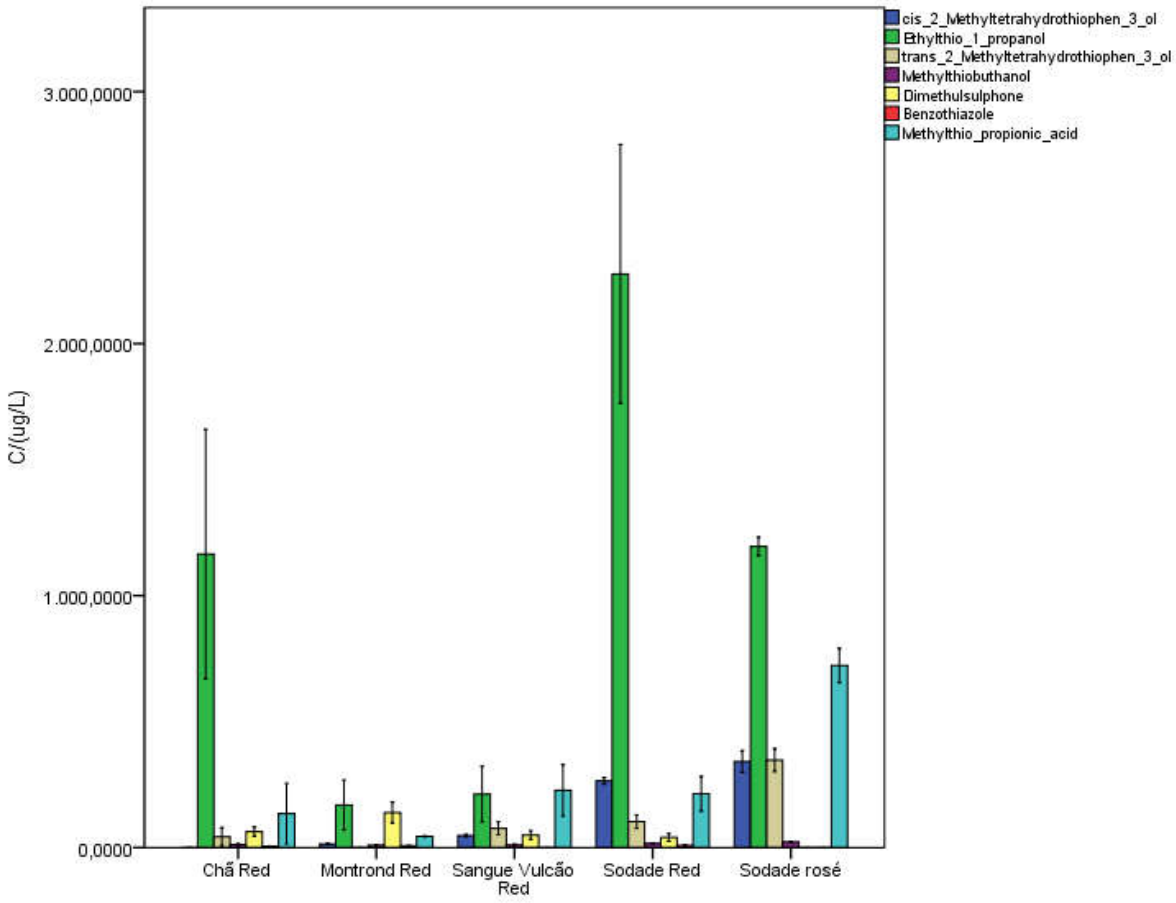


Figure 7.10 - Graphical comparison of sulfur compounds determined in red and rosé wines of Fogo Island.

The 3-methylthio-1-propanol (methionol) is the main heavy sulfur compound in the wines and this compound normally has the highest concentration among heavy sulfur compounds in wines. Its production is associated with degradation of methionine amino acid by yeast as shown the figure 7.11 (Perestrelo et. al., 2006; Seow et. al., 2010; Yin et al., 2015). Its limit of perception in the wines varies between 1.2 – 4.5 $mg.L^{-1}$ and at high concentration it confers to the wines a bad aroma, potato, cauliflower, cooked cabage (Mestres et. al., 2000).

In the white wines of Cape Verde, Montrond, Chã and Sodade, the concentration of methionol varied between 452 ± 6 to 1.61E03 ± 9.1E01 $\mu g.L^{-1}$ and the maximum was found in the Chã white wine. These concentrations of methionol are normal in the wines according to the literature and so not affecting the quality of these white wines (Moreira et. al., 2011).

In the red wines, the concentration of methionol determined varied between 626 ± 111 to 2.03E03 ± 8.0E01 μg.L^{-1} where the minimum and maximum belong to Montrond and Sodade red wines respectively. These concentrations are usually found in the wines according to the literature (Mestres et. al., 2000).

The Sodade rosé wine has the highest concentration of methionol determined in the wines of Cape Verde, 6.03E03 ± 3.8E01 μg.L^{-1}. This value is above the threshold value of perception in the wines and it may affect the quality of aroma of this (Mestres et. al., 2000; Moreira et. al., 2011).

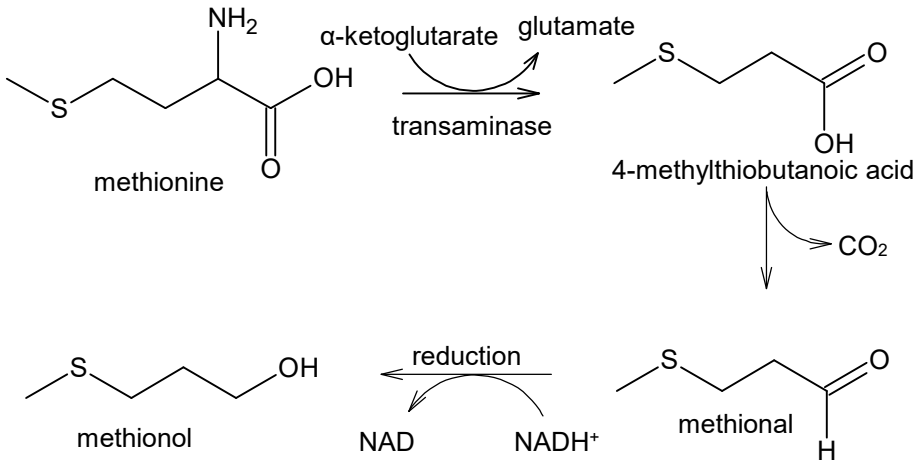


Figure 7.11 – Mechanism of formation of methionol from methionine by yeast (Perestrelo et al., 2006).

The analysis of *cis*-2-methyltetrahydrothiophen-3-ol and *trans*-2-methyltetrahydrothiophen-3-ol showed they were detected in all white wines samples. They are usually found in the white wines and when the concentrations are above their threshold values contribute to a bad aroma in the wines (Moreira et. al., 2010).

The 4-methylthio-1-butanol was detected in all wines samples. It presences is common in wines and the concentration determined in all samples are below the threshold values in wines.

The dimethyl sulfone was detected in all wines except in rosé Sodade wine. The Montrond red wine has the highest value of concentration, 138 ± 41 μg.L^{-1}, but because dimethyl sulfone is odorless, the concentration of it does not affect the quality of wine.

The benzothiazole was detected in four wines sample, Montrond white wine, Chã red, Sodade red and Montrond red wines, but the concentrations are below of LOD.

The 3-ethylthio-1-propanol was detected in all wines samples with the concentration relatively high. The red wine Sodade has the highest concentration and its quality can be affected by this compound.

In addition to the identified compounds, the chromatograms of wine extracts displayed several unidentified peaks as shown in figure 6.5. These peaks in some wines are relatively intense, mainly the ones detected with retention times 64 and 70.5 minutes. In red wines Sangue de Vulcão and Montrond, the peaks for the time 70.5, were the most intense compared to other wines analysed.

The sulfur compounds 2-mercaptoethanol and 3-mercapto-propanol were not detected in any wines samples.

7.2.2. Chemometrics analysis for sulfur compounds in the wines

The PCA analysis was made to view in general the data and to verify data cluster. The two principal components, PC1 and PC2, explain 52% of variance and with PC3 77% of variance. The figure 7.12, with distribuition of variables on the PC1 and PC2 represent the PCA.

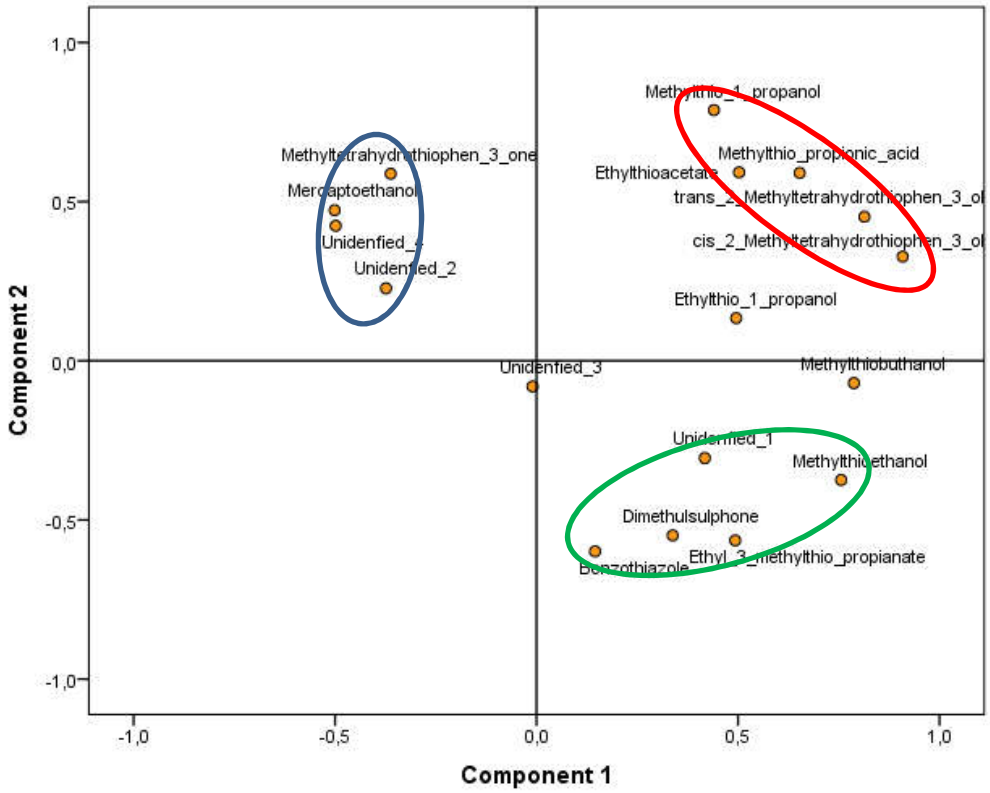


Figure 7.12 - Plot of PCA for heavy sulfur compounds in the wines.

In the figure 7.12 is possible to identify some cluster of variables on the two principal components. Some variables also are out of those clusters.

The discriminant analysis to verify the separation of wines according with heavy sulfur compounds is presented in the figure 7.13.
The analysis of figure 7.13 which represent the two discriminant function, allow to verify there is a clear separation between the Sangue Vulcão red wine and Sodade rosé wine from others wines. The other wines have a slight separation between them but not as Sodade rosé wine and Sangue Vulcão wine.

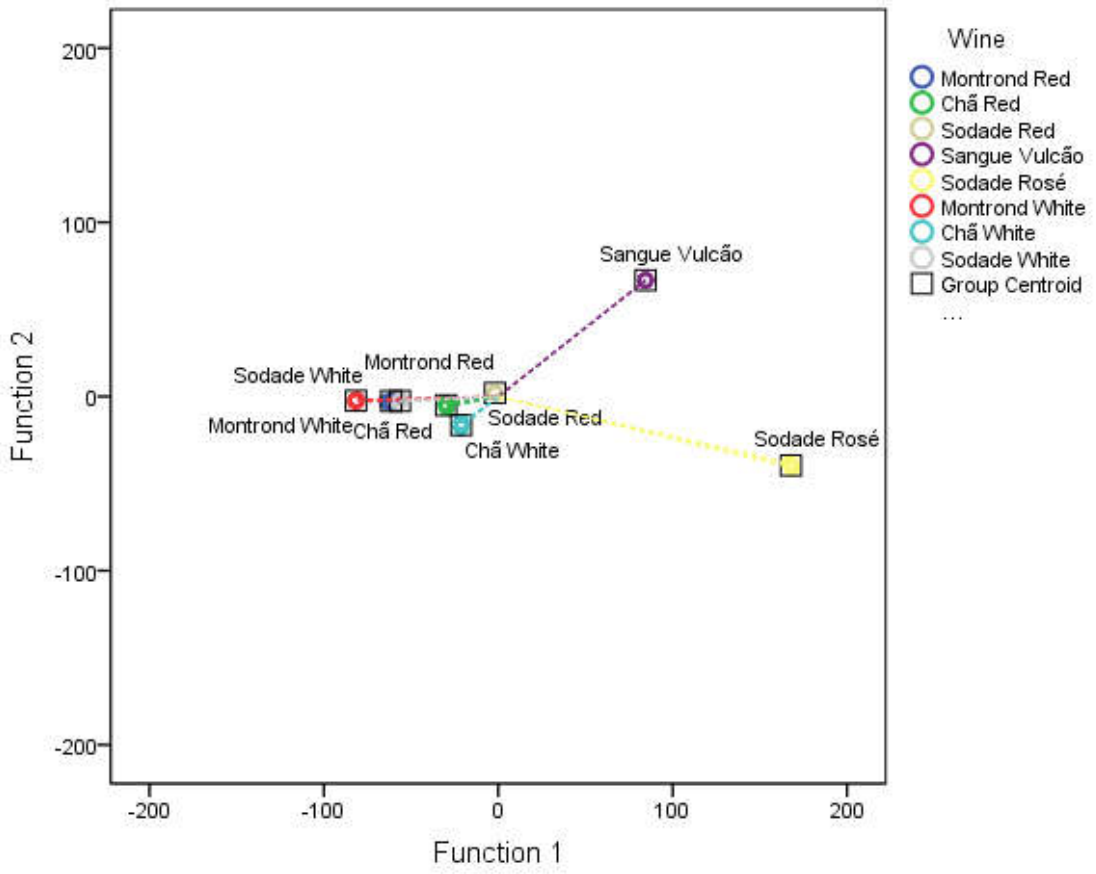


Figure 7.13 - 2D scatterplot of canonical scores of discriminant functions with heavy sulfur compounds.

The main discriminating variables determined were presented on the tables 7.12 and 7.13. Nine variables participated to the differentiation the wines of Cape Verde according to the sulfur compounds.

The table 7.14 presents the validation of functions and as it shows, 100% of samples were correctly classified to the seven functions.

Table 7.11 - Standardized canonical discriminant function coefficients.

	Function						
	1	2	3	4	5	6	7
Ethylthioacetate	-0.573	0.174	2.014	0.019	0.331	0.157	0.617
Mercaptoethanol	8.158	3.005	-1.028	0.067	0.055	0.244	-0.088
2-Methyltetrahydrothiophen-3-one	2.840	4.942	0.946	0.316	0.090	-0.128	0.072
Methylthioethanol	0.308	-1.810	-0.169	1.282	-0.196	-0.310	0.135
Ethyl-3-methylthiopropianate	-2.897	1.357	-0.098	-0.672	1.318	0.308	0.423
3-Methylthio-1-propanol	7.573	-1.357	-0.267	-0.011	0.059	-0.176	0.048
c-2-Methyltetrahydrothiophen-3-ol	4.494	0.641	4.313	0.285	-0.685	-1.328	-1.510
Ethylthio-1-propanol	-3.150	-1.210	1.056	-0.641	-0.369	0.891	0.004
t-2-Methyltetrahydrothiophen-3-ol	-3.767	0.089	-4.217	-0.165	0.529	1.816	0.542

Table 7.12 - Classification function coefficients.

	Wine							
	Montrond Red	Chã Red	Sodade Red	Sangue Vulcão	Sodade Rosé	Montrond White	Chã White	Sodade White
Ethylthioacetate	-6.744	-18.007	11.843	-43.814	-77.764	5.878	-19.899	-2.187
Mercaptoethanol	141.061	344.866	518.042	1270.623	1560.614	5.672	372.379	170.714
2-Methyltetrahydrothiophen-3-one	7.408	19.889	44.934	134.966	86.764	-1.101	15.383	10.697
Methylthioethanol	2.084	2.717	2.445	-5.148	14.683	1.749	5.981	2.350
Ethyl-3-methylthiopropianate	-12.147	-27.167	-40.399	-67.024	-131.149	-2.040	-35.815	-14.482
3-Methylthio-1-propanol	1.058	2.453	3.570	6.882	11.355	0.164	2.914	1.268
c-2-Methyltetrahydrothiophen-3-ol	1.744	3.890	8.713	13.691	19.037	0.448	4.701	2.389
Ethylthio-1-propanol	-0.219	-0.500	-0.700	-1.888	-2.298	-0.008	-0.551	-0.253
t-2-Methyltetrahydrothiophen-3-ol	-1.848	-3.922	-8.989	-12.684	-19.280	-0.574	-4.879	-2.534
(Constant)	-304.091	-1543.206	-4017.678	-16406.831	-32794.177	-96.324	-2236.219	-465.011

Fisher's linear discriminant functions

Table 7.13 - Classification matrix for heavy sulfur compounds.

		Wine	Predicted Group Membership								Total
			Montrond Red	Chã Red	Sodade Red	Sangue Vulcão	Sodade Rosé	Montrond White	Chã White	Sodade White	
Original	Count	Montrond Red	3	0	0	0	0	0	0	0	3
		Chã Red	0	3	0	0	0	0	0	0	3
		Sodade Red	0	0	3	0	0	0	0	0	3
		Sangue Vulcão	0	0	0	3	0	0	0	0	3
		Sodade Rosé	0	0	0	0	3	0	0	0	3
		Montrond White	0	0	0	0	0	3	0	0	3
		Chã White	0	0	0	0	0	0	3	0	3
		Sodade White	0	0	0	0	0	0	0	3	3
	%	Montrond Red	100	0	0	0	0	0	0	0	100
		Chã Red	0	100	0	0	0	0	0	0	100
		Sodade Red	0	0	100	0	0	0	0	0	100
		Sangue Vulcão	0	0	0	100	0	0	0	0	100
		Sodade Rosé	0	0	0	0	100	0	0	0	100
		Montrond White	0	0	0	0	0	100	0	0	100
		Chã White	0	0	0	0	0	0	100	0	100
		Sodade White	0	0	0	0	0	0	0	100	100

a. 100,0% of original grouped cases correctly classified.

This classification is more easily seen in the dendogram of figure 7.15. There is a clear separation of Sodade rosé wine from the others. Through the dendogram, its possible to verify that there are samples of Sangue Vulcão and Chã red wines in the same group that was not possible to check by LDA.The Sodade white wine and Montrond red wine are in the same class with some proximity of Chã white wine. It can also be seen that some some samples of Chã and Sangue Vulcão red wines are mixed in two distinct classes.

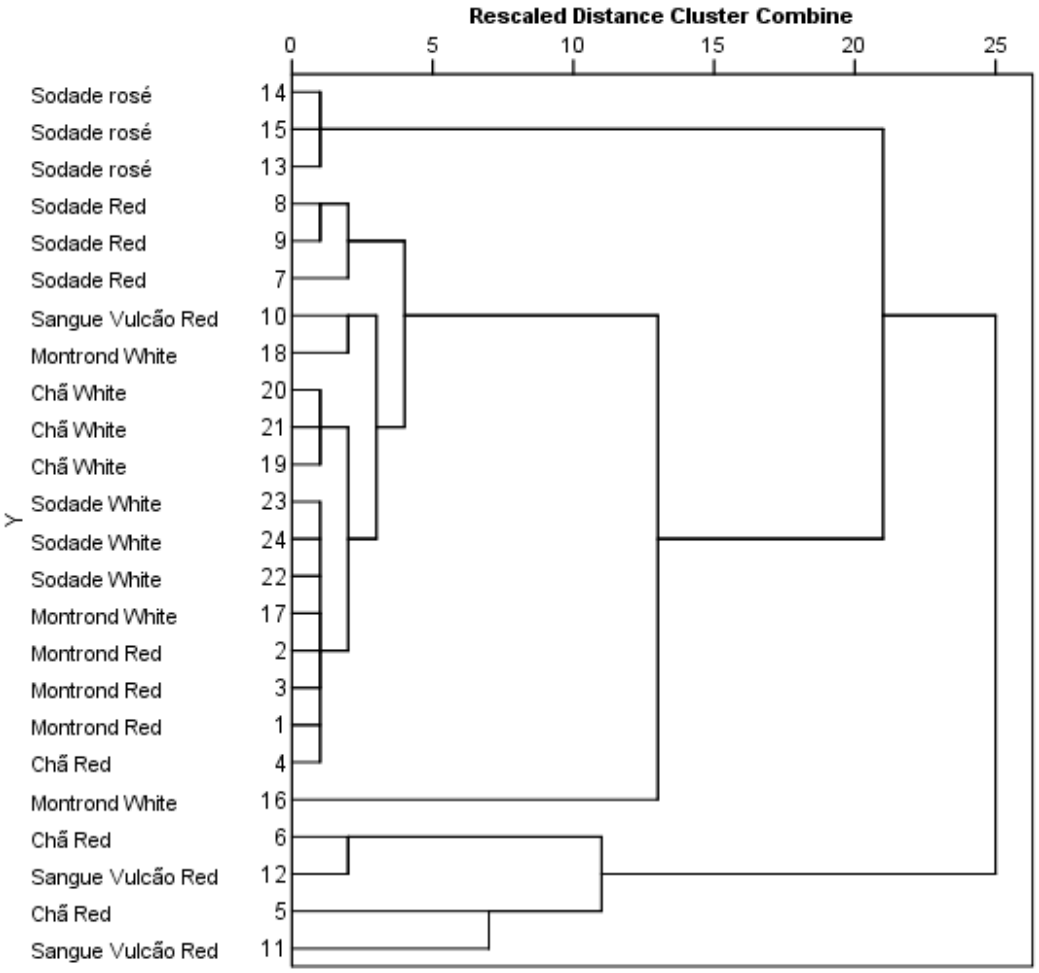


Figure 7.14 - Dendogram of cluster analysis obtained with heavy sulfur compounds in the wines.

7.3. PRESENTATION OF RESULTS FOR VOLATILES AROMA COMPOUNDS

The analysis of wine samples by SPME-HS-GC-MS show many organic compounds such as esters, terpenes, alcohols, sesquiterpene, nor-isoprenoids and acids. The table 7.15 shows these compounds detected in wines of Fogo Island. Those compounds were identified by comparing their retention times with standard compounds and comparison of the retention indices (as Kovats indices) with literature data. The comparison of MS fragmentation pattern with standard compounds and mass spectra database search was performed using the National Institute of Standards and Technology (NIST) 14 spectral database, considering fit and retrofit values higher than 70 %.

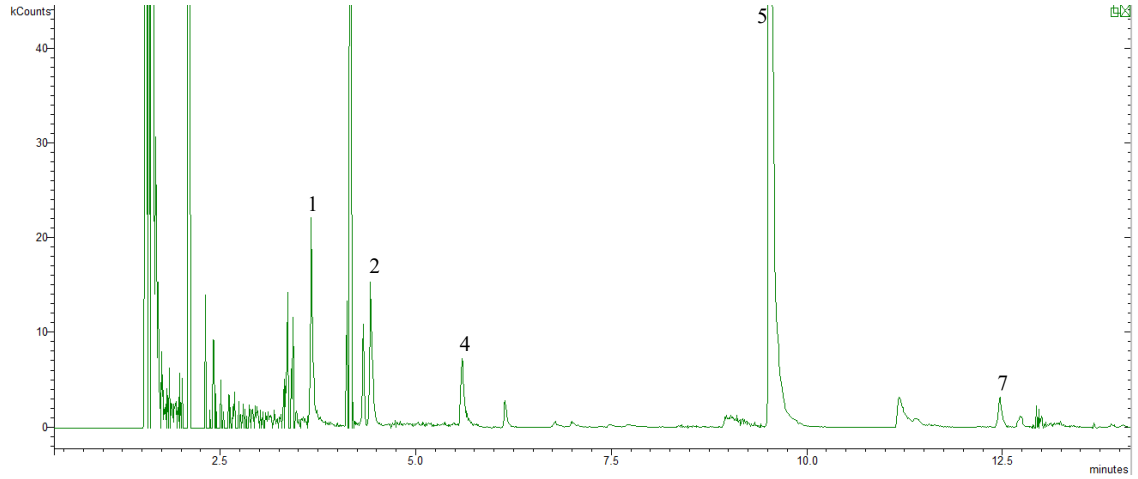
Calibration curves were made with the standard compounds available. Other compounds without standard their concentrations were expressed as $\mu g.L^{-1}$ or $mg.L^{-1}$ equivalents of compounds with similar chemical structure for which standards were available (Lukić et. al., 2016).

Table 7.14 - Retention time (RT), retention indices (RI), identification method (ID), selected ions used as m/z identifiers of volatile compounds in wines.

RT (min)	RI_{calc}[a]	RI_{lit}[b]	Compounds	ID [c] (fit/retrofit, %)	Identifier Ions (m/z)[d]
			Esters		
3.66	798	-	Ethyl 2-methylpropanoate (Ethyl isobutyrate)	MS (80.4/81.1)	43/71/88*/116
4.41	807	802	Ethyl butanoate	STD, MS	43/71/88*
4.65	817	815	Ethyl lactate	STD, MS	45*
5.59	857	854	Ethyl 3-ethylbutanoate (Ethyl isovalerate)	MS (86.7/87.3)	57/85/88*
6.13	880	876	Isoamyl acetate	STD, MS	43/55*/70
9.56	1005	1000	Ethyl hexanoate	STD, MS	43/88*/99
9.93	1017	1011	Hexyl acetate	STD, MS	43*/55/56
12.47	1103	1097	Ethyl heptanoate	MS (80.0/85.4)	88*/101/113
14.86	1185	1182	Diethyl succinate	STD, MS	101*/129*
16.83	1256	1252	Isoamyl hexanoate	MS (83.5/85.6)	43/70*/71/99
16.92	1259	1252	Isoamyl butanoate	MS (79.3/87.3)	43/70*/71/99
16.95	1260	1258	Phenylethyl acetate	STD, MS	43/104*
17.18	1268	1244	Diethyl malate	MS (72.5/73.8)	43/71*/89/117
18.09	1301	1296	Ethyl nonanote	MS (79.8/83.9)	88*/101
20.78	1404	1396	Ethyl decanoate	STD, MS	88*/101
25.58	1603	1595	Ethyl dodecanoate	STD, MS	88*/101
33.84	2000	1993	Ethyl hexadecanoate	MS (82.7/84.3)	88*/101
			Alcohols		
5.97	873	868	1-Hexanol	MS (80.2/82.7)	56*/69
10.66	1042	1036	Benzyl alcohol	MS (87.6/89.0)	77/79*/107/108
12.91	1118	1116	2-Phenylethanol	STD, MS	91*/92*
			Terpenes		
8.43	965	937/933	α-Pinene	STD, MS	92/93*/121*
11.20	1060	1031	Limonene	STD, MS	67/68/93*
11.31	1064	1060	γ-Terpinene	STD, MS	77/91/93*/121*/136
12.88	1117	1088	Terpinolene	MS (80.1/89.40)	91/93*/121*/136
13.28	1131	1099	Linalool	STD, MS	43/55/71/93*/121*
16.30	1237	-	Unidentified terpene 1	-	91/93*/121*
16.53	1245	-	Unidentified terpene 2	-	93*/121*/136
17.13	1267	-	Unidentified terpene 3	-	91/93*/121*/136
			Norisoprenoids		
17.70	1287	-	Unidentified ionone	-	91/93*/121*/136/177/192
20.32	1386	1386	β-Damascenone	STD, MS	69*/121*/190
			Sesquiterpen		
23.83	1528	-	Unidentified sesquiterpene	-	105/161*/204*
			Acids		
9.39	999	990	Hexanoic acid	STD, MS	60*/73
19.98	1373	1373	Decanoic acid	STD, MS	55/60*/73/129

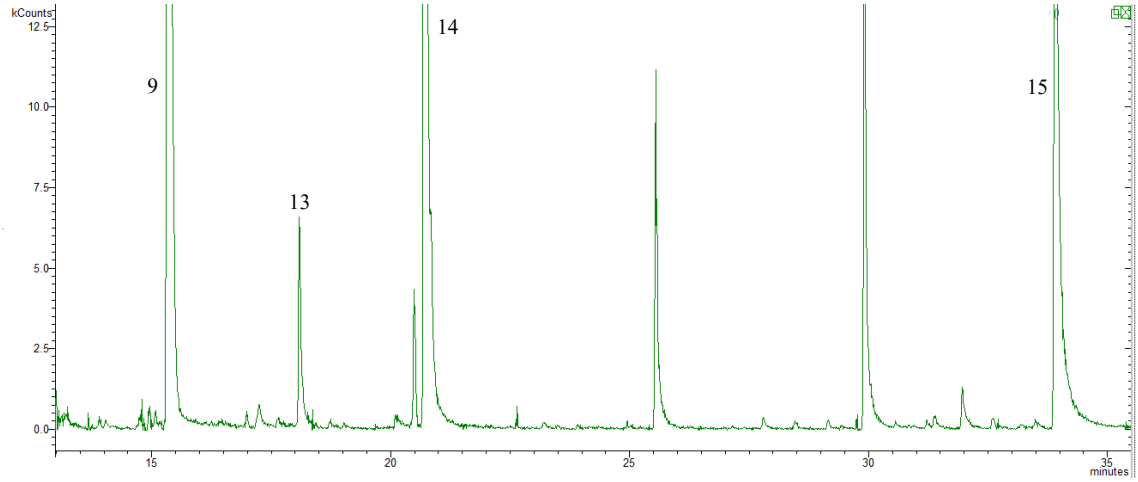
[a]RI_{calc}: retention indices calculated from C8 to C20 n-linear alkanes with VF-5 ms capillary column. [b]RI_{lit}: retention indices reported in the literature for VF-5 ms capillary column or equivalent. [c] ID: identification methods. Compounds were identified by comparing (i) their retention times with those of authentic compounds (STD), (ii) the retention indices with those from literature data and (iii) the MS fragmentation pattern with those of STD and mass spectra database performed using NIST 14 spectral database, considering fit and retrofit values >70%; [d] quantitative ions are mark with superscript *

The figures 7.15 to 7.18 are chromatograms that reveal peaks of some compounds identified in wines samples. The chromatograms presented were obtained at different ion current as show in the figures.



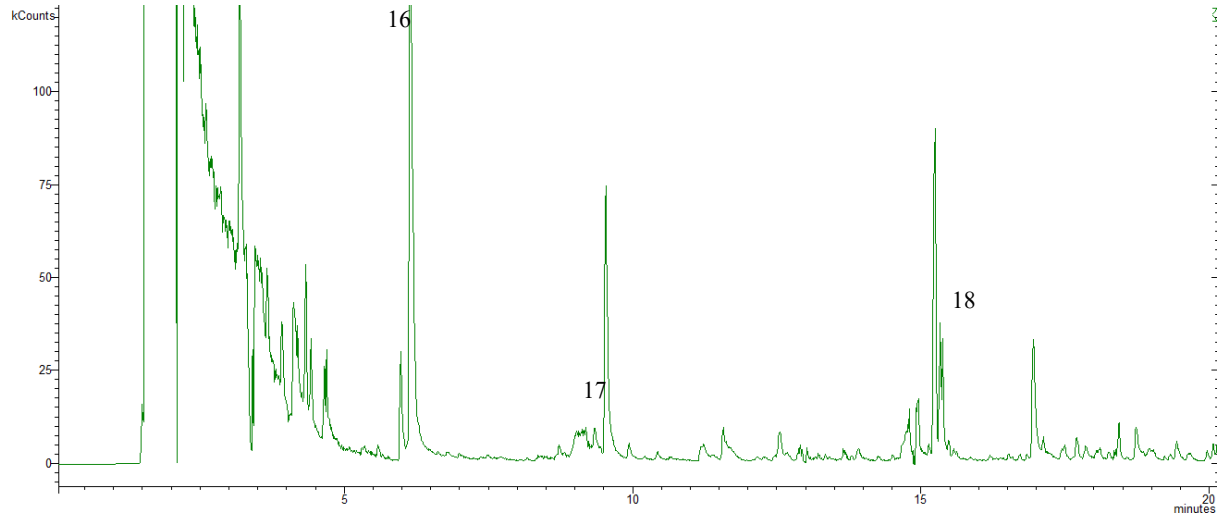
1 - ethyl isobutanate; **2** - ethyl butoanate; **4** - ethyl isovalerate; **5** - ethyl hexanoate; **7** - ethyl heptanoate

Figure 7.15 - Chromatogram of esters at 88 m/z ion current for Chã red wine sample.



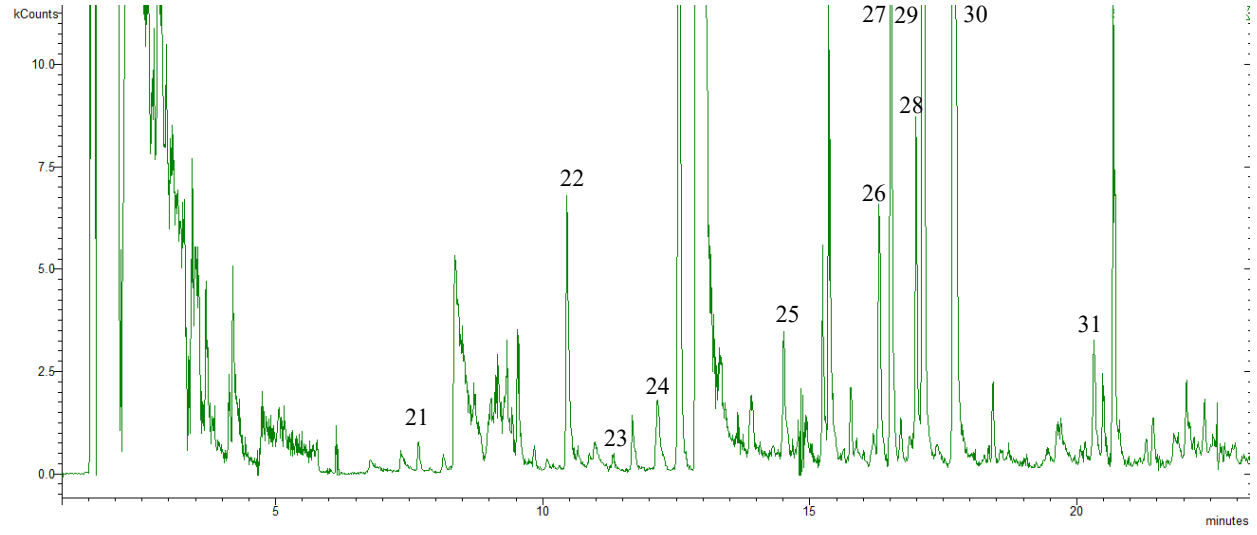
9 - ethyl octanoate; **13**-ethyl nonanoate; **14** - ethyl decanoate; **15** - ethyl hexadecanoate

Figure 7.16 - Chromatogram of esters at 88 m/z ion current for Chã red wine sample.



16: isoamyl acetate; **17**: hexyl acetate; **18**: phenylethyl acetate

Figure 7.17 - Chromatogram for acetate esters at 43 m/z ion current for Chã red wine sample.



21: α-pinene; **22**: limonene; **23**: γ- terpinene; **24**: terpinolene; **25**: linalool; **26**: UT; **27**: UT; **28**: L-α-terpineol; **29**: UT; **30**: UT; **31**: damascenone

Figure 7.18 - Chromatogram for terpenes at 93/121 m/z ion current for Chã red wine sample.

The concentrations, mean and standard deviations, of compounds determined in white, red and rosé wine samples from Fogo Island are presented in table 7.16 and 7.17.

Table 7.15 – Concentration mean value and standard deviations (± SD) of volatiles aroma compounds determined in white wine.

Compounds	Montrond	Chã	Sodade
	Mean ± SD	Mean ± SD	Mean ± SD
Esters (mg.L^{-1})			
Ethyl isobutyrate[A]	7.10E-2^{b} ± 4.00E-3	0.101^{c} ± 0.000	5.69E-2^{a} ± 1.00E-3
Ethyl butanoate	5.46E-2^{a} ± 3.00E-3	1.66^{b} ± 0.03	9.21E-2^{a} ± 3.00E-3
Ethyl lactate[B]	5.70^{b} ± 0.81	1.59^{a} ± 0.26	1.94^{a} ± 0.17
Ethyl isovalerate[B]	0.174^{a} ± 0.011	0.277^{b} ± 0.017	0.232^{ab} ± 0.050
Ethyl hexanoate	2.35^{a} ± 0.16	4.80^{b} ± 0.63	4.02^{b} ± 0.01
Hexyl acetate	0.753^{c} ± 0.040	0.415^{b} ± 0.05	0.164^{a} ± 0.005
Ethyl heptanoate[C]	8.10E0-3^{a} ± 2.00E-3	1.70E-2^{b} ± 1.00E-3	1.50E-2^{b} ± 1.00E-3
Diethyl succinate	1.44^{a} ± 0.05	1.62^{b} ± 0.01	1.63^{b} ± 0.02
Ethyl octanoate	2.33^{a} ± 1.15	1.65^{a} ± 0.05	1.52^{a} ± 0.11
Isoamyl hexanoate[C]	6.30E-2^{a} ± 1.1E-2	0.275^{c} ± 0.029	0.147^{b} ± 0.009
Phenylethyl acetate	3.18^{a} ± 0.82	9.73^{b} ± 0.90	1.70^{a} ± 0.05
Diethyl malate[D]	3.40E-2^{a} ± 4.00E-3	0.178^{b} ± 0.005	0.218^{b} ± 0.008
Ethyl nonanote[C]	5.61E-2^{a} ± 5.00E-3	0.153^{b} ± 0.026	0.137^{b} ± 0.001
Ethyl decanoate[C]	5.96^{a} ± 2.31	3.82^{a} ± 0.47	3.11^{a} ± 0.11
Ethyl hexadecanoate[C]	0.935^{a} ± 0.091	2.08^{c} ± 0.05	1.36^{b} ± 0.00
Isoamyl acetate	4.73^{b} ± 0.15	4.67^{b} ± 0.36	2.21^{a} ± 0.06
Alcohols (mg.L^{-1})			
1-Hexanol*	0.139^{a} ± 0.003	0.825^{b} ± 0.003	1.29^{c} ± 0.05
Benzyl alcohol*	4.01E-2^{a} ± 0.000	3.90E-2^{b} ± 8.00E-3	2.77E-2^{b} ± 5.00E-3
2-Phenylethanol	6.42^{a} ± 0.47	13.2^{a} ± 1.7	6.49^{b} ± 0.14
Terpenes (µg.L^{-1})			
α-Pinene[F]	0.631^{c} ± 0.018	0.306^{a} ± 0.012	0.388^{b} ± 0.021
Limonene	4.60^{a} ± 0.82	3.42^{a} ± 0.30	3.81^{a} ± 0.30
γ-Terpinene[F]	0.453^{a} ± 0.078	0.570^{a} ± 0.054	0.993^{b} ± 0.054
Terpinolene[F]	0.640^{a} ± 0.133	0.799^{a} ± 0.147	0.984^{a} ± 0.158
Linalool	0.954^{b} ± 0.033	1.42^{c} ± 0.05	ND^{a}
α-Terpineol	4.06^{b} ± 0.22	2.50^{a} ± 0.70	7.24^{c} ± 0.23
Unidentified terpene 1*	5.00E-3^{a} ± 0.000	4.11E-2^{b} ± 5.00E-3	5.10E-2^{c} ± 2.00E-3
Unidentified terpene 2*	7.01E-3^{a} ± 1.0E-3	0.116^{b} ± 0.005	0.125^{c} ± 0.001
Unidentified terpene 3*	2.10E-2^{a} ± 2.00E-3	0.274^{b} ± 0.017	0.282^{b} ± 0.018
Nor-isoprenoids (µg.L^{-1})			
β-Damascenone	ND^{a}	12.8^{b} ± 0.6	ND^{a}
Unidentified ionone*	0.158^{a} ± 0.010	1.42^{b} ± 0.07	4.05^{c} ± 0.09
Sesquiterpene* (µg.L^{-1})	9.90E-3^{a} ± 1.0E-4	3.90E-2^{b} ± 5.0E-3	8.20E-2^{c} ± 1.0E-2
Acids (µg.L^{-1})			
Hexanoic acid*	8.91E-2^{a} ± 1.0E-4	1.09^{b} ± 0.02	8.20E-2^{b} ± 1.0E-2
Decanoic acid*	3.01E-2^{a} ± 3.01E-2	1.20E-2^{a} ± 4.0E-3	ND^{a}

*peak area/10^{7}.SD: standard deviations from three determinations; ND-not detected. Values not sharing the same superscript letter (a–d) within the horizontal line are different according to the Tukey test. [A]Calibration curve of ethyl butoanate. [B]Calibration curve of ethyl hexanoate/100. [C]Calibration curve of ethyl octanoate. [D]Calibration curve of diethyl succinate/100. [F]Calibration curve of limonene.

Table 7.16 - Concentration mean value and standard deviations (± SD) of volatiles aroma compounds determined in red and rosé wines from Fogo Island.

	Chã red			Montrond red			Sodade rosé			Sodade red			Sangue Vulcão red		
Esters (mg.L^{-1})	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Ethyl isobutyrate [A]	6.00E-2 [a]	±	2.0E-3	3.80E-2 [b]	±	1.0E-3	0.112 [c]	±	0.013	7.30E-2 [a]	±	3.0E-3	3.61E-2 [b]	±	0.000
Ethyl butanoate	5.50E-2 [a]	±	3.0E-3	5.80E-2 [a.b]	±	1.2E-3	7.31E-2 [b]	±	5.0E-3	4.41E-2 [a.b]	±	3.0E-3	5.80E-2 [a]	±	2.0E-3
Ethyl lactate [B]	4.93 [a]	±	1.30	3.00 [b]	±	0.013	1.60 [b]	±	0.44	2.25 [b]	±	0.06	6.37 [a]	±	0.57
Ethyl isovalerate [B]	0.138 [a]	±	0.012	0.125 [a]	±	0.016	0.304 [b]	±	0.006	0.238 [c]	±	0.004	0.122 [a]	±	0.001
Ethyl hexanoate	2.35 [a]	±	0.20	2.05 [a]	±	0.14	3.33 [b]	±	0.11	2.37 [a]	±	0.09	2.76 [c]	±	0.17
Hexyl acetate	8.20E-2 [a]	±	1.0E-3	0.118 [b]	±	0.010	0.160 [c]	±	0.001	6.40E-2 [d]	±	6.0E-3	0.102 [e]	±	0.004
Ethyl heptanoate [C]	4.70E-2 [a]	±	1.0E-3	9.40E-2 [b]	±	7.0E-3	4.01E-3 [c]	±	4.00E-3	3.81E-2 [a]	±	2.0E-3	8.81E-2 [b]	±	3.0E-3
Diethyl succinate	0.957 [a]	±	0.043	1.74 [b]	±	0.13	1.55 [b]	±	0.04	1.04 [a]	±	0.10	2.18 [c]	±	0.05
Ethyl octanoate	2.15 [a]	±	0.20	2.78 [b]	±	0.07	2.43 [a]	±	0.00	2.26 [a]	±	0.16	2.93 [b]	±	0.01
Isoamyl hexanoate [C]	3.60E-2 [a]	±	2.0E-3	2.90E-2 [a.c]	±	4.0E-3	0.208 [b]	±	0.006	2.61E-2 [a.c]	±	1.60E-2	5.69E-2 [a.d]	±	4.0E-3
Phenylethyl acetate	1.74 [a]	±	0.134	1.77 [a]	±	0.11	5.08 [b]	±	0.53	1.16 [a]	±	0.09	1.59 [a]	±	0.07
Diethyl malate [D]	8.00E-3 [a]	±	5.00E-3	1.21E-2 [a]	±	7.0E-3	0.249 [b]	±	0.027		ND		1.39E-2 [c]	±	2.0E-3
Ethyl nonanote [C]	0.169 [a]	±	0.005	0.151 [a]	±	0.016	4.70E-2 [b]	±	9.00E-3	0.109 [c]	±	0.003	0.160 [a]	±	0.004
Ethyl decanoate [C]	4.30 [a]	±	0.17	3.30 [b]	±	0.07	3.20 [b.d]	±	0.07	3.61 [b.e]	±	0.24	4.73 [c]	±	0.03
Ethyl hexadecanoate [C]	0.911[a]	±	0.095	1.12 [a]	±	0.06	1.273 [a]	±	0.594	0.978 [a]	±	0.089	2.19 [b]	±	0.30
Isoamyl acetate	1.87 [a]	±	0.08	2.31 [b]	±	0.13	2.37 [b]	±	0.30	1.35 [c]	±	0.04	2.66 [b]	±	0.09
Alcohol (µg.L^{-1})															
1-Hexanol*	0.167 [a]	±	0.006	0.206 [b]	±	0.006	0.164 [a]	±	0.017	0.256 [c]	±	0.019	0.225 [d.c]	±	0.008
Benzyl alcohol*	3.20E-2 [a]	±	7.0E-3	0.169 [b]	±	0.012	3.40E-2 [a]	±	1.0E-3	0.184 [b]	±	0.013	0.139 [c]	±	0.008
Phenylethanol	6.25 [a]	±	0.67	6.64 [a.c]	±	1.20	10.7 [b]	±	0.1	8.09 [c]	±	0.23	8.30 [c]	±	0.49

Terpenes (µg.L^{-1})															
α-Pinene [F]	0.341 [a]	±	0.022	0.383 [a]	±	0.020	0.246 [b]	±	0.032		ND		0.473 [c]	±	0.043
Limonene	3.59 [a]	±	0.08	3.26 [a]	±	0.02	3.13 [a]	±	0.48	2.27 [b]	±	0.34	4.20 [a]	±	0.03
γ-Terpinene [F]	0.043 [a]	±	0.043	3.20E-2 [a]	±	3.20E-2	0.288 [a]	±	0.288		ND			ND	
Terpinolene [F]	1.12 [a]	±	0.13	0.505 [b]	±	0.024	0.505 [b]	±	0.044	0.812[c]	±	0.023	0.194 [d]	±	0.125
Linalool	6.301[a]	±	0.128	4.54 [b]		0.17	0.475 [c]	±	0.475	3.34 [d]	±	0.76	3.60 [b,d]	±	0.11
Unidentified terpene 1*	1.50E-2 [a]	±	1.0E-3	1.81E-2 [b]	±	1.0E-3	7.00E-3 [c]	±	1.0E-4	1.70E-2 [b]	±	1E-4	1.80E-2 [b]	±	1.0E-3
Unidentified terpene 2*	4.61E-2[a]	±	2.0E-3	5.40E-2[b]	±	4.0E-3	1.00E-2 [c]	±	1.0E-3	3.60E-2 [d]	±	1E-4	6.00E-2 [b]	±	1.0E-3
L-α-Terpineol	13.4 [a]	±	1.1	11.4 [a]	±	1.1		ND		12.6 [a]	±	0.3	7.43 [b]	±	0.05
Unidentified terpene 3*	0.107 [a]	±	0.002	0.119 [b]	±	0.006	2.20E-2 [c]	±	3.0E-3	8.31E-2 [d]	±	1.0E-3	0.136 [e]	±	0.002
Norisoprenoids (µg.L^{-1})															
Unidentified Ionone*	0,136 [a]	±	0,003	0,190 [b]	±	0,017	0,142 [a]	±	0,013	0,231 [c]	±	0,009	0,138 [a]	±	0,003
Damascenone	10,7 [a]	±	0,8	17,4 [a]	±	1,1	12,3 [a]	±	0,7		ND		22,9 [c]	±	0,1
Sesquiterpene* (µg.L^{-1})	5,00E-3 [a]	±	2,00E-3	2,00E-3 [a]	±	2,00E-3	6,00E-3 [a]	±	2,00E-3	7,00E-3 [a]	±	2,00E-3	2,60E-2 [b]	±	9,0E-3
Acids (µg.L^{-1})															
Hexanoic acid		ND			ND			ND			ND			ND	
Decanoic acid*	3,00E-3 [a]	±	1,00E-3		ND			ND		2,50E-2 [a]	±	1,20E-2	9,00E-3 [a]	±	1,00E-3

*peak area/10^7.SD: standard deviations from three determinations; ND - not detected. Values not sharing the same superscript letter (a–d) within the horizontal line are different according to the Tukey test. [A]Calibration curve of ethyl butoanate. [B]Calibration curve of ethyl hexanoate/100. [C]Calibration curve of ethyl octanoate. [D]Calibration curve of diethyl succinate/100. [F]Calibration curve of limonene.

7.3.1. Discussion of results for volatiles compounds

The analysis of esters detected in white wines reveals many compounds of this organic family. The figure 7.19 and 7.20 are a graphic representation of esters in white and red wines of Fogo Island.

In the white wine Chã, phenylethyl acetate has higher concentration, 9.73 ± 0,.90 mg.L^{-1} among esters. The Montrond and Sodade white wines have respectively 3.18 ± 0.82 and 1.70 ± 0.05 mg.L^{-1}. These values of concentration are very high when compared with other determined in wines (Barros et. al., 2012).This compound is very important to wine quality due to the floral pleasant odor which it gives to wines (Jiang & Zhang, 2010; Sumby et. al., 2010).

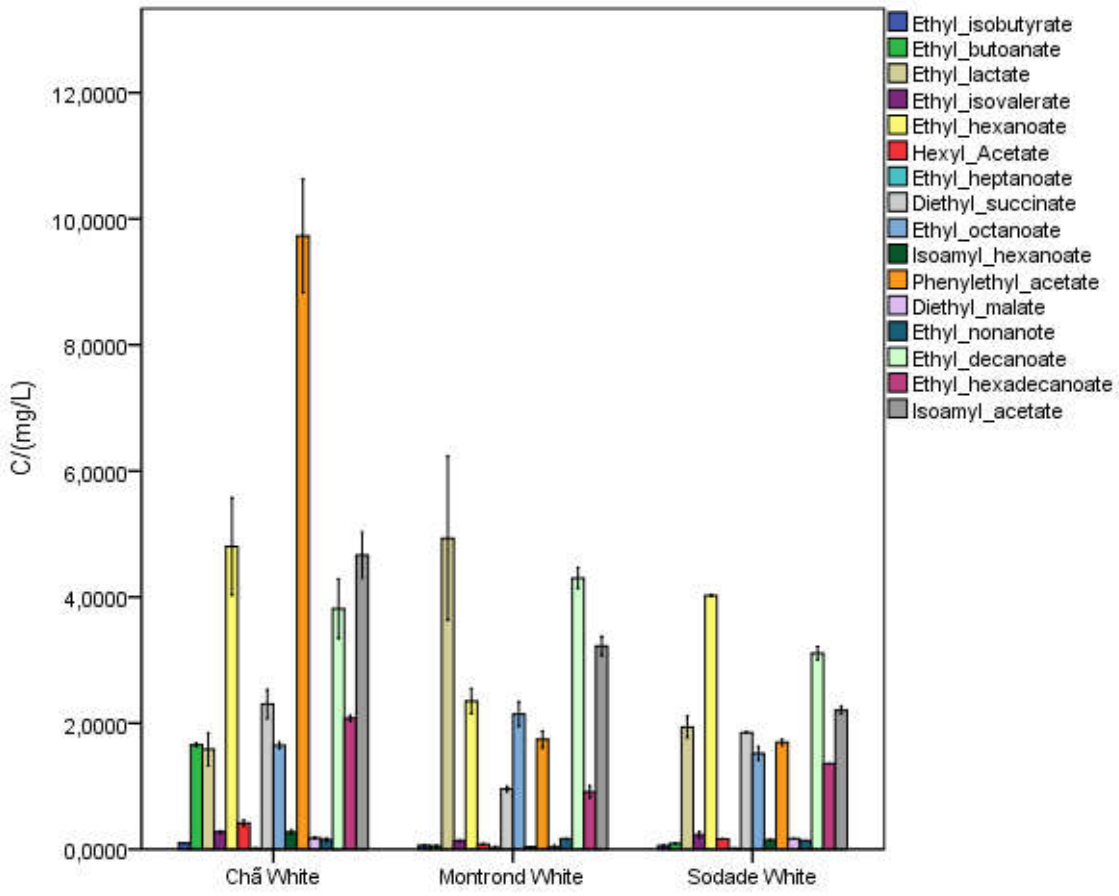


Figure 7.19 - Graphic representation of esters determined in white wines of Fogo Island.

In Chã, Montrond, Sangue Vulcão and Sodade red wines, the phenylethyl acetate has similar values of concentrations according to Tukey test. The Sodade rose wine presented high concentration of this compound, 5.08 ± 0.53 $mg.L^{-1}$. This compound is common in wines, representing in some wines 0.53% of ester (Bakker & Clarke, 2012). All these determined values are relatively high compared with wines from other regions or countries (Antalick et. al., 2010; Wang et. al., 2016).

The analysis of ethyl hexanoate determined in white wines show significant difference according to Tukey test. This ester are always present in the wines and it normally represent 0,55% of total esters (Bakker & Clarke, 2012). In white wines the concentration determined of ethyl hexanoate varied between 2.35 ± 0.16 to 4.80 ± 0.63 $mg.L^{-1}$. These values are very high compared with other wines (Antalick et. al., 2010; Barros et. al., 2012). But compared with Australian Verdelho wines, with ~2 $mg.L^{-1}$ the maximum concentration, the concentration in Cape Verde white wines are not very different (Sonni et. al., 2016).

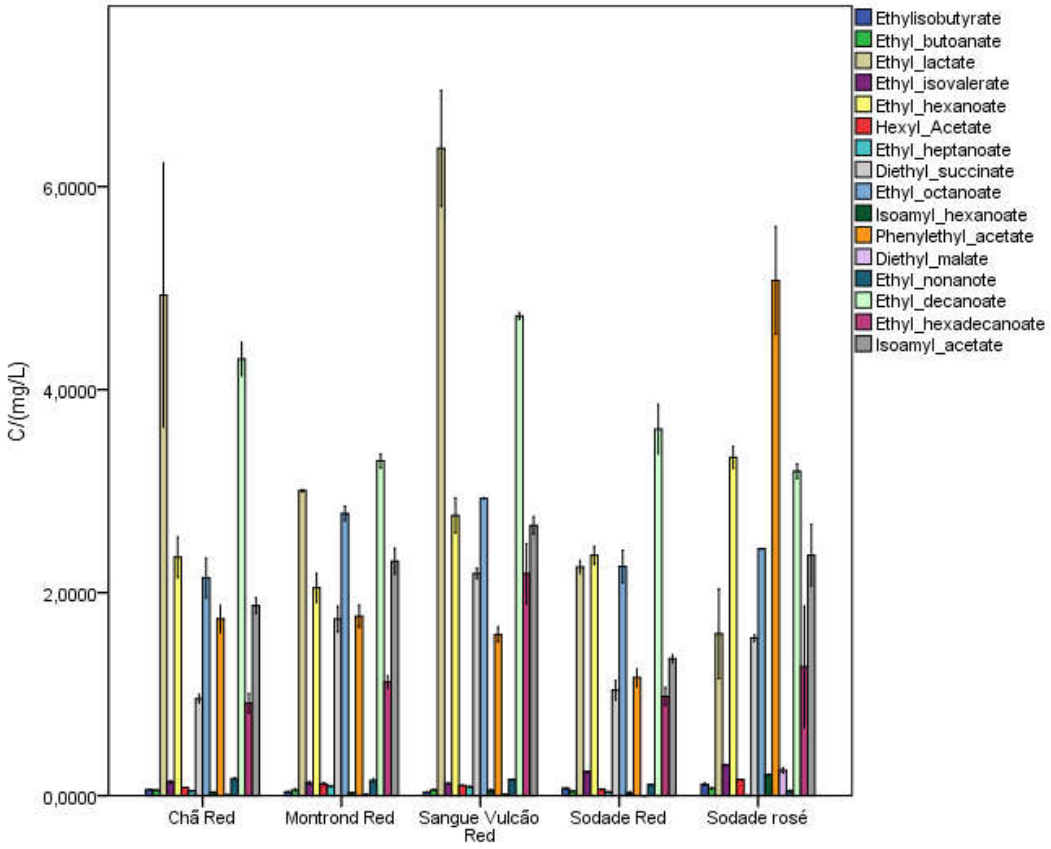


Figure 7.20 – Graphic representation of esters determined in red and rosé wines of Fogo Island.

In the red wines, the ester ethyl hexanoate has similar concentration in all samples. Sodade rosé wine has 3.33 ± 0.11 $mg.L^{-1}$ concentration. The concentration of this compound determined in the red and rosé wines are lower than for white wines but they are similar to wines from Cabernet Sauvignon, Cabernet Gernischet and Chardonnay Varieties grown in the Loess Plateau Region of China and Australian (Jiang & Zhang 2010; Sonni et. al., 2016).

Ethyl octanoate is an ester which concentration can be found 1.10 to 5.10 $mg.L^{-1}$ in white wines and 1,00 to 6,00 $mg.L^{-1}$ in red wines its common in the wines (Bakker & Clarke, 2012). It represent 3.78% of esters in some wines and it attribute to the wines an apple, ethereal and vinous odor (Bakker & Clarke, 2012). The concentration determined in the wines from Cape Verde varied 1.52 ± 0.11 to 2.33 ± 1.15 $mg.L^{-1}$. These concentration are similar those found in Australian Verdelho wines and Portuguese Arinto wines (Barros et. al., 2012; Sonni et. al., 2016).
In the red wines the concentration of ethyl octanoate are quite similar between them and these concentration are normally found in the wines (Bakker & Clarke, 2012; Jiang & Zhang, 2010; Sonni et. al., 2016).

For ethyl decanoate, a semi-quantitavive analysis in the white wines, the concentration of this ester varied between 3.11 ± 0.11 to 5.96 ± 2.31 $mg.L^{-1}$ equivalent of ethyl octanoate. In the red wines, Chã and Sangue Vulcão have the highest determined values of ethyl decanoate, 4.30 ± 0.17 and 4.73 ± 0.03 $mg.L^{-1}$ equivalent of ethyl octanoate respectively. The other red wines and rosé wine have similar concentrations around 3.20 ± 0.07 to 3.61 ± 0.26 $mg.L^{-1}$ equivalent of ethyl octanoate as show the table 6.10. This organic volatile compound is important to wines aroma because of the fruity and floral odor that it attribute to the wines and it represents 4.68% of esters (Antalick et. al., 2010; Bakker & Clarke, 2012; Sumby et. al., 2010).

Isoamyl acetate, a volatile compound which contribute with banana aroma to the wines, has a higher concentration in white wines than red and rosé wines. The Montrond and Chã white wines have respectively 4.73 ± 0.15 $mg.L^{-1}$ and 4.67 ± 0.36 $mg.L^{-1}$ equivalent of ethyl octanoate, and Sodade white wine has half of these values, 2.21 ± 0.06 $mg.L^{-1}$ equivalent of ethyl octanoate. This concentration is similar to red and rosé wines, except Sodade red wine which has 1.35 ± 0.04 $mg.L^{-1}$.This compound is common in all wines, red, white and rosé (Antalick et. al., 2010; Bakker & Clarke, 2012; Barros et. al., 2012; Wang et. al., 2016).

The diethyl succinate has similar concentration in all white, red and rosé wines analysed. It very common in wines with pleasant fruit odor, representing 12,90% ot total esters formed in some wines (Bakker & Clarke, 2012). The concentration determined in all wines from Cape Verde are similar to Portuguese and China wines (Barros et. al., 2012; Jiang & Zhang, 2010; Wang et. al., 2016).

All other detected esters, ethyl isobutyrate, ethyl butanoate, ethyl lactate, hexyl acete, diethyl malate, ethyl isovalerate, isoamyl hexanoate and ethyl hexadecanoate play an important rool in the characterization of wines with their fruit and floral aroma (Sonni et. al., 2016; Vilanova et. al., 2013; Wang et. al., 2016).

The analyses of alcohols in wines like show the tables 7.15 and 7.16, were detected hexanol, benzyl alcohol and 2-phenylethanol. The hexanol and benzyl alcohol were detected in all white wines, although they were not detected in Montrond red wine and Sodade rosé wine.
The 2-phenylethanol, compound with rose-like aroma, was detected in all analysed wines. The Chã white wine has higher concentration among white wines with 13.2 ± 1.7 $mg.L^{-1}$. In the red wines, Sangue Vulcão and Sodade have the highest concentration, 8.30 ± 0.49 and 8.09 ± 0.23 $mg.L^{-1}$ respectively. The concentration in the Sodade rosé wine, 10.7 ± 0.10 $mg.L^{-1}$ is higher than values determined in red wines. The values determined in all wines are similar to those found in Chinese and Spain wines but lower than Portuguese white wines (Barros et. al., 2012; Jiang & Zhang, 2010; Vilanova et. al., 2013; Wang et. al., 2016).

The acids detected were hexanoic and decanoic acids. The hexanoic acid gives a sweet like odor to wines, but decanoic acid gives a rancid and fat odor (Bakker & Clarke, 2012). Hexanoic acid was detected in all white wines, but in red wines it was not detected. Decanoic acid was detected in Chã and Montrond white wines. In the red wines it was detected Chã, Sodade and Sangue Vulcão red wines. These two acids were detected in Chinese, Australian and Spain wines (Sonni et. al., 2016; Vilanova et. al., 2013; Wang et. al., 2016).

The figures 7.21 and 7.22 are a comparison of alcohols and acids determined in white, red and rosé wines of Fogo Island.

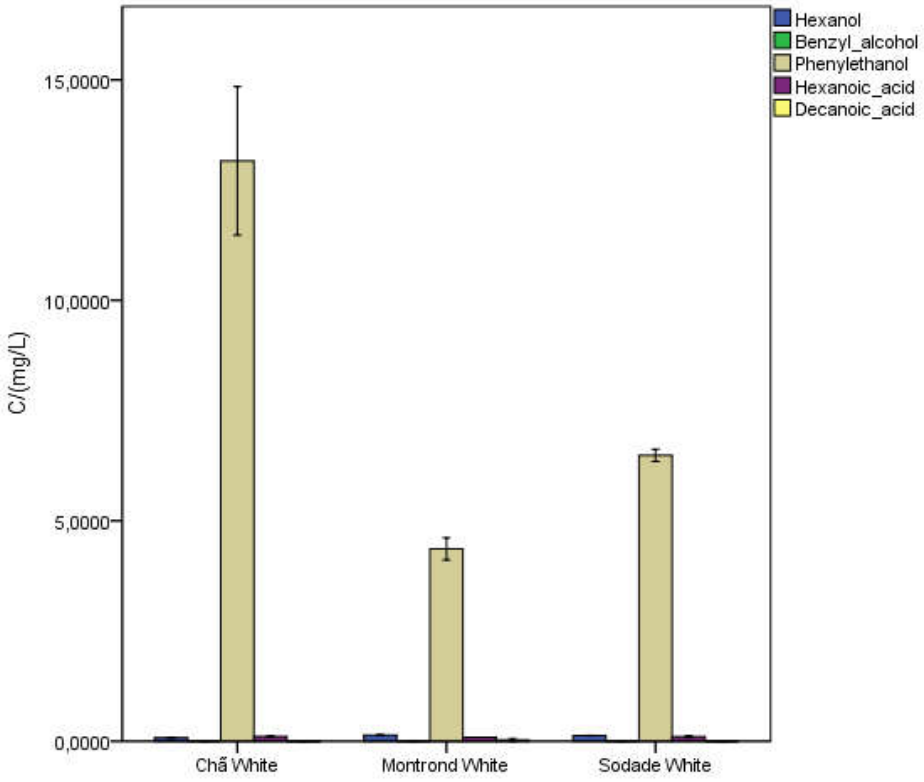


Figure 7.21 – Graphic representation of alcohols and acid detected in white wines of Fogo Island.

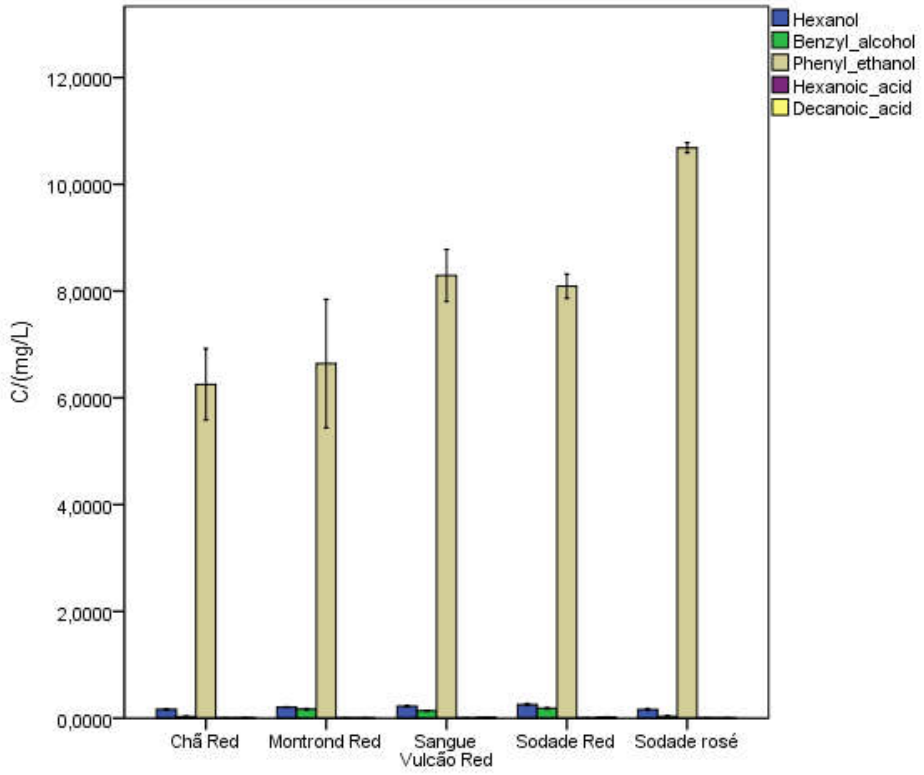


Figure 7.22–Graphic representation of alcohols and acid detected in red and rosé wines of Fogo Island.

The terpenes analysis, reveal the presence of α-pinene, limonene, γ-terpinene, terpinolene, linalool and α-terpineol. The values of concentration to α-terpineol in white wines are lower than the Australian and Portuguese wines but similar with Pinot Blanc from NW Spain (Barros et. al., 2012; Sonni et al., 2016; Vilanova et. al., 2013). The concentration determined of limonene in white wines varied between 3.81 ± 0.30 to 4.60 ± 0.82 $\mu g.L^{-1}$. These values of limonene in Cape Verdean white wines are lower than some Portuguese white wines (Barros et. al., 2012). In the red wines, the values of concentration of limonene determined are much lower than wines from Cabernet Gernischet and Chardonnay varieties grown in the Loess Plateau Region of China (Jiang & Zhang, 2010).

Also three terpenes were detected, but could not correctly identify which terpenes were they.

The figures 7.23 and 7.24 are a graphic comparison of all terpenes, norisoprenoides and sesquiterpenes detected in white, red and rosé wines.

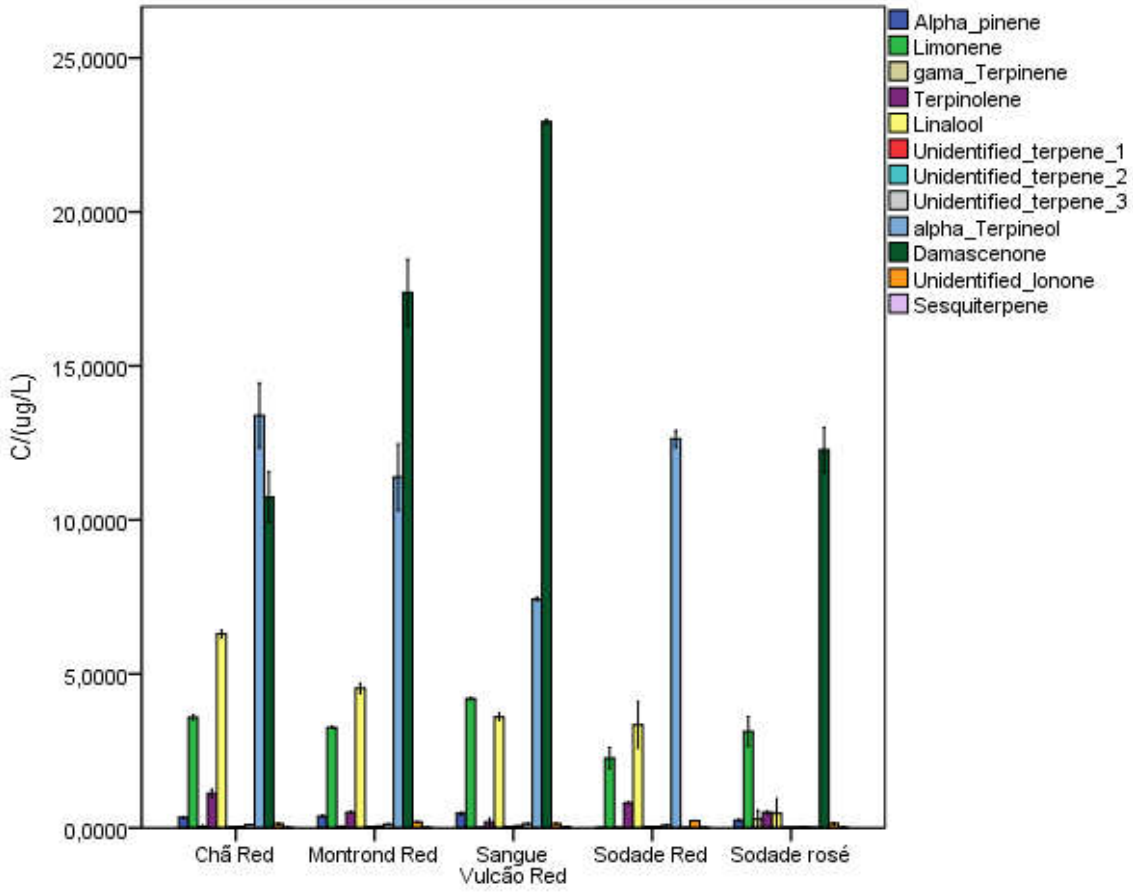


Figure 7.23 – Graphic representation of terpenes, norisoprenoids and sesquiterpenes in red and rose wines of Fogo Island.

In relation to norisoprenoids compounds, two compounds were identified, the β-damascenone and an ionone compound that was not possible to identify. β-damascenone was detected only in Chã red wine, while others wines such as Sodade red wine it was not detected. This compound was also detected in Madeira wines and French wines (Pereira et. al., 2014; Pineau et. al., 2007).

The others compounds, unidentified ionone and a sesquiterpene were detected in all analysed wines. All these identified compounds are responsible to varietal aroma wines and important to wines qualities (Coelho et. al., 2006).

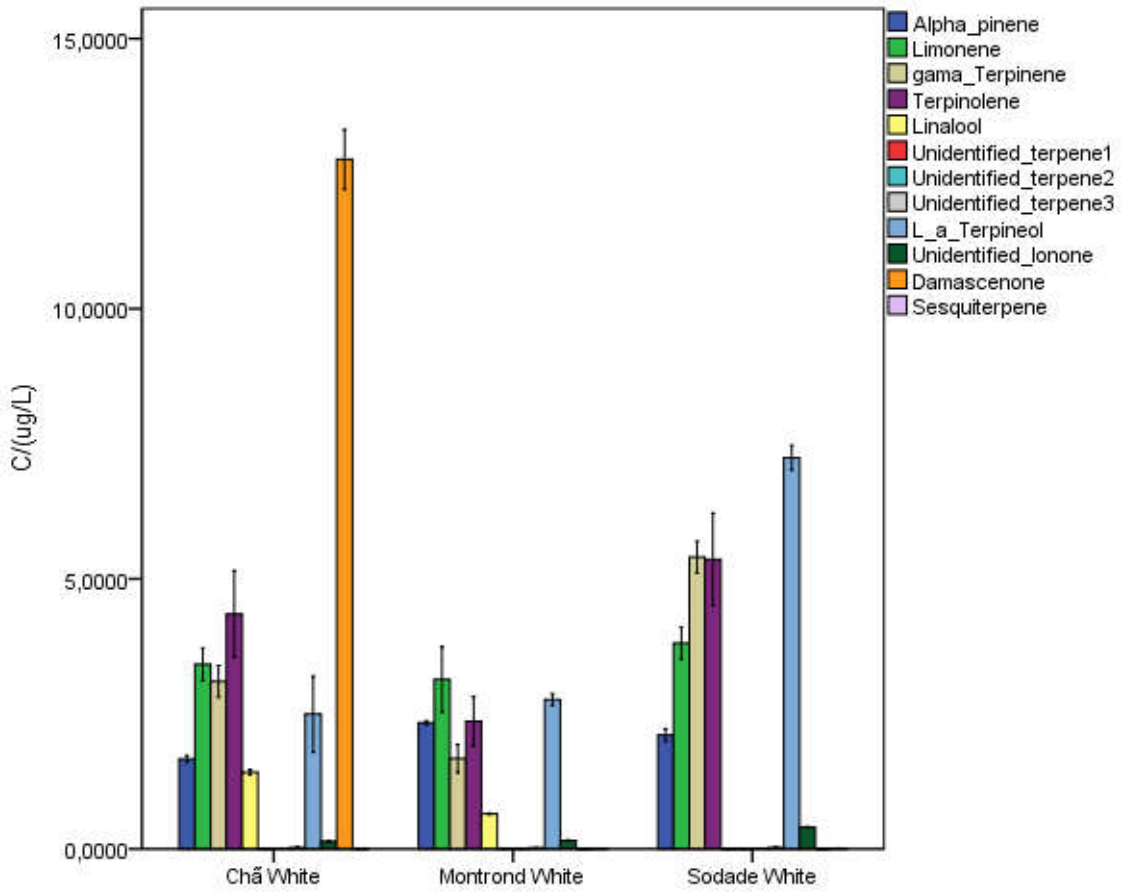


Figure 7.24 – Graphic representation of terpenes, norisoprenoids and sesquiterpenes in white wines of Fogo Island.

The total number of volatile aroma compounds detected in all wines is represented in the figure 7.25. The Chã white wine with 33 compounds, presented the majority number of compounds. The Chã red and Montrond white wines have similar number of compounds, 32 of total compounds, while Sangue Vulcão red wine has 31 compounds.

The Sodade wines, white and red, have 30 compounds. The Sodade rosé and Montrond red wines have lower numbers of volatile compounds 29 and 28 detected compounds.

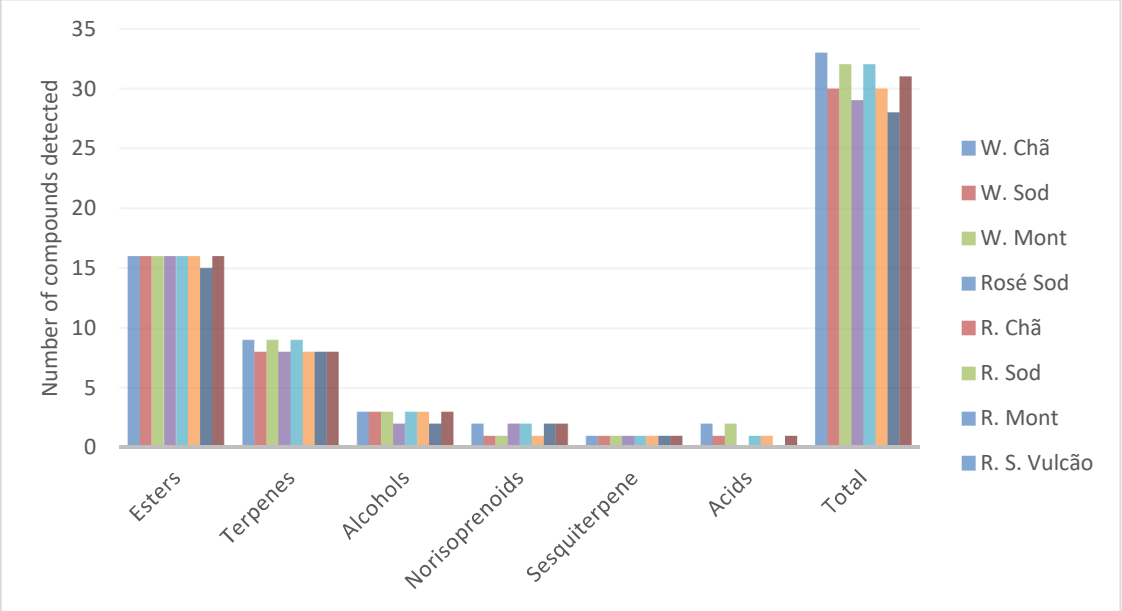


Figure 7.25 – Graphic representation of number of compounds detected in all analysed wines from Fogo Island.

7.3.2. Chemometric analysis of volatiles aromas compounds in the wines

For volatiles aroma compounds, in the PCA, the two principal component obtained, PC1 and PC2, explain 62% of variance and with PC3 77% of variance. The PCA is represented in the figure 7.26.

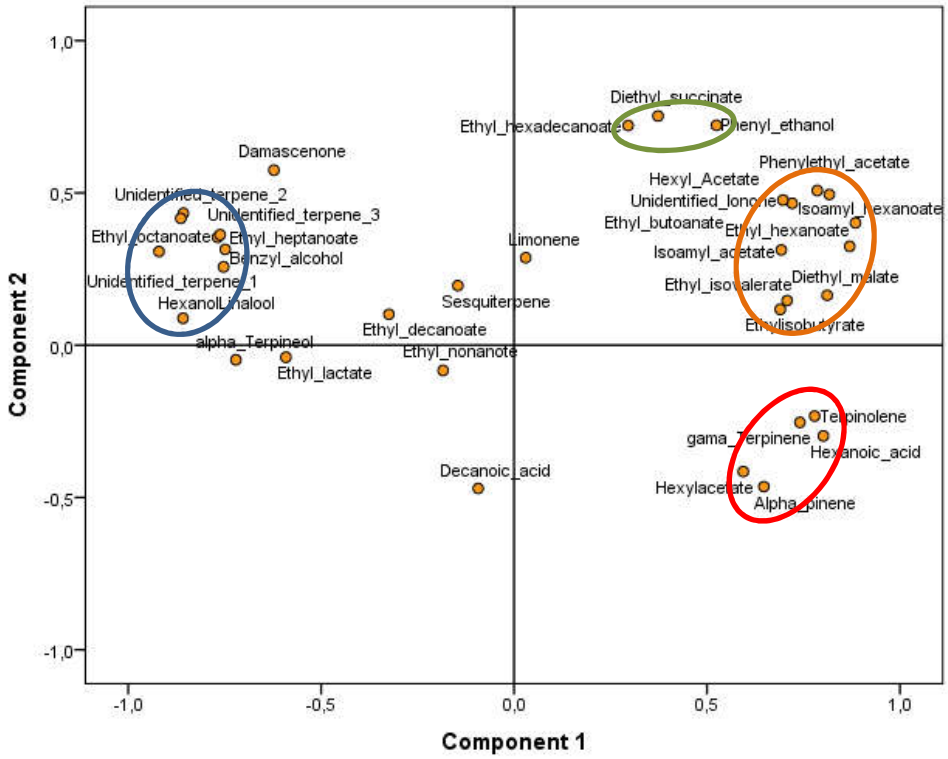


Figure 7.26 - PCA for volatiles compounds in the wines of Cape Verde

With the PCA is possible to verify some cluster of variables presented in the figure 7.26. Also some variables are completely dispersed one of the other.

The discriminant analysis presented in the figure 7.27 allow to classified the wines. All wines were classified according to the volatile compounds.

For the volatiles compounds were calculated seven discriminant functions, however the first and the second function explain 100% of variance. The main discriminant variables were nine esters presents on the table 7.17 and 7.18 with their respective coefficients.

The Chã white wine clearly stands out from the other wines. Despite all wines were classified as show the values on the table 7.19, it was not possible to verify separation of other wines in the figure 7.27.

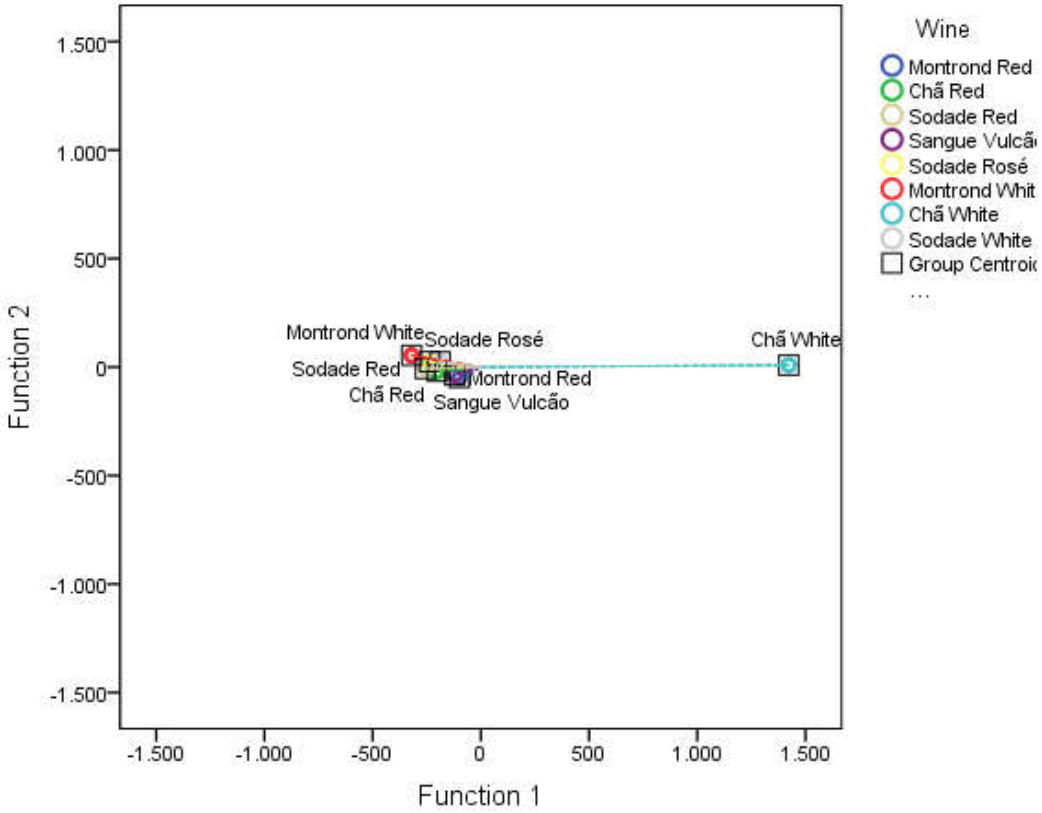


Figure 7.27 - 2D scatter plot of canonical scores of discriminant functions with volatiles aroma compounds.

Table 7.17 - Standardized canonical discriminant function coefficients

	Function						
	1	2	3	4	5	6	7
Ethyl isobutyrate	-3.156	1.116	1.273	0.547	0.642	0.269	0.038
Ethyl butanoate	11.339	-0.355	-0.062	-0.074	-0.148	0.146	-0.041
Ethyl lactate	-0.301	0.251	0.648	1.358	0.928	0.789	0.273
Ethyl isovalerate	-3.444	0.742	0.618	-0.171	-0.250	0.573	-0.378
Ethyl hexanoate	-4.176	1.255	-1.126	-0.769	-2.011	1.304	0.445
Hexyl acetate	12.624	-0.878	-0.289	-0.405	1.961	-1.582	0.438
Ethyl heptanoate	8.305	-4.812	-0.332	-0.270	0.169	0.620	-0.082
Diethyl succinate	0.975	0.908	1.411	1.901	-0.279	-0.138	-0.257
Hexyl acetate	-7.457	4.745	-0.414	0.864	0.092	0.026	-0.193

Table 7.18 - Classification Function Coefficients

	Wine							
	Montrond Red	Chã Red	Sodade Red	Sangue Vulcão	Sodade Rosé	Montrond White	Chã White	Sodade White
Ethyl isobutyrate	-107117.360	-31961.184	8257.404	-86156.104	7968.460	64835.111	-1113908.731	-30713.250
Ethyl butoanate	165100.818	64879.240	11824.903	143120.908	30119.994	-51324.403	1633895.384	77108.872
Ethyl lactate	22.327	70.637	92.716	70.577	128.796	164.749	-599.487	78.816
Ethyl isovalerate	-28155.203	-9859.094	32.711	-23705.022	-2004.431	12187.053	-279299.453	-10526.103
Ethyl hexanoate	-2538.206	-969.789	-154.299	-2186.740	-424.990	914.309	-23036.124	-961.425
Hexyl acetate	123554.451	46864.610	5601.120	105201.994	19316.181	-42828.596	1228664.719	53799.806
Ethyl heptanoate	170621.758	59424.362	1500.383	143146.540	1739.517	-91244.653	1564310.522	47638.066
Diethyl succinate	2265.272	1396.922	1052.631	2481.958	1862.927	1099.059	17034.434	2126.494
Hexyl acetate	-29645.979	-9979.748	131.964	-24597.885	244.586	17244.592	-272360.649	-7567.369
(Constant)	-15690.348	-3139.273	-1247.681	-12725.569	-3630.966	-5658.606	-1433697.008	-5745.260

Fisher's linear discriminant functions

The table 7.19 shows the values of validation of classification and 100% of samples were correctly classified relating to the functions.

Table 7.19 - Classification matrix for volatiles compounds

		Wine	Predicted Group Membership								Total
			Montrond Red	Chã Red	Sodade Red	Sangue Vulcão	Sodade Rosé	Montrond White	Chã White	Sodade White	
Original	Count	Montrond Red	3	0	0	0	0	0	0	0	3
		Chã Red	0	3	0	0	0	0	0	0	3
		Sodade Red	0	0	3	0	0	0	0	0	3
		Sangue Vulcão	0	0	0	3	0	0	0	0	3
		Sodade Rosé	0	0	0	0	3	0	0	0	3
		Montrond White	0	0	0	0	0	3	0	0	3
		Chã White	0	0	0	0	0	0	3	0	3
		Sodade White	0	0	0	0	0	0	0	3	3
	%	Montrond Red	100	0	0	0	0	0	0	0	100
		Chã Red	0	100	0	0	0	0	0	0	100
		Sodade Red	0	0	100	0	0	0	0	0	100
		Sangue Vulcão	0	0	0	100	0	0	0	0	100
		Sodade Rosé	0	0	0	0	100	0	0	0	100
		Montrond White	0	0	0	0	0	100	0	0	100
		Chã White	0	0	0	0	0	0	100	0	100
		Sodade White	0	0	0	0	0	0	0	100	100

a. 100,0% of original grouped cases correctly classified.

The discrimination of wines is confirmed by hierarchical cluster analysis, presented by dendogram in the figure 7.28. In the dendogram, there is the unique classification to Chã white wine too far away from other wines. All the other wines despite some proximity are classified into different groups that previously was not possible to properly display in the figure 7.27.

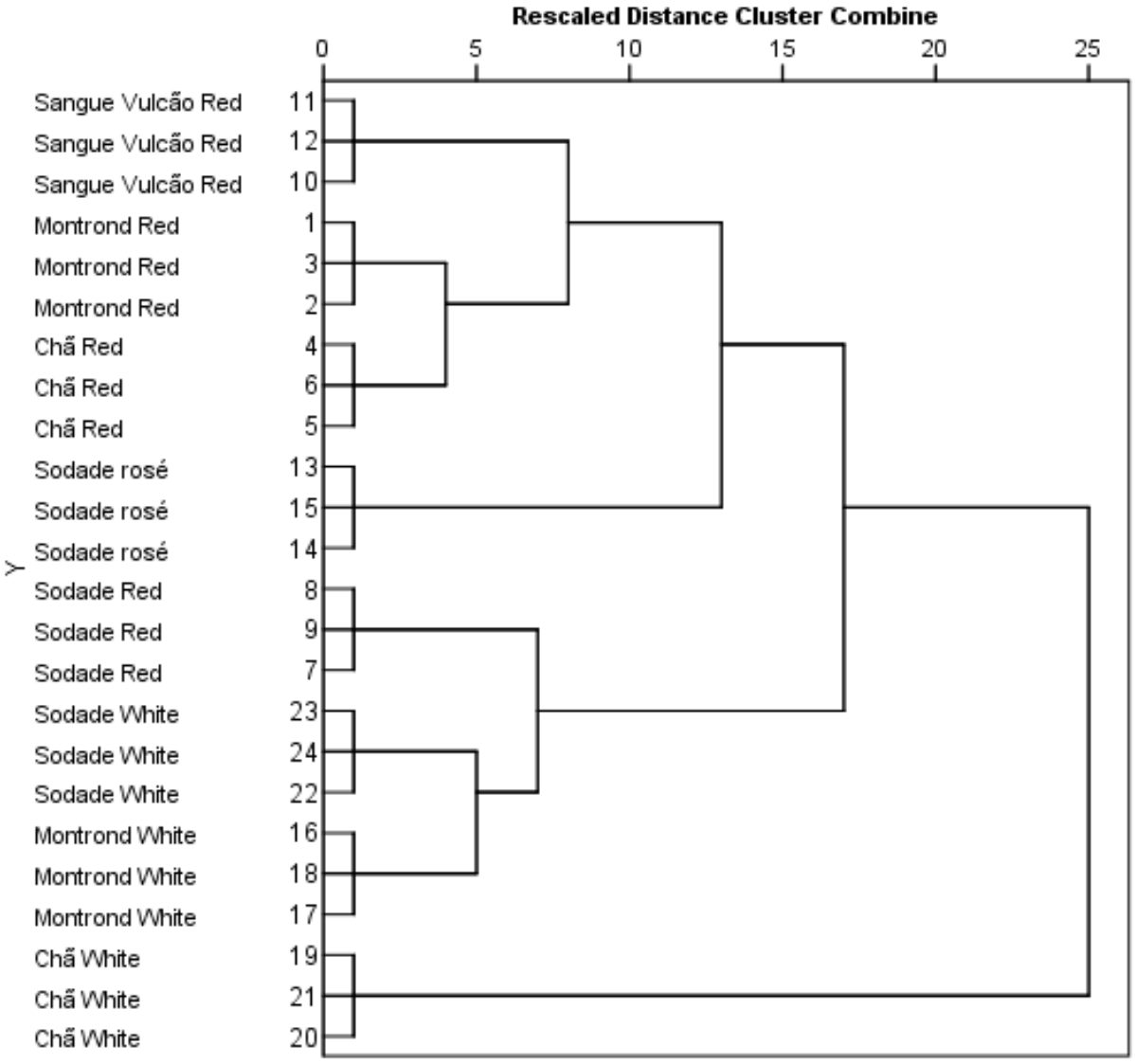


Figure 7.28 – Dendogram of cluster analysis obtained with volatiles aroma compounds in the wines

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.

The chemometric analysis through the principal components analysis, linear discriminant analysis and cluster analysis were successfully applied to make distinction of the wines according to their phenolic compounds. Through phenolics compounds there are a clear distinction between the four red wines, Chã, Sodade, Sangue Vulcão and Montrond. Specially the Chã red wine was clearly distintict from the other wines by chemometric analysis through phenolic compounds. For white wines it was not possible to make the differentation with the phenolic compounds.

The wines from Fogo Island analysed presented various types of sulfur compounds and beyond the standards used, appeared others compounds with intense peaks in the chromatogram which were not identified. The diversity of sulfur compounds in wines and their high concentrations may be related to the presence of sulfur in natural volcanic soil that is used as germicide, allowing an increased of sulfur compounds.
The concentrations of some sulfur compounds are relatively high compared to the amounts normally found in wines, mainly methionol, which concentration is very high in Sodade rosé wine. The unidentified compounds in the chromatogram have an intense peaks in the chromatograms, suggesting that possibly have relatively high concentrations in the wines.

Generally, all compounds detected in some wine arise are present in another wines, which can be explained by the use of grapes from the same region and the application of the same production techniques.The application of chemometrics analysis with heavy sulfur compounds, allowed the distinction of Sodade and Sangue Vulcão red wines from other wines analysed. Some wines, Montrond red wine and Sodade white wine, are in the same class or group and they have similarities with Chã white wine.

Within volatiles compounds, the wines from Fogo Island have presented a great number of esters, terpenes, some norisoprenoids, alcohols, sesquiterpenes and acids. A qualitative analysis of the red, rosé and white wines analysed showed a profile of volatiles compounds not very different among of them. With the exception of hexanoic and decanoic acid that contribute to unpleasant odor to the products, all the identified volatiles compounds give a good and pleasant odor to the wines. The esters and terpenes are the major volatiles compounds present in all analysed wines, because of their fruity and flower aroma they are very important to the quality of wines.

The Chã white wine presented highest concentration of volatiles aroma than other white wines, mainly 2-phenylethyl acetate and damascenone, which concentrations were very high. Through the volatiles aroma compounds, all the wines were been distinguished by chemometric analysis mainly Chã white wine which is very distincted from other wines.

Despite some similarities between of the red and white wines, they are in different groups or class according to their volatiles aroma compounds.

The trade of the wines in Cape Verde is very important to the economy of Fogo Island. This was the first study to chemical level of the wines, but more should be done to evaluate and to improve the quality of this product.

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