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COMPOSITION INVESTIGATION AND CHARACTERIZATION OF SOME ROMANIAN WINE VARIETIES BY CHROMATOGRAPHIC AND SPECTRAL METHODS

PhD Thesis
Summary

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Keywords: Romanian wine, volatile compounds, $^{18}O/^{16}O$ isotope ratio, metals, GC/MS, IRMS, ICP-MS

Contents

Introduction

Wine is a popular alcoholic beverage consumed worldwide, known since the early periods of civilization. Moderate consumption of wine, especially red wine, has been shown to have beneficial effects on health and longevity. Its commercial value derives in most of the geographical location of vineyard, the grape variety and year of winegrape production. Knowing this fact has led to regulations in most wine producing regions, and the concern to develop different methods to verify in particular the its origin and harvest year [1]. Wine is a complex matrix, its major components being water, alcohol and sugars. In addition it contains a large number of chemical compounds such as amino acids, phenols, volatile compounds, stable isotopes and trace and ultra-trace metals. Many of these chemicals can be used as a parameter to distinguish the origin of the wine. The concentrations of these compounds are significantly influenced by many factors as: wine region, agricultural practice, grape variety, soil type, climate and, not least, the winemaking process. Accordingly, these factors could play an important role in the characterization and differentiation between wines [2]. Wine characterization depending on the grape variety and geographical origin can be done effectively by determining of some parameters such as, the composition of volatile compounds, elemental composition and isotopic ratios of oxygen and hydrogen. Wine is one of the agricultural products whose flavor and taste are influenced by where it originates. Specific environment in which the grape-vine grew plays a very important role, which is reflected in the flavor and aroma of the vineyard from which the wine belong [3]. The volatile composition can be very useful in the identification and certification of wine quality categories such as *mono varietal wine*, *young*, *aged* or *wines with Denomination of Origin* (DO) [4]. Gas chromatography coupled with mass spectrometry (GC-MS) is a commonly used method for the isolation and identification of volatile compounds in wine and food due to its high sensitivity and reproducibility. In recent years, the isotopic ratios of oxygen and hydrogen in wine are used both to detect adulteration and to identify or verify the geographic area they come from, because their values are predictable considering the region, the climate and the level of rainfall. This spatial variation in rainfall is recorded in plant material because plants take water from the soil (which comes mostly from local precipitation) and incorporates hydrogen and oxygen atoms in the products of photosynthesis. Also, the metal content of wines is of great interest because of their contribution to its stability, due to possible toxicological concern and the fact that some metals were regulated by law. The main interest, however, is to use metal content in order to characterize wines according to their geographical origin, taking into account the relationship between wine and metals present in the composition of the soil [5].

Consequently, the goal of this thesis is to analyze the chemical composition of a large number of wines from the major wine regions of Romania. We investigated the volatile content, metal content and the oxygen isotope ratio. A thorough study of their values, depending on the geographical origin, variety, and year of production, may be a useful tool for differentiation and to establish a possible criteria of authenticity.

The thesis is divided into two distinct parts, a bibliographic one describing the parameters involved and the experimental methods used and the part containing the results and their analysis. Thus, Chapter 1 presents general aspects of wine features, details about wine-making technology of red and white wines, their chemical composition and phases of evolution of the wine. Chapter 2 describes in more detail in the wine chemical compounds which can be used as parameters to characterize and establish their geographical origin. Chapter 3 contains a description of instrumental analytical methods used. In Chapter 4 we present the volatile compounds identified by GC-MS and their analysis. Chapter 5 contains the determination of the oxygen isotopic ratios ($^{18}O/^{16}O$) using *isotope ratio mass spectrometry* (IRMS), and in Chapter 6, we present an analysis of the metal composition of investigated wines determined by *mass spectrometry inductively coupled plasma* (ICP-MS). This summary of the thesis contain only the experimental part and personal contributions.

CHAPTER 1

Determination of volatile compounds of wine by gas chromatography coupled with mass spectrometry

1.1 Samples Preparation

In this study we investigated 27 samples of commercial white wines of the four most important wine regions in Romania: Oltenia (Oprişor and Vânju-Mare vineyards), Muntenia (Cramele Halewood and Ceptura vineyards), Moldova (Huşi vineyard) and Transylvania (Jidvei vineyard). Wine varieties chosen were *Sauvignon Blanc*, *Feteasca Albă* şi *Riesling* from three consecutive years: 2008, 2009 and 2010 (Table 1.1).

Liquid-Liquid Extraction. The protocol used in this work for the extraction of volatile compounds from wine samples was adapted from that described by *I. Andujar-Ortiz et al.* [6]. Thus, 25 mL sample of wine and 5 ml of methylene chloride (Merck) were transferred to a flask equipped with ground stopper which was then placed on the magnetic stirrer. The extraction was carried out under continuous stirring in an ice bath (at about 0°C) for one hour. Subsequently, the mixture was kept for 15 minutes in an ultrasonic bath at the same temperature of about 0°C, to avoid possible formation of emulsions. After separation, the organic phase was collected using a Pasteur pipette and centrifugated. In order to remove any water, it was filtered through a layer anhydrous sodium sulfate. The obtained extract was concentrated by rotary evaporation at about 200μL Subsequently 1μL of extract was injected into the GC-MS spectrometer using the "splitless" mode.

Table 1.1 *White wine samples investigated.*

No. crt.	Wine Variety	Code	Vineyard	Production year
1	Sauvignon Blanc	SBPV	Muntenia (Vineyard I) (Cramele Halewood, Dealurile Munteniei)	2008
2		SBPV		2009
3		SBPV		2010
4		SBO	Oltenia (Vineyard I) (Oprişor, Dealurile Olteniei)	2008
5		SBO		2009
6		SBO		2010
7		SBCS	Oltenia (Vineyard II) (Mehedinţi, Vânju-Mare)	2008
8		SBCS		2009
9		SBCS		2010
10	Fetească Albă	FAH	Moldova (Dealurile Huşilor)	2008
11		FAH		2009
12		FAH		2010
13		FACH	Muntenia (Vineyard I) (Cramele Halewood, Dealurile Munteniei)	2008
14		FACH		2009
15		FACH		2010
16		FASC	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2008
17		FASC		2009
18		FASC		2010
19	Riesling	RSC	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2008
20		RSC		2009
21		RSC		2010
22		RCS	Oltenia (Vineyard II) (Mehedinţi, Vânju-Mare)	2008
23		RCS		2009
24		RCS		2010
25		DRJ	Transylvania (Jidvei)	2008
26		DRJ		2009
27		DRJ		2010

1.2 GC-MS Analysis

The wine samples were analyzed using the gas chromatograph system (*Trace GC*) coupled with a mass spectrometer (*Polaris Q*) (Thermo-Finnigan). For separation, a capillary column was used with the stationary phase from methyl-phenyl siloxane class (5%) type HP 5-MS with a length of 30 m, the inner diameter of 0.25 mm and thickness of the stationary phase of 0.25μm. Helium was used as eluent, at a constant flow rate of 1.5 mL/min. The column temperature program was: the initial temperature of 50°C was maintained for two minutes, and then was increased linearly at a rate of 10°C/min

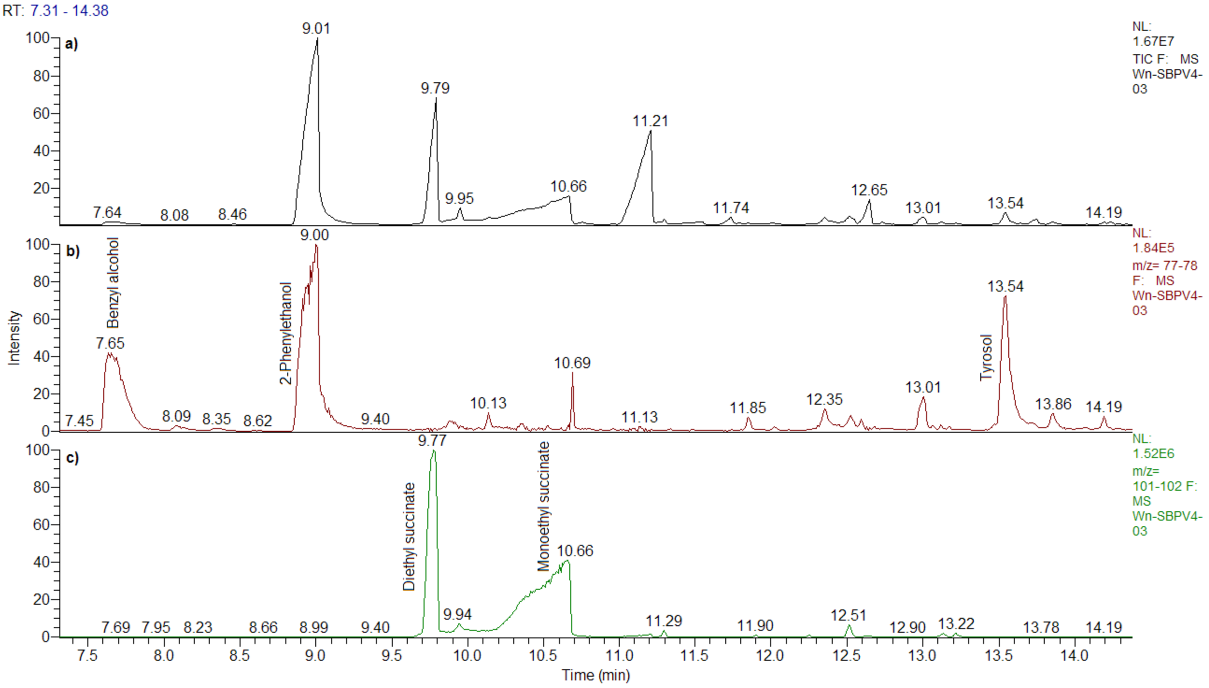

Figure 1.1 *GC-MS chromatogram of a sample of Sauvignon Blanc originating from Muntenia: a) total ions chromatogram (TIC); b) m/z=77; c) m/z=101.*

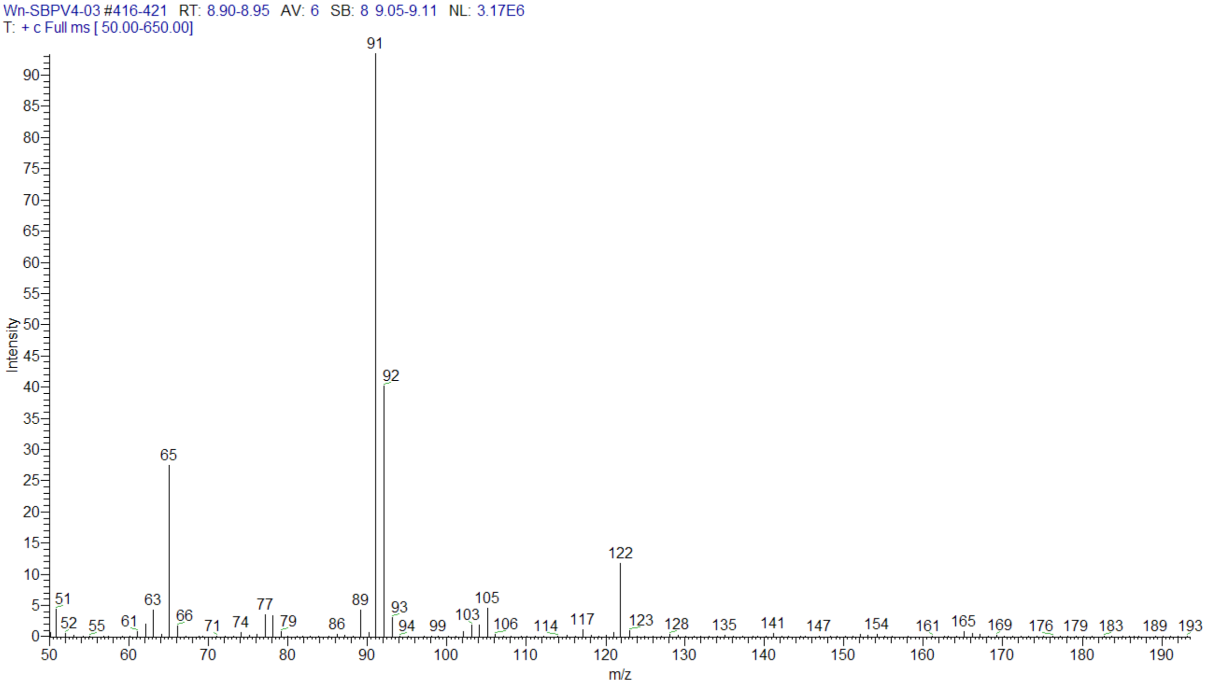

Figure 1.2 *Mass spectrum obtained for 2-phenyl-ethanol (Sauvignon Blanc wine sample, Muntenia)*

up to 300°C where it was maintained for 10 minutes. The temperature of the injector was 250°C. The mass spectrometer is equipped with an electron impact ionization source

(EI) with 70 eV ionization energy. The temperature at the interface and at the ion source were 300°C and 250°C. The acquisition was performed in the *"full scan"* mode and the mass domain scanned was from 50 to 650 Daltons.

1.3 Results

During GC-MS analysis, forty eight volatile compounds were identified belonging to eight different classes, such as: alcohol, ethyl esters, fatty acids, phenolic compounds, aldehydes, ketones, terpenes and lactones. In most cases the identification of compounds was achieved by comparing measured mass spectra with existing *NIST* library spectra and the more difficult cases also with those published in the literature [7, 8]. Volatile compounds detected by GC-MS were grouped in classes of chemical structures and are reported as percent area (%-Area). Compounds identified were consistent with other results obtained by different authors in recent years [9–16].

To perform the quantitative analysis of identified volatile compounds we considered the chromatographic areas of each compound separately, using the basic ion intensity, and then, the percentage concentrations were calculated for each compound. The study of the structures and concentrations of detected compounds indicates the following features:

(a) *Higher alcohols* and *ethyl esters* are the most important group of volatile compounds representing more than 90 % of the volatile fraction identified in the analyzed wines, a situation reported also by other authors [9].

(b) *Fatty acids*, *phenols*, *aldehydes*, *ketones*, *lactones* and *terpenes* were found in small quantities. These compounds are produced by yeast during alcoholic fermentation and have an essential role, influencing wines aroma.

(c) From the identified alcohols the most abundant were the aromatic alcohols: *2-phenyl-ethanol*, *tyrosol* and *triptophol* formed during alcoholic fermentation by enzymatic degradation of amino acids or by catabolic Ehrlich reaction.

(d) The most abundant *ethyl esters* were the *esters of diprotic acids* (diethyl succinate, monoethyl succiate and diethyl malate).

In Figure 1.1 we present the characteristic chromatogram of *aromatic alcohols* and *esters of diprotic acids* in a *Sauvignon Blanc* sample originating from *Muntenia* region and in figure 1.2 the mass spectrum of 2-phenyl-ethanol. The chromatogram obtained with the selected ion $m/z = 77$ (figure 1.1 b) contain phenol type compounds (benzyl

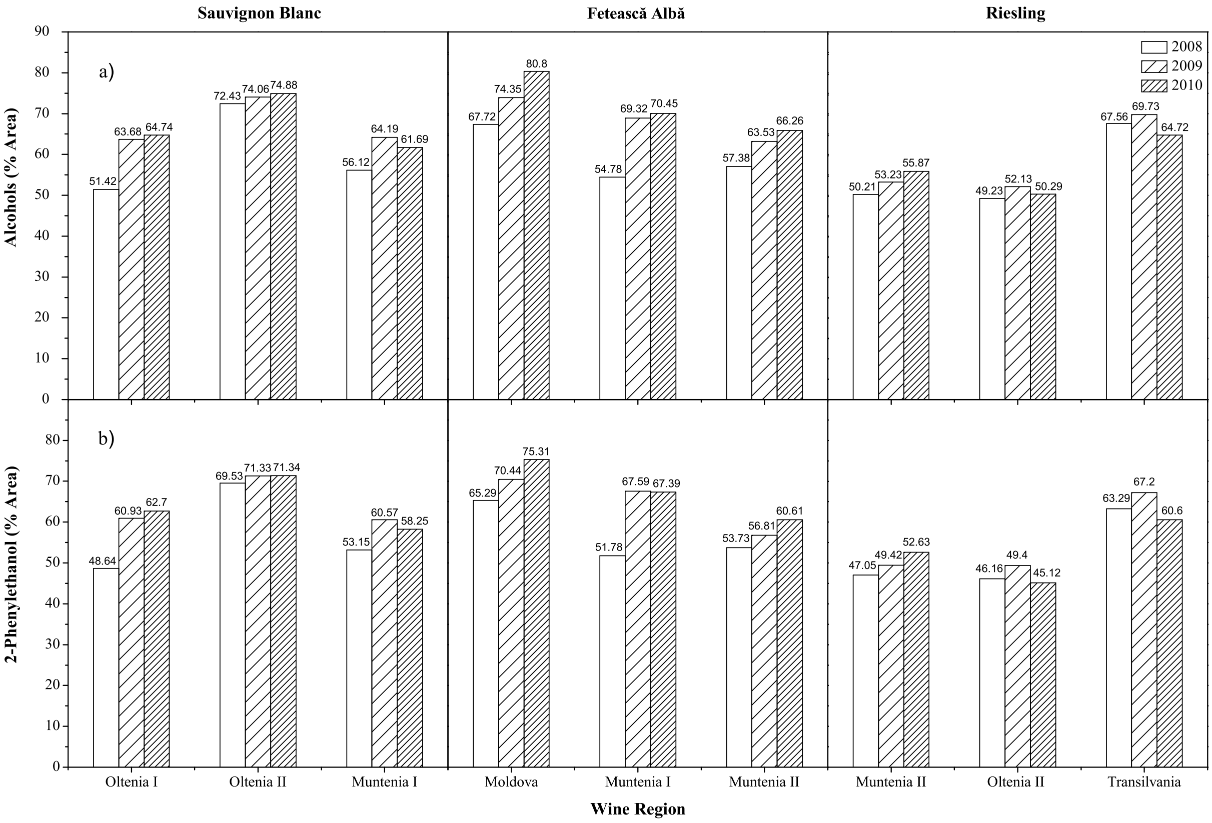

Figure 1.3 *The amount of a) alcohol and b) 2-phenyl-ethanol determined from wine varieties, Sauvignon Blanc, Fetească Albă and Riesling from three consecutive years.*

alcohol, 2-phenyl-ethanol and tyrosol), and the the chromatogram with selected ion m/z $= 101$ (Figure 1.1 c) is characteristic for the *esters of diprotic acids* (diethyl succinate and monoethyl succiate).

1.3.1 Study of geo-climatic influence on volatile fraction of wines

To emphasize the geo-climatic influence on the volatile fraction of wines, we used three types of wines, *Sauvignon Blanc*, *Fetească Albă* and *Riesling*, from the most important wine regions of Romania: *Oltenia*, *Muntenia*, *Moldova* şi *Transylvania*.

1.3.1.1 Higher Alcohols

Higher alcohols are quantitatively the most important group of volatile compounds determined and accounted for more than 50% of the volatile components in wine case, situation known and confirmed in the literature [17]. However, their content vary from brand to brand and from one vineyard to another, which can be observed in Figure 1.3.

The most important alcohols in the investigated wines were the **aromatic** ones: *2-phenyl-ethanol*, *tyrosol*, *triptophol* and *benzyl alcohol*. From the class of **aliphatic alcohols** was detected only the *3-methyl butanol*, but in a low amount, in some samples being less than 1% of the alcohols identified, a situation also found in the literature [11].

1.3.1.2 Ethylic esters

Thirteen esters were identified in the investigated white wine samples, three of which being fatty acid ethyl esters, nine esters of carboxylic acids (succinic, malic, citric) and an ethyl ester of a heterocyclic carboxylic acid (2-furoic acid).

In Figure 1.4 is represented the total content of ethyl esters measured for three different types of wines for three consecutive years and also the evolution of the most abundant diprotic esters. This time there is a decreasing trend of their contribution from year to year, contribution correlated with the upward trend observed for alcohol. This correlation is due to the fact that the esters are the second class of compounds in the order of abundance, the average value in the investigated wines being of approximately 30% from the total of identified compounds.

1.3.1.3 Fatty Acids

Seven fatty acids were identified in the investigated wines (butanoic, hexanoic, octanoic, decanoic, dodecanoic, tetradecanoic and hexadecanoate acids). Their level was very low in all three types of wine but in concordance with that found in other different wine varieties [18].

In Figure 1.5 we present the characteristic chromatogram of fatty acids and esters for a sample of *Riesling* in Transylvania, and in figure 1.6 the mass spectrum of octanoic acid. chromatograms obtained when selecting ions with m/z=60 (figure 1.5 b) and m/z=61 (figure 1.5 c) are compunds of fatty acid type (butanoic, hexanoic, octanoic and decanoic acids) the corresponding esters.

We note that they are equidistant in time, hovering at about three minutes of each other, differing in mass by two methylene groups (CH_2). In the case of fatty acids, due to their high polarity, there is a widened shape of the peak due to interaction with non-polar stationary phase.

Hexanoic and *octanoic* acids accounts for the largest contribution to the total amount of fatty acids, whose average values are about 0.9% and 1.3%. Of these, *octanoic acid* has a slightly higher level in all three types of wine, being consistent with other results reported in the literature [9, 18, 19].

Figure 1.4 shows the percentage contribution of fatty acids in the wines investigated.

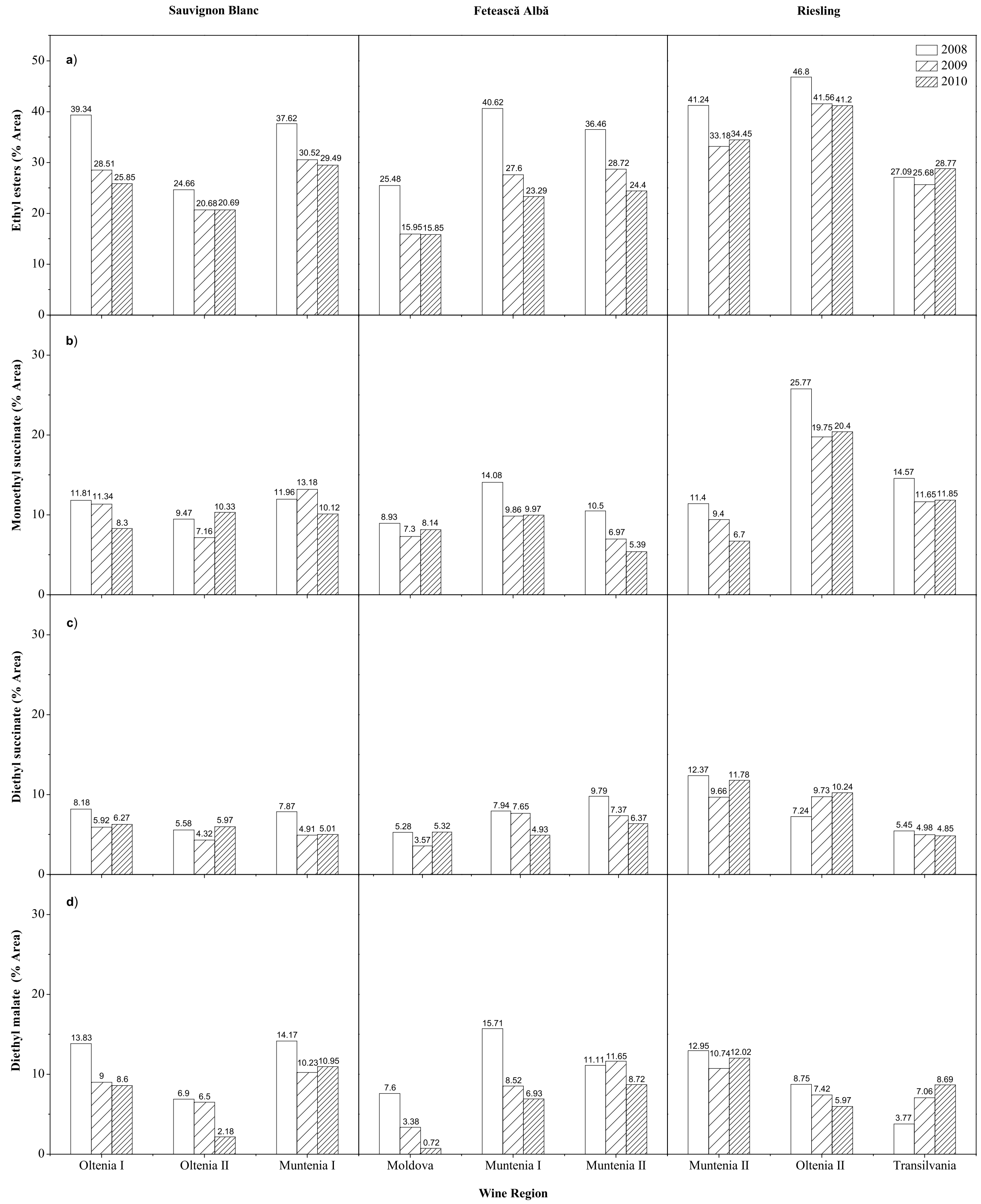

Figure 1.4 *The total content of ethyl esters in different types of wine measured for three consecutive years (a) and the esters of the most abundant diprotic acids: (b) monoethyl succinate, (c) diethyl succinate and (d) diethyl malate.*

1.3.1.4 Phenolic volatile compounds

Fourteen phenolic compounds were found in the investigated wines, the most important being: *4-vinyl guaiacol*, *styrene*, *vanillic acid methyl ester*, ethyl esters of *vanilic*,

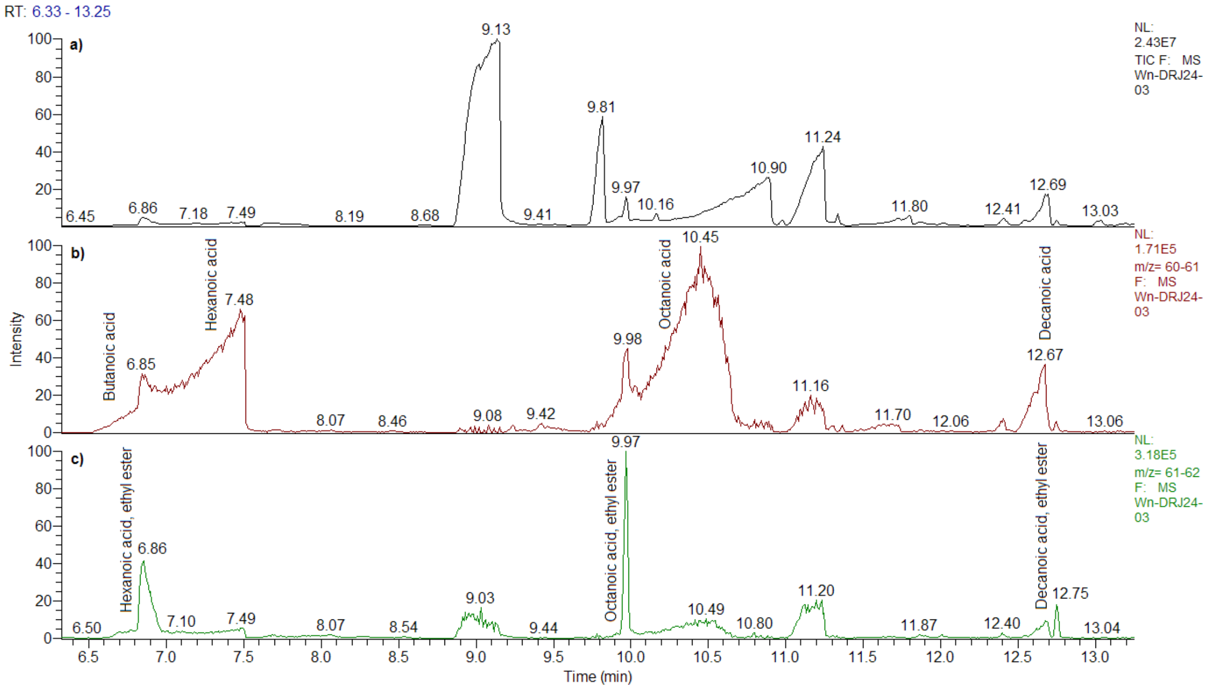

Figure 1.5 *GC-MS chromatogram of a sample of Riesling in Transylvania: a) TIC; b) m/z=60 (acids); c) m/z=61 (esters).*

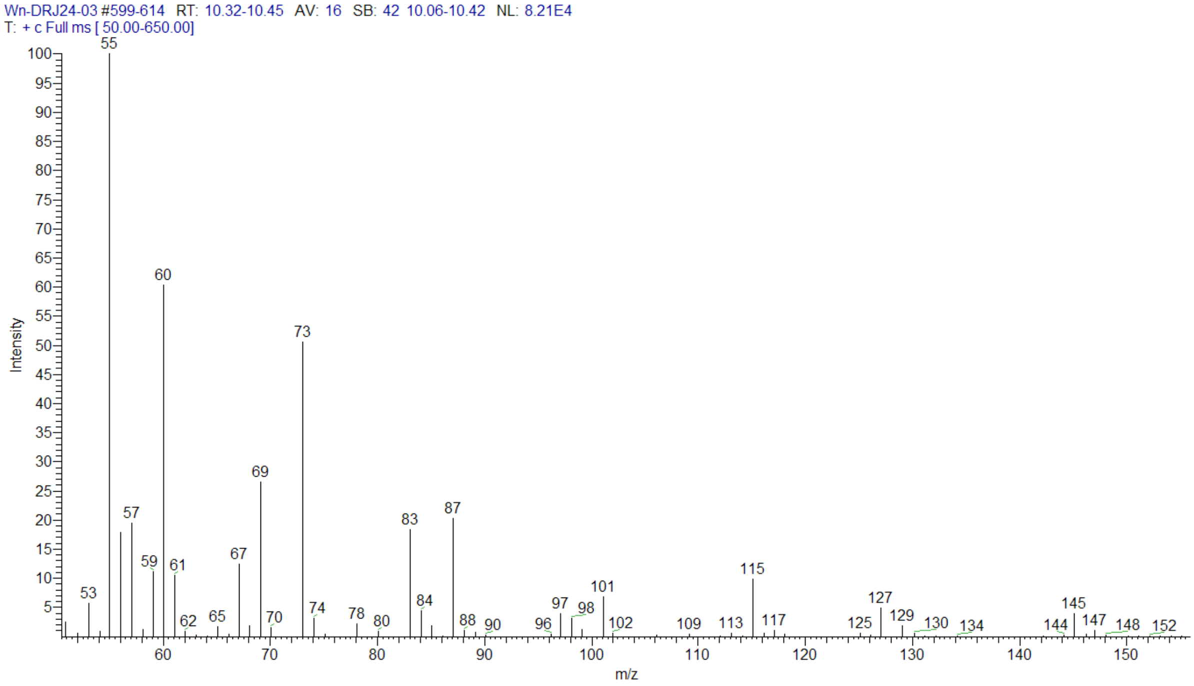

Figure 1.6 *Mass spectrum obtained for octanoic acid in a sample of Riesling wine from Transylvania.*

ferulic, *p-hydroxy cinnamic*, *o-* and *m-hydroxy cinnamic* acids. According to the literature a higher content of *ethyl p-coumarate* or *ethyl ferulate* can be an indicator that the

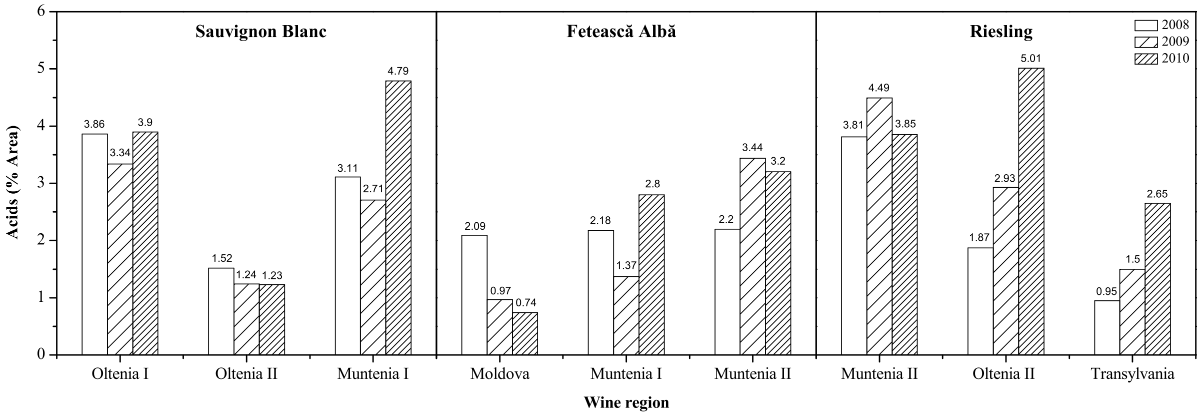

Figure 1.7 *The amount of fatty acids for the three wine varieties originating from different regions and from three consecutive years.*

wine was aged in barrels [20]. Also, the *4-vinyl guaiacol* is found only in whites wine and its presence in the red and rosé wines can serve as an indicator for recognizing the mixture with white wines [4]. In the Figure 1.8 the total phenolic compounds profile is presented in the three types of wine analyzed.

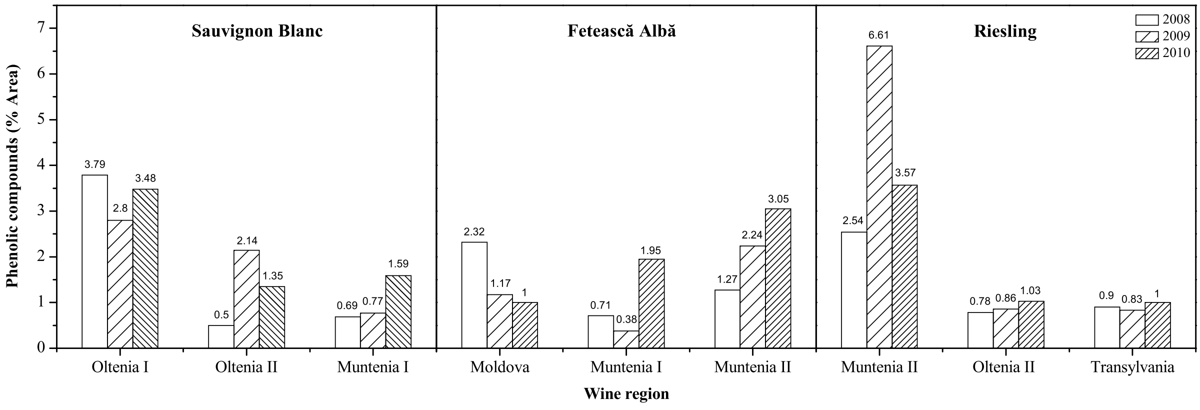

Figure 1.8 *The content of phenolic compounds.*

A higher content of *ethyl p-coumarate* was obtained in *Sauvignon Blanc* from Oltenia (vineyard I) in 2008. The level of *4-vinyl guaiacol* is low, its values being below 0.1% with *Fetească Albă* being an exception, as in Moldova and Muntenia (vineyard II) where values close to 0.2% from the total volatile compounds identified were obtained.

1.3.1.5 Aldehydes

Four aromatic aldehydes were detected namely *phenylacetaldehyde*, *4-hydroxy-2-methoxy benzaldehyde*, *benzene butanal* and *syringic aldehyde*. Of these the most important in wine aroma are *phenylacetaldehyde* and *syringic aldehyde*. *Syringic aldehyde* belongs to the group of phenolic aldehydes and is one of the major components together with vanillin and its derivatives which are formed by thermal degradation of lignin during burning oak wood [10, 21]. Since its level is low in the majority of wines in the majority of wines not exceeding 0.01%, and in some *Sauvignon Blanc* samples is even missing, its contribution to total aldehydes is low. *Phenylacetaldehyde* has the largest contribution to the total aldehyde content [22].

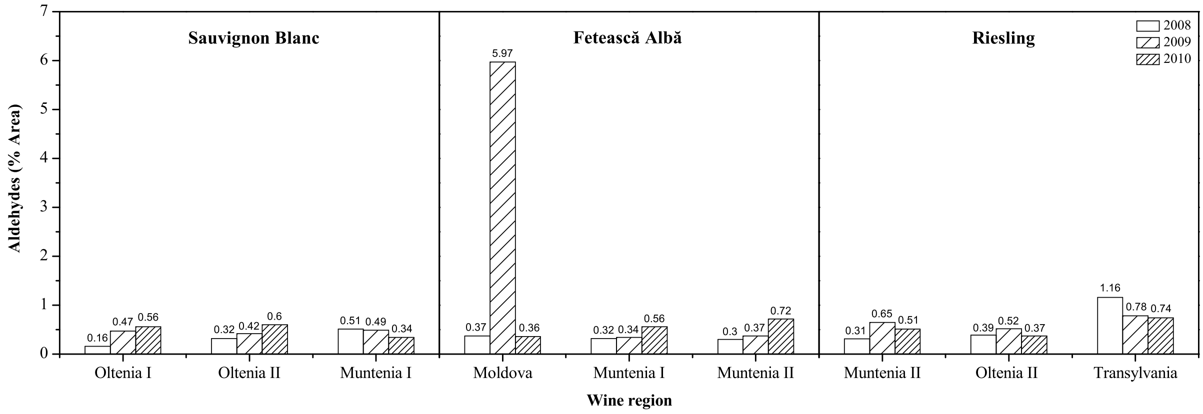

Figure 1.9 *Aldehyde content on three types of wine investigated.*

In the Figure 1.9 we present the aldehyde content. We can note an unusually large value for *Feteascǎ Albǎ* in the year 2009 from Moldova region, due mostly to a higher content of *benzene butanal.* Although this compound was identified with a high probability using the *NIST* library spectra, it is not reported in other papers. It is likely that it arise from the material container in which was aging.

1.3.1.6 Ketones

Ketones found in the samples of wine were: *acetovanillone* (4'-hydroxy-3'-methoxy acetophenone) and *acetosyringone* (4'-hydroxy-3',5'-dimetoxy acetophenone). These, together with Syringic aldehyde and *4-vinyl guaiacol* occur during wine aging in oak barrels, an old tradition that is still used to enhance the sensory quality of wines [10, 21, 23, 24].

GC-MS chromatogram of these compounds in a wine sample of *Feteascǎ Albǎ* from Muntenia is presented in Figure 1.10, and in Figure 1.11 the mass spectra obtained for *acetovanillone.*

GC-MS chromatogram obtained with the selected ion m/z=135 (Figure 1.10 b) is characteristic of the compound *4-vinyl guaiacol*, and the chromatogram obtained with the selected ion m/z=151 (Figure 1.10 c) for vanillin derivatives (acetosyringone, acetophenone, ethyl vanillin, methyl vanillin).

The total kenone content in the investigated samples is presented in Figure 1.12.

1.3.1.7 Lactones

Two lactones were identified in the analyzed wines: *dihydro-3-(phenylmethyl)-2(3H)-furanone* and γ-*decalactone*, the most abundant being γ-*decalactone*. γ-decalactons are

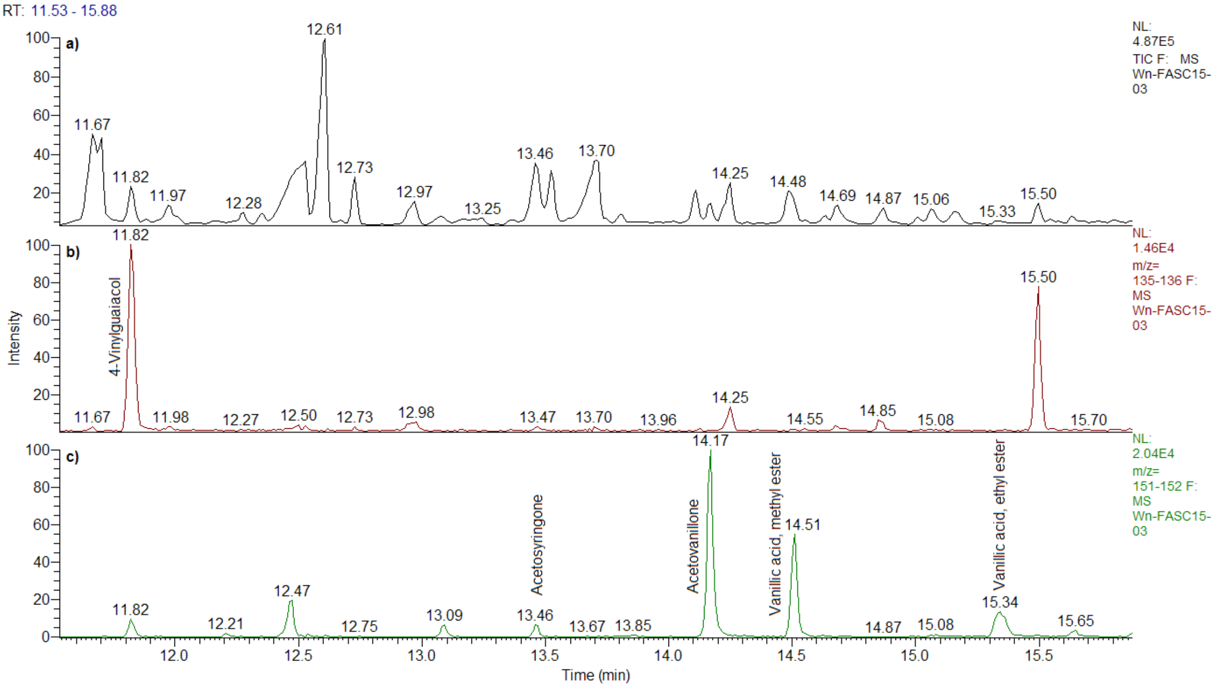

Figure 1.10 *GC-MS chromatogram of a sample of Fetească Albă originating from Muntenia: a) total ions chromatogram (TIC); b) m/z=135; c) m/z=151.*

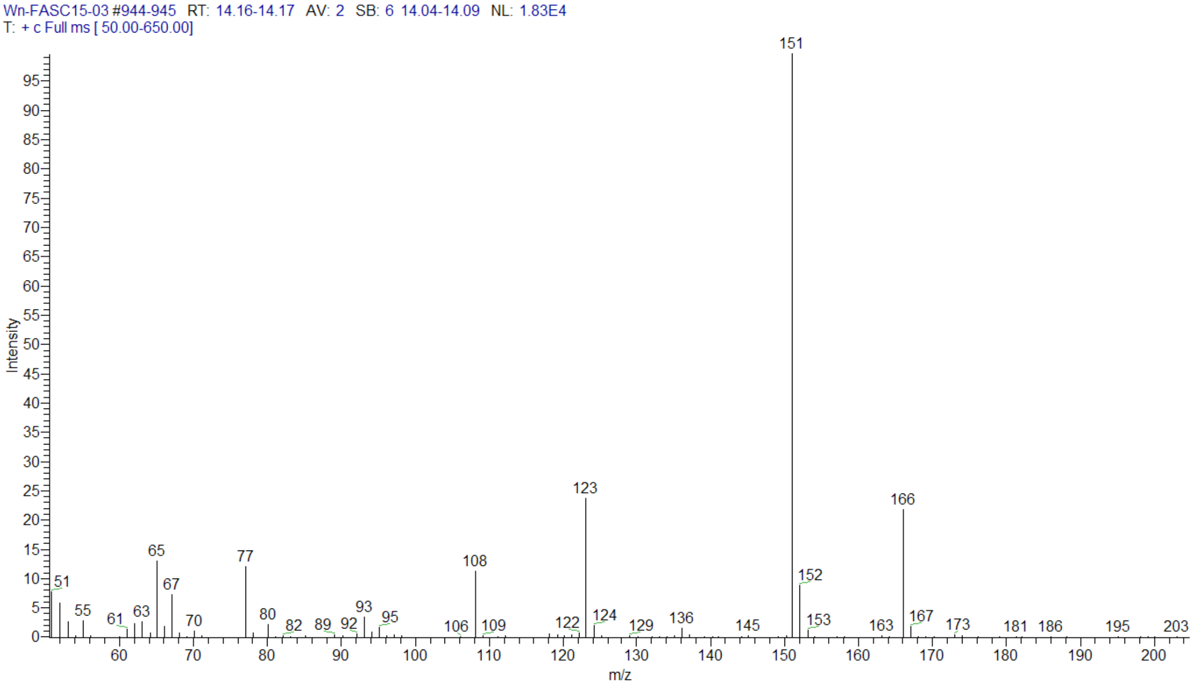

Figure 1.11 *Mass spectra obtained for acetovanillone (Fetească Albă from Muntenia).*

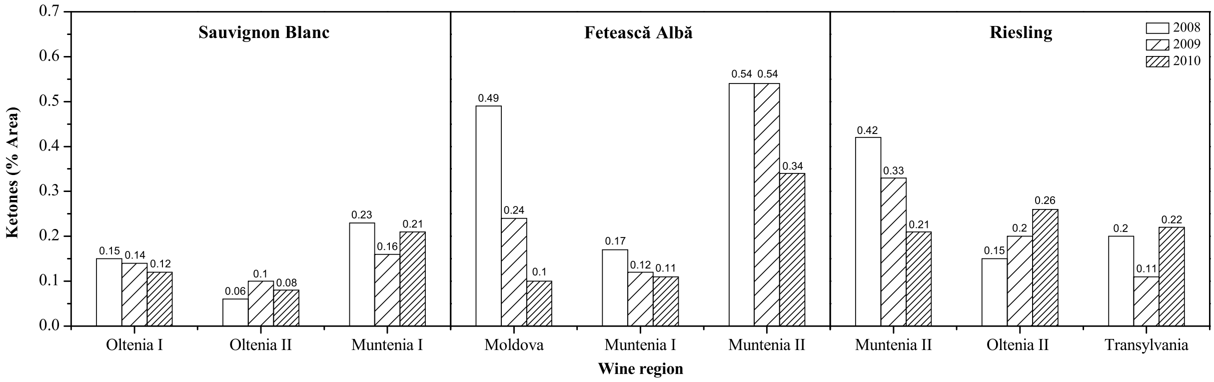

Figure 1.12 *Total ketone content investigated in three wine varieties.*

among the most important components contributing to sensory characteristics of wines aged in oak [25–27].

In Figure 1.13 we present the content in lactones in the investigated three wine varieties.

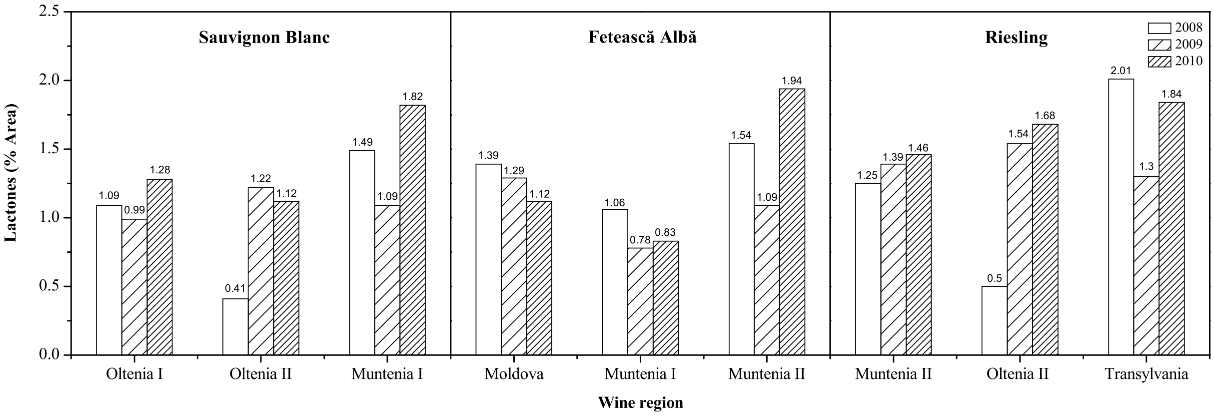

Figure 1.13 *Total lactone content investigated in three wine varieties.*

1.3.1.8 Terpenes

Since most of the terpenoid compounds occur in micro-concentrations in grapes, must and wine, their quantification is often difficult [28]. Thus, in our samples only one terpene was identified, *acetyl carene*, a bicyclic monoterpene. Its percentage in the three types of wine is generally very small, between 0.02 – 0.3%, but consistent with data reported in literature [11].

In Figure 1.14 we present the evolution of *acetyl carene* in the wine samples studied.

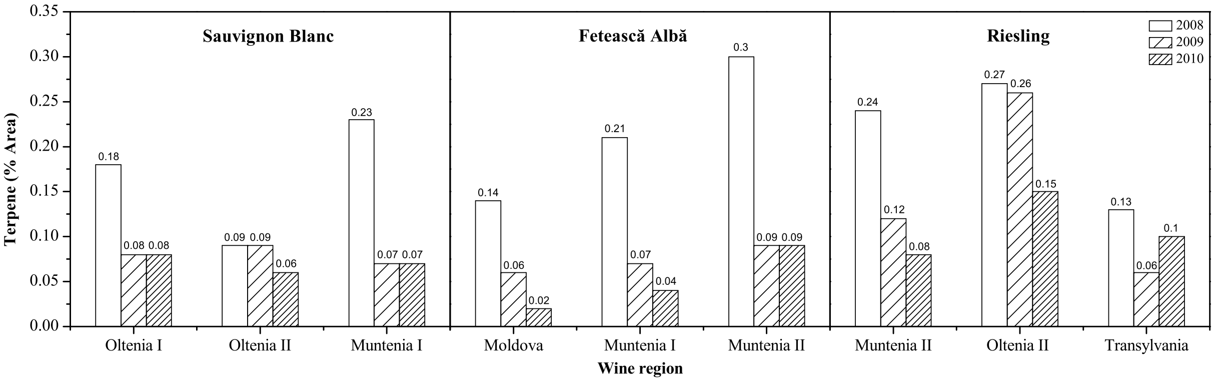

Figure 1.14 *Total acetyl carene content investigated in three wine varieties.*

1.3.2 Study of grape variety influence on volatile fraction of wines

To determine the influence of grape variety on the wine volatile composition we analyzed three types of wine, *Sauvignon Blanc*, *Fetească Albă* and *Riesling* produced in the region of Muntenia. In this respect we followed the behavior of the major volatile compounds in these wine varieties, namely, higher alcohols and ethyl esters. The obtained results are presented in Figure 1.15.

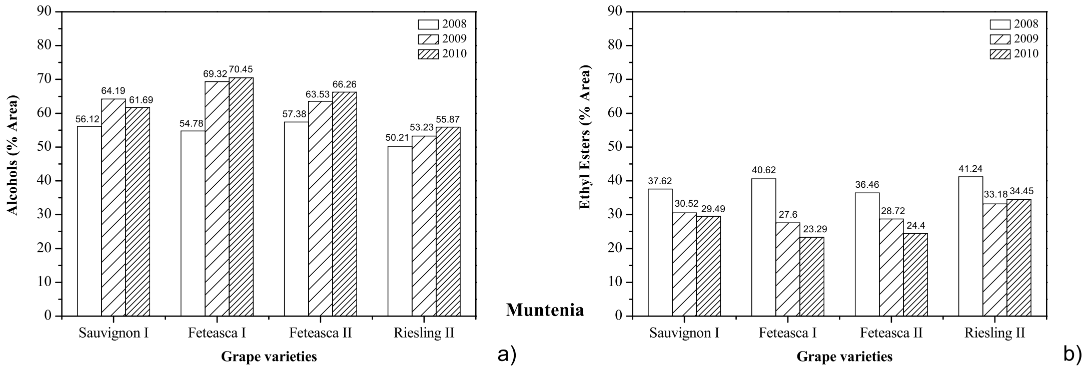

Figure 1.15 *Content in a) alcoohols and b) ethylic esters in three varieties of wine from Muntenia.*

A first observation is the upward trend from year to year which appearing the case of total content of alcohols with an exception for *Sauvignon*. This increase is of course correlated with a decrease of ethyl esters, as the wine is younger, with few exceptions attributable to the contribution of minor compounds.

1.4 Conclusions

In conclusions the level of *higher alcohols* varies widely from one vineyard to another and from year to year. Differences arising between one region to another or within the same vineyards are influenced by the original fermentation conditions and initial physico-chemical composition of must and on the grape variety [18] and, as a result, of the hydrolysis of esters or evaporation during wine maturation in wood [26].

As for the contribution of *esters* we observe that in the majority of wines it increases with age. This can be explained by the major contribution of diprotic ethyl esters, whose concentration increases significantly with time of storage due to chemical esterification during the course of aging [26]. Some variations which occur can be caused by complex reactions of hydrolysis, esterification or oxidations by processes related to hydroxyl radical oxidation [29]. Furthermore, in some vineyards the level of esters differ significantly from year to year, while in other vineyards values are almost at the same level, but, however, it is not possible derive a relation between them. We assume, therefore, that their level depends substantially on grape variety and on the fermentation conditions in each vineyard. It seems that the type of enzymes and the factors influencing their action (such as temperature, oxygen, pH and nitrogen sources) are essential. Also, some technological processes, such as, maceration in contact with the grape skin, or wine clearing before fermentation increase the amount of *fatty acids* in wine [30], while aging on yeast decreases their quantity [9].

Comparing the evolution of *phenolic compounds* content from year to year in each vineyard we observe a lack of a trend. This behavior seems to be an indication that the contribution of phenols depends to a greater extent on the vinification technology used than on geo-climatic conditions. For example, in the technology for producing white wines, the contact with the grapes skin is a pre-fermentation process to enhance floral and fruity character of wine [31, 32]. Consequently, a higher content of phenolic compounds found in some vineyards can be explained by the fact that contact with the grapes skin has occurred a longer time [33].

Phenylacetaldehide level increase rapidly after alcoholic fermentation in wines made from botrytized grapes [34], a higher content in some wines may be the result of their production from this type of grapes.

As concerns the content of *ketones* notable differences are observed between the values obtained in two vineyards from the same region, which leads to the thought that the process of wine making had a major influence. As concerns the temporal evolution, although in some cases there is a noticeable increase in the content of ketones as older the wine, in others it is almost constant or we observe even a decrease of them, which may reinforce the idea mentioned above.

A different behavior was observed for the investigated terpene, *acetyl carene*, as its level seemed to depend to a greater extent on geo-climatic conditions than on the processing conditions.

Therefore we believe that, the so diverse evolution of the amount of volatile compounds will be used in the future to establish criteria for differentiation of wines according to their origins, taking into account the climatic conditions or technology of vinification, after a study carried on over the years and through the development of a database at the national level and integration of such a project with those already started at the European level and even globally.

CHAPTER 2

Determination of the isotopic compositions of wines by isotope ratio mass spectrometry

2.1 Determination of oxygen isotope ratio $^{18}O/^{16}O$ from white wines samples

In this study we investigated 27 samples of commercial white wines originating from the four most important wine regions in Romania: Oltenia (Oprişor and Vânju-Mare vineyards), Muntenia (Cramele Halewood and Ceptura vineyards), Moldova (Huşi vineyard) and Transylvania (Jidvei vineyard). Wine varieties chosen were *Sauvignon Blanc*, *Feteasca Albă* and *Riesling* from three consecutive years: 2008, 2009 and 2010 (Table 2.1).

2.1.1 Samples preparation for isotopic analysis of ^{18}O

Samples preparation for ^{18}O analysis in wine was performed using the method of isotopic equilibration of CO_2 and water from analyzed wines.

The equlibration has been done by introducing 5 ml of wine sample in a flask and freezing it at -80°C. After removing the air from the container, we introduced CO_2 of high purity and with known isotopic composition, at a pressure a bit lower that the atmospheric one ($\approx$ 600 torr). Vials containing the samples of wine and carbon dioxide were then placed in a thermostatic bath equipped with a stirring system at a temperature of 25 $\pm$ 0.1°C, for 24 hours. After the isotopic equilibration reaction, the CO_2 from the vial was extracted and purified cryogenically, and then we measured the value $\delta^{18}O$.

2.1.2 Measurement of isotopic ratio $^{18}O/^{16}O$

The isotopic ratio determination $^{18}O/^{16}O$ were carried out in the *Departament of mass spectrometry, cromatography and applyed physics* at INCDTIM, Cluj-Napoca, using a spectrometer, *Delta V Advantage* (ThermoFinnigan, Bremen, Germany), equipped with "Dual Inlet" sample introduction mechanism.

Measurements of $^{18}O/^{16}O$ were made toward a laboratory standard (water) that was previously calibrated using a reference material (water) with known isotopic value, $\delta^{18}O_{VSMOW}$ = -1.52‰. Their value was expressed using following "delta value", $\delta^{18}O$ [35]:

$$\delta^{18}O = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \tag{2.1}$$

where, R_{sample} and $R_{standard}$ represents the isotopic ratios $^{18}O/^{16}O$ of the sample and of the carbon dioxide use as standard. Reproducibility of the measurements was 0.3 ‰.

$\delta^{18}O$ for samples of *white wines* investigated by isotope ratio mass spectrometry are presented in Table 2.1.

Isotopic differences that occur in the same sort of wine produced in the same vineyard, but different production years are due to weather conditions that characterized those years. For example, in the case of *Sauvignon Blanc* originating form vineyard I in *Oltenia* region, $\delta^{18}O$ value vary from 3.7 ‰ (2008) to 1.1 ‰ (2009) and respectively to 1.6 ‰ (2010). These differences are mainly due to different amounts of precipitation that fell in these years, which influence the isotopic composition $\delta^{18}O$ with a greater extent than temperature variations of those years.

Varieties of wine from *Muntenia* region were: *Sauvignon Blanc*, *Fetească Albă* and *Riesling* and in Figure 2.1 we present the evolution of the measured values. In this region the lowest $\delta^{18}O$ values were obtained for wines produced in the year 2010. Instead, samples for 2009 presented in most cases the highest $\delta^{18}O$ values, 2008 having fairly homogeneous average values in all investigated vineyards. This behavior may be due to the fact that climatic conditions (temperature, humidity, amount of precipitation) were different, 2010 being a rainy year in Muntenia region, while 2009 was hot and dry.

If we analyze two types of wine, *Sauvignon Blanc* and *Riesling*, from the same vineyard of *Oltenia* region, whose $\delta^{18}O$ values are shown in Figure 2.2, we observe the same trend of $\delta^{18}O$ variation from year to year, and in addition, the values for wines produced in the same year are very close. These comparable values seem to suggest that oxygen isotopes are more effective parameters to determine the geographical origin of the wine than to identify its type.

Table 2.1 *$\delta^{18}O$ values for samples of white wines investigated originating from three consecutive years.*

No. crt.	**Wine variety**	**Cod**	**Vineyard**	**Production year**	$\delta^{18}O$ [‰]
1	Sauvignon Blanc	SBPV	Muntenia (Vineyard I) (Cramele Halewood, Dealurile Munteniei)	2008	2.8
2		SBPV		2009	3.4
3		SBPV		2010	2.4
4		SBO	Oltenia (Vineyard I) (Oprişor, Dealurile Olteniei)	2008	3.7
5		SBO		2009	1.1
6		SBO		2010	1.6
7		SBCS	Oltenia (Vineyard II) (Mehedinţi, Vânju-Mare)	2008	1.7
8		SBCS		2009	2.1
9		SBCS		2010	1.1
10	Feteascǎ Albǎ	FAH	Moldova (Dealurile Huşilor)	2008	4.2
11		FAH		2009	-0.6
12		FAH		2010	0.4
13		FACH	Muntenia (Vineyard I) (Cramele Halewood, Dealurile Munteniei)	2008	2.5
14		FACH		2009	1.5
15		FACH		2010	-0.7
16		FASC	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2008	3.0
17		FASC		2009	3.9
18		FASC		2010	1.4
19	Riesling	RSC	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2008	3.5
20		RSC		2009	4.1
21		RSC		2010	-0.8
25		RCS	Oltenia (Vineyard II) (Mehedinţi, Vânju-Mare)	2008	1.3
26		RCS		2009	1.8
27		RCS		2010	1.1
22		DRJ	Transilvania (Jidvei)	2008	1.7
23		DRJ		2009	-1.2
24		DRJ		2010	1.8

2.2 Determination of oxygen isotope ratio $^{18}O/^{16}O$ from red wines samples

In this study we investigated 27 samples of commercial red wines originating from the four most important wine regions in Romania: *Banat* (Recaş vineyard), *Oltenia* (Vânju-Mare and Oprişor vineyards), *Muntenia* (Dealu Mare vineyard with centers: Urlaţi, Ceptura, Zoreşti) and Dobrogea (Babadag vineyard). Wine varieties chosen were: *Merlot*,

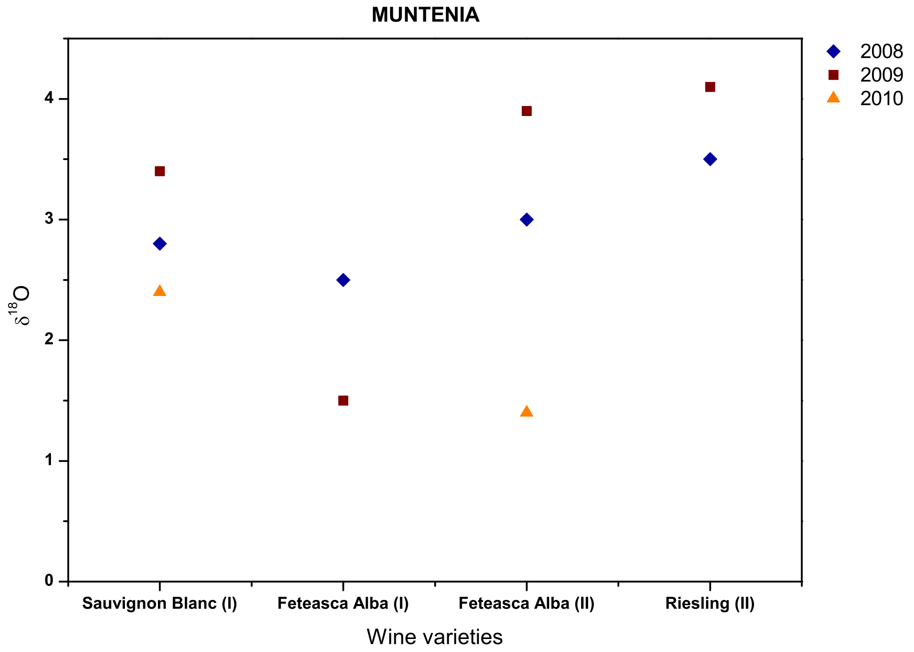

Figure 2.1 *Influence of wine varieties on the values of* ^{18}O *obtained from the same region.*

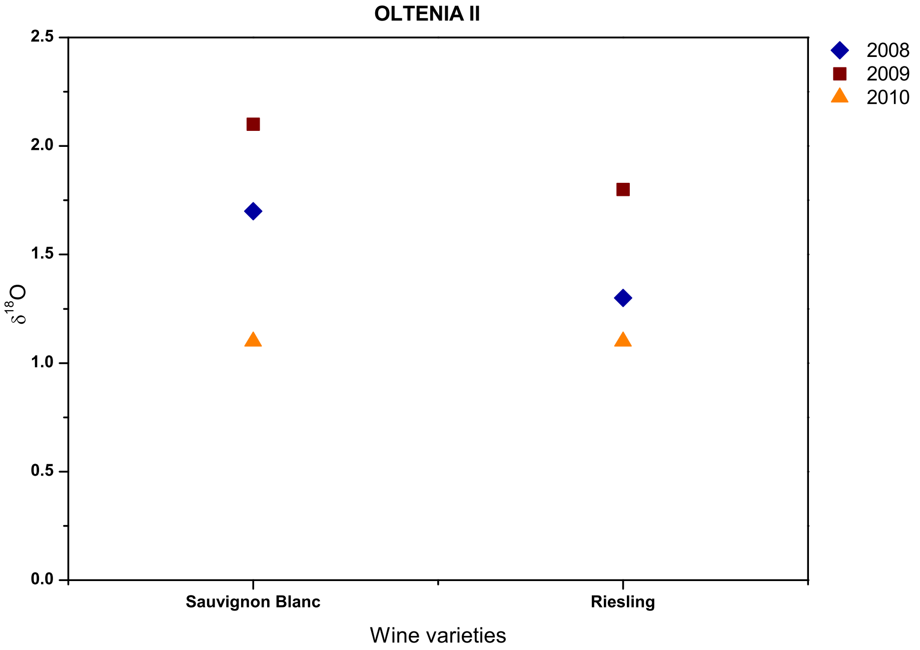

Figure 2.2 *Influence of wine varieties on the values of* ^{18}O *from the same vineyard.*

Cabernet Sauvignon and *Fetească Neagră* from three consecutive years 2006, 2007 and 2008 (Table 2.2).

Sample preparation and measurement of isotopic ratios of oxygen was performed according to the methods described in the previous paragraphs, 2.1.1 and 2.1.2.

Table 2.2 presents measured $\delta^{18}O$ values of investigated wines.

Table 2.2 *$\delta^{18}O$ values obtained for investigated red wines.*

No. crt.	Wine variety	Code	Vineyard	Production year	$\delta^{18}O$ [‰]
1	Merlot	MR	Banat (Recaş, Viile Timişului)	2006	1.7
2		MR		2007	5.6
3		MR		2008	2.6
4		MVC	Dobrogea (Ceptura, Colinele Dobrogei)	2006	0.1
5		MVC		2007	5.7
6		MVC		2008	3.3
7		MPM	Oltenia (Vineyard II) (Vânju-Mare, Mehedinţi)	2006	1.8
8		MPM		2007	2.5
9		MPM		2008	3.7
10	Cabernet Sauvignon	CSURL	Muntenia (Vineyard III) (Dealu-Mare, Urlaţi)	2006	0.5
11		CSURL		2007	2.6
12		CSURL		2008	2.6
13		CSO	Oltenia (Vineyard I) (Oprişor, Dealurile Olteniei)	2006	1.6
14		CSO		2007	3.2
15		CSO		2008	3.1
16		CSVM	Oltenia (Vineyard II) (Vânju-Mare, Mehedinţi)	2006	2.3
17		CSVM		2007	3
18		CSVM		2008	1.7
19	Fetească Neagră	FNSC	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2006	0.9
20		FNSC		2007	1.7
21		FNSC		2008	2.7
22		FNURL	Muntenia (Vineyard III) (Dealu-Mare, Urlaţi)	2006	0.3
23		FNURL		2007	2
24		FNURL		2008	1.5
25		FNDM	Muntenia (Vineyard IV) (Dealu-Mare, Zoreşti, Buzău)	2006	1.1
26		FNDM		2007	2.1
27		FNDM		2008	3.6

For *Merlot* we observe that $\delta^{18}O$ values obtained in Banat and Dobrogea regions are higher in 2007 compared with 2006 and 2008 values (Figura 2.3). These values are certainly due to excessively hot and dry climate of the year 2007 [36], low in precipitations, 479.02 l/mp, and with thermal values over multiannual mean since May until mid-September [37]. Also, $\delta^{18}O$ value for *Merlot* wine produced in Dobrogea is much higher compared with the characteristic isotopic value of the same wine in 2006. The year 2008 was, also, a hot year, but with more precipitations, 666.2 l/mp [37], than in 2007, and the thermal regime was 1.4°C over the climatological normal compared to 2007, when the thermal regime was 1.6-2°C higher, and this is reflected in lower values than in 2007 [38].

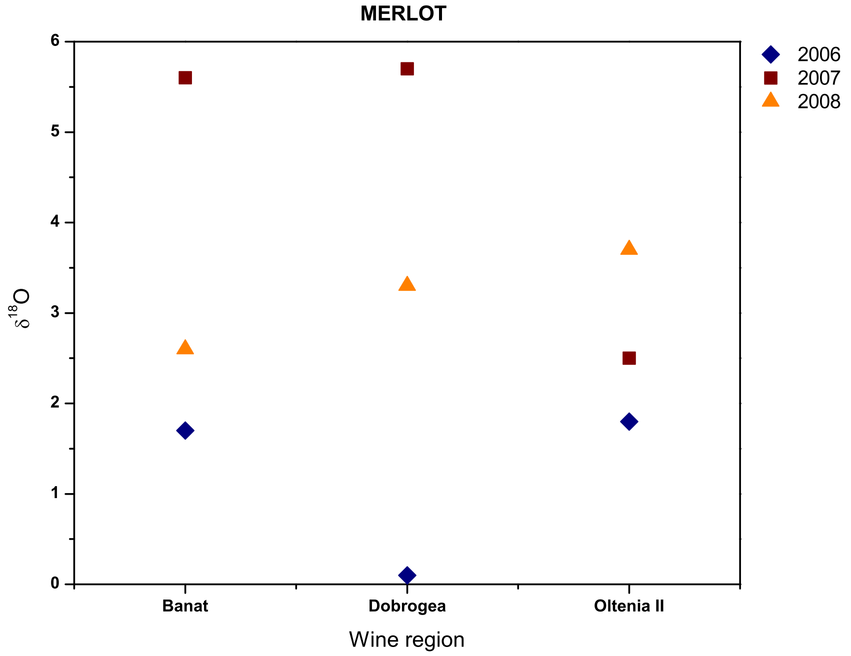

Figure 2.3 *Variation from year to year of* $\delta^{18}O$ *values of Merlot wine from different regions.*

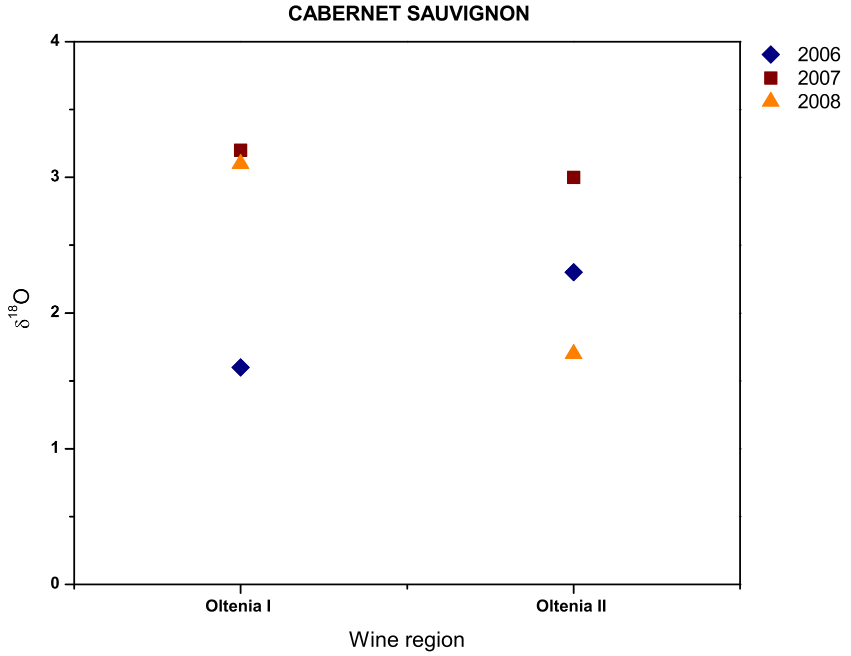

Figure 2.4 *Comparison between* $\delta^{18}O$ *values for Cabernet Sauvignon in two vineyards for Oltenia region.*

The smallest $\delta^{18}O$ values obtained in 2006 are also a consequence of meteorological conditions that prevailed this year. The year 2006 was a rainy year, which was directly related to the degree of sweat evaporation and thus the degree of enrichment of the $\delta^{18}O$ values was lower. The reported results by *J. Dunbar* (1982) showed that the enrichment in $\delta^{18}O$ value of water in grape juice is due to the physical process of evapo-transpiration, which is directly related to ambient air temperature and humidity [39].

For *Cabernet Sauvignon* originating from the two *Oltenia* vineyards, isotopic values obtained were comparable in the three years of production as shown 2.4. Small isotopic differences may occur due to different soil types, involving different water retention.

In Figure 2.5 we represented $\delta^{18}O$ values obtained for *Fetească Neagră* (an old Romanian variety prevalent in Moldova and Muntenia), produced in the three vineyards of Dealu-Mare positioned on the territory of two counties (Prahova and Buzău).

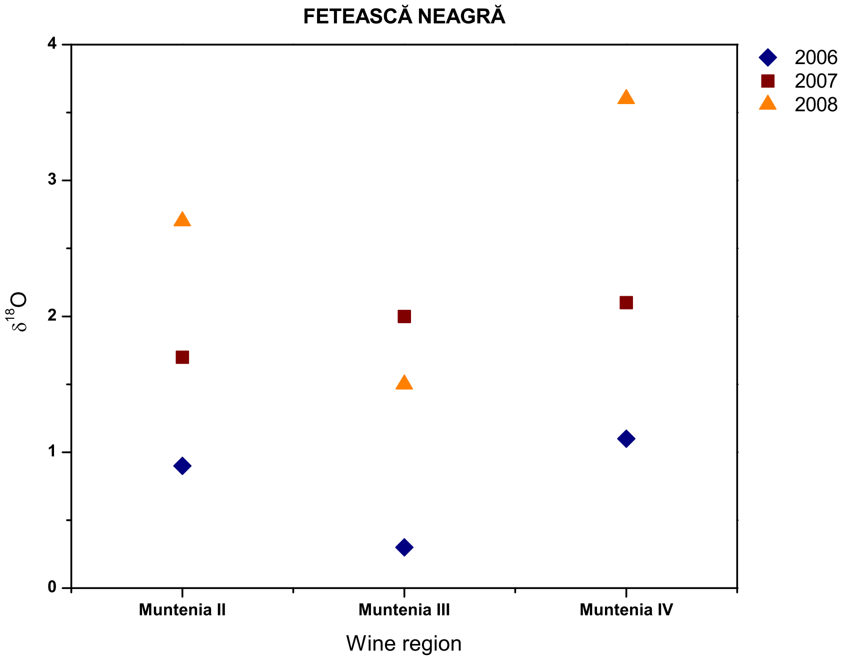

Figure 2.5 *$\delta^{18}O$ values comparison obtained for Fetească Neagră in Muntenia vineyards.*

We can see that the highest values were obtained for wine produced in Vineyard IV. This might be because vineyard IV is located in another county, at distance of about 50 km from the other two, so that the soil type and specific weather conditions (the microclimate) are probably different. A similar evolution of $\delta^{18}O$ values depending on years of production can be observed also for wines in Vineyard II.

A comparison between *Cabernet Sauvignon* and *Fetească Neagră* originating from vineyard III from Muntenia shows the fact that $\delta^{18}O$ values obtained for the two types of wine are quite similar (Figure 2.6). This is likely the consequence of the fact that the isotopic

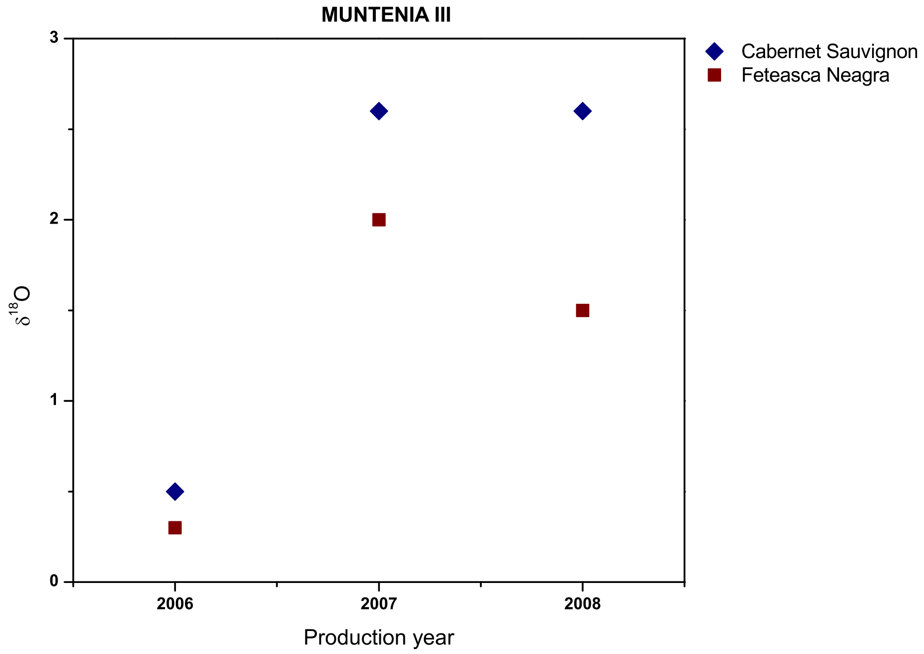

Figure 2.6 *Influence of wine variety on the value of* ^{18}O.

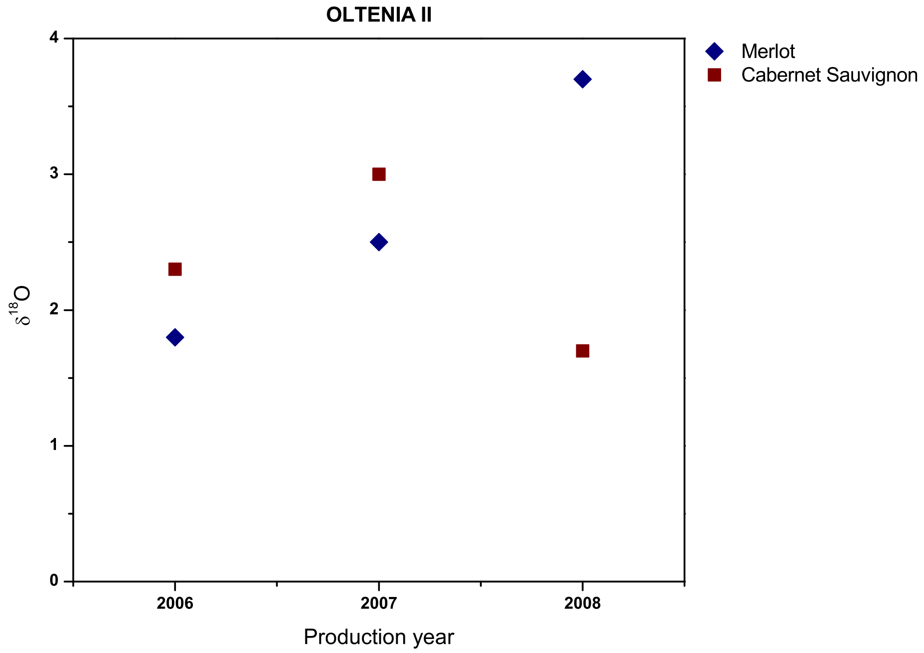

Figure 2.7 *Influence of wine variety on the value of* ^{18}O.

ratio of oxygen depends in particular on climatic and geographical conditions in which the plant grew and developed and to a lesser extent the photosynthetic metabolism of plants, so, consequently, on the variety of grapes [40].

The same situation is observed for *Cabernet Sauvignon* and *Merlot* originating from *Oltenia* vineyard, in the years 2006 şi 2007, as we can see in Figure 2.7. The different evolution observed in 2008 could be due to the grape harvest time and weather conditions during harvest. For high quality red wines, grape harvesting is done when it reaches full maturity, i.e. phenolic maturity. The quality of red wines is in a tightly correlation with the qualitative evolution of grape skin (phenolic compounds, flavors), phenolic maturation analysis being the only way to properly assess the optimal time to harvest the grapes. In comparison with *Merlot*, *Cabernet Sauvignon* supports in a better way supramaturation and due to lower losses of anthocyanins, can be harvested over a longer period of time [41].

The table 2.3 contains a comparative study in terms of oxygen isotope ratio between *white* and *red* wines. Thus, we chose white and red wine varieties from the same vineyard and production year.

Table 2.3 *$\delta^{18}O$ values for white and red wines in the year 2008 obtained in different vineyards.*

No. crt.	**Wine variety**	**Kind of wine**	**Vineyard**	**Production year**	$\delta^{18}O$ **[‰]**
1	Sauvignon Blanc	white	Oltenia (Vineyard I) (Oprişor, Dealurile Olteniei)	2008	3.7
2	Cabernet Sauvignon	red			3.1
3	Sauvignon Blanc	white	Oltenia (Vineyard II) (Vânju-Mare, Mehedinţi)	2008	1.7
4	Riesling	alb			1.3
5	Cabernet Sauvignon	red			1.7
6	Merlot	red			3.7
7	Fetească Albă	white	Muntenia (Vineyard II) (Ceptura, Dealurile Munteniei)	2008	3.0
8	Riesling	white			3.5
9	Fetească Neagră	red			2.7

We can observe that vineyard I from Oltenia and vineyard II from Muntenia present $\delta^{18}O$ values of about 3‰, while in vineyard II from Oltenia a value of 2‰ was obtained. This time too, the oxygen isotopic composition is influenced mainly by the location where the grapes grew (geographical origin) and is not largely influenced by grape variety. An exception is observed for *Merlot* which in vineyard II from Oltenia region has the highest value, 3.7‰, probably due, as we already mentioned, to the grapes harvest time and weather conditions during it.

2.3 Conclusions

In this study, oxygen istope ratio was investigated for 54 commercial wines (27 white and 27 red) originating from the most important wine regions in Romania. If , in the case of *white wines*, the minimal $\delta^{18}O$ values were obtained in 2010, for *red wines* the minimal values were obtained in 2006. In both cases these values are due to similar weather conditions, mainly to a large amount of rainfall and temperatures close to normal. The driest years 2007, 2008 and 2009, were reflected in high specific isotope $\delta^{18}O$ values. Oxygen isotopic composition is mainly influenced by geo-climate and in a smaller extent by grape variety.

CHAPTER 3

Wine analysis using inductively coupled plasma mass spectrometry

3.1 ICP-MS Analysis

All measurements were performed with an inductively coupled plasma mass spectrometer *Perkin-Elmer Elan DRC(e)*, equipped with an nebulizer *Meinhard* and with a cyclonic spray chamber. Experimental conditions were: argon flow on nebulizer - 0.86 L/min; auxiliary gas flow - 1.2 L/min; argon flow in plasma - 15 L/min; lens voltage - 7.25 V; RF power in plasma - 1100 W; $CeO/Ce = 0.027$; $Ba^{++}/Ba^{+} = 0.025$.

Analysis was performed using a semi-quantitative method available in the software of the ICP-MS spectrometer, *Total Quant III.* This method is used to explore the full range of masses, excluding only the masses around the oxygen and argon which could cause signal saturation. Mass range explored was $m/z = 6 - 14$, $m/z = 22 - 37$ şi $m/z = 40 - 238$. Initially we determined the background signal generated by the spectrometer (blank) by aspirating ultrapure water. Subsequently, using an external calibration standard we measured a solution of known concentration 10 μg/L Mg, Rh, Pb, Cu, Cd, In, Ba, Ce, U in 1% HNO_3 (Atomic Spectroscopy Standard, Perkin Elmer), identifying elements in the calibration solution. Intensities were determined for each of them and the reponse of the spectrometer was calculated comparing the measured concentrations those specified.

3.2 Samples preparation

Preparation of wine samples for ICP-MS analysis consists of their mineralization and dilution to avoid clogging the nebulizer. For measurements, following a method described by *W. Diegor şi colab.* [42], 2.5 ml ultrapure nitric acid were added to the 2.5 ml of wine in a Teflon container which was then sealed. Six such vessels were inserted in a device consisting of six cylinders of stainless steel mount between two flanges, ensuring a pressure-resistant armor. The entire system was placed in an oven at 200°C for 12 hours. Following this treatment have resulted colorless solutions over which ultrapure water was added and the volume was brought up to a volume of 50 ml, and samples were diluted 1:20 v/v.

3.3 Experimental Results

In our study using ICP-MS spectrometer we measured concentrations of 27 metals for 54 samples of commercial wines (27 white wines and 27 red wines). The metals analyzed were: *Na*, *Mg*, *K*, *Ca*, *Al*, *Cr*, *Mn*, *Fe*, *Ni*, *Ti*, *V*, *Co*, *Cu*, *Zn*, *As*, *Mo*, *Cs*, *Ba*, *W*, *Pb*, *Cd*, *La*, *Ce*, *Au*, *Hg*, *Sr* and *Rb*.

Depending on their concentration in wine these elements are divided into three groups: *macro-elements* or *major elements* (*Na*, *Mg*, *K* and *Ca*) with concentration $>$ 10 mg/L; *micro-elements* or *oligo-elements* (*Al*, *Cu*, *Fe*, *Mn*, *Zn*, *Rb*, *Sr*, etc.) with concentrations in the domain 0.1-10 mg/L and *trace elements* (*Pb*, *Cd*, *Cs*, etc.) whose concentrations are under 0.1 mg/L [43, 44].

Maximum allowed concentrations in wines for toxic metals are given in Decision no. 1134 from 10 October 2002 for approval of Methodological norms to apply the Law of vine and wine in the system of the common organization of the wine market, no. 244/2002 [45]. For all wine samples analyzed toxic metal content was within the maximum limits allowed by law.

In Figures 3.1 and 3.2 we represent the major elements distribution in the investigated samples of white and red wine.

From the major elements, *potassium* showed the highest concentrations, followed by *magnesium*, *calcium* and *sodium*, values consistent with those found in literature [44, 46, 47]. Although the mean values in the two types of studied wines (white and red) are different, as we will see below, this order is preserved.

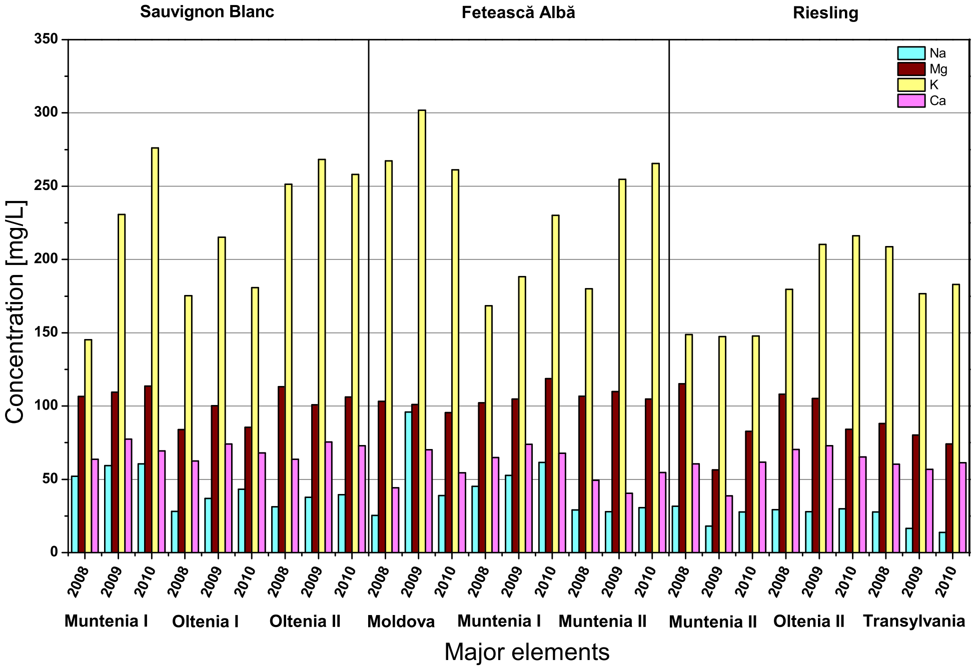

Figure 3.1 *Distribution of major elements in white wines.*

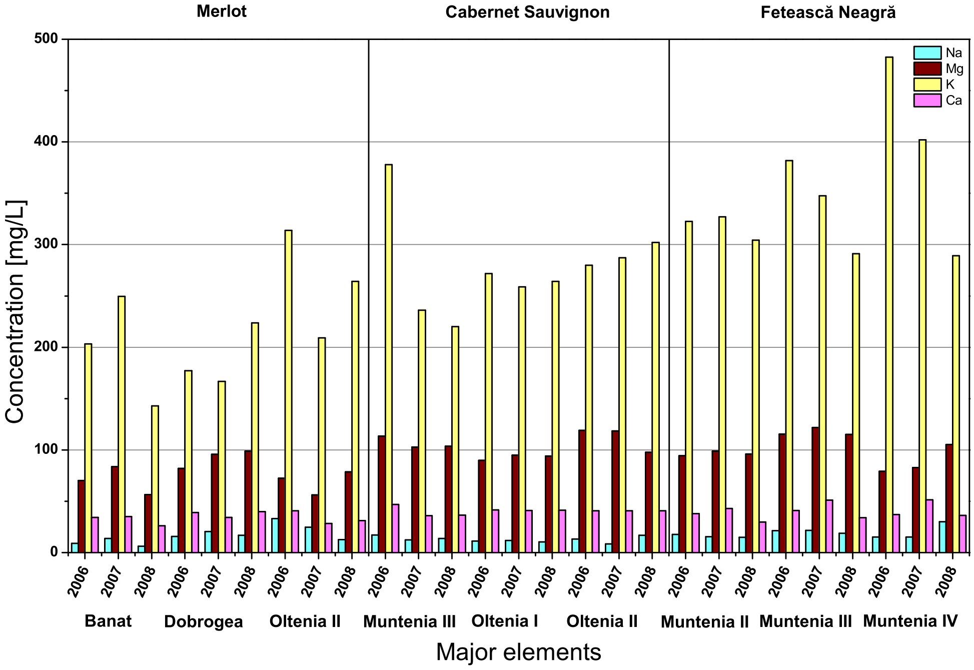

Figure 3.2 *Distribution of major elements in red wines.*

For an overview, which we will try to detail, in table 3.1 we present the average of the major elements determined in all wine samples as well as theirs minimum and maximum values:

Table 3.1 Concentration of major elements in wines studied.

Element	Minimal value [mg/L]	Maximal value [mg/L]	Mean value [mg/L]
	WHITE WINES		
Na	13.84	95.88	37.73
Mg	56.38	118.68	98.52
K	145.22	301.84	212.46
Ca	38.74	77.36	62.77
	RED WINES		
Na	6.04	33.1	16.2
Mg	56.08	121.96	93.94
K	142.76	482.56	281.36
Ca	26.18	51.46	38.37

Comparing the *white* and *red* wines in terms of the content of major elements we observe that in the white wines we have found higher concentrations of *Na* and *Ca*, while *K* was more abundant in the red wines. *Mg*, being the central atom in the porphyrin ring of chlorophyll, occur at similar concentrations in both types of wines. Higher values of *Na* and *Ca* obtained for *white wines* are probably caused by the fact that they are more “treated“ chemically for clearing.

Elements considered to be of particular interest due to their effect on organoleptic properties of the wine, called *micro-elements*, such as *Fe*, *Al*, *Mn*, *Zn* and *Cu* appear in lower concentrations.

Total average concentration of these elements obtained for the investigated *white wines* was: 1.42 mg/L for *Fe*, 0.94 mg/L for *Al*, 0.82 mg/L for *Mn*, 0.32 mg/L for *Zn* and 0.1 mg/L for *Cu*, and for *red wines* overall average was: 2.83 mg/L for *Fe*, 1.51 mg/L for *Al*, 0.93 mg/L for *Mn*, 0.87 mg/L for *Zn* and 0.11 mg/L for *Cu*. We observe for all trace elements higher average concentrations for *red wines*.

These values are due primarily to the fact that, in their case, the wine making technology requires a longer contact with the grape skin during fermentation. As will be noted below, at the level of vineyard, other endogenous or exogenous factors can affect essentially the content of iron, or, more generally, the metal content as a whole.

Given the much lower share of *copper* which, as noted, is at the lower end of the *micro-elements* group, for a better clarity of the representation, will be treated with the group of trace elements.

In Figure 3.3 and 3.4 we represent the concentrations of more abundant trace elements in our samples.

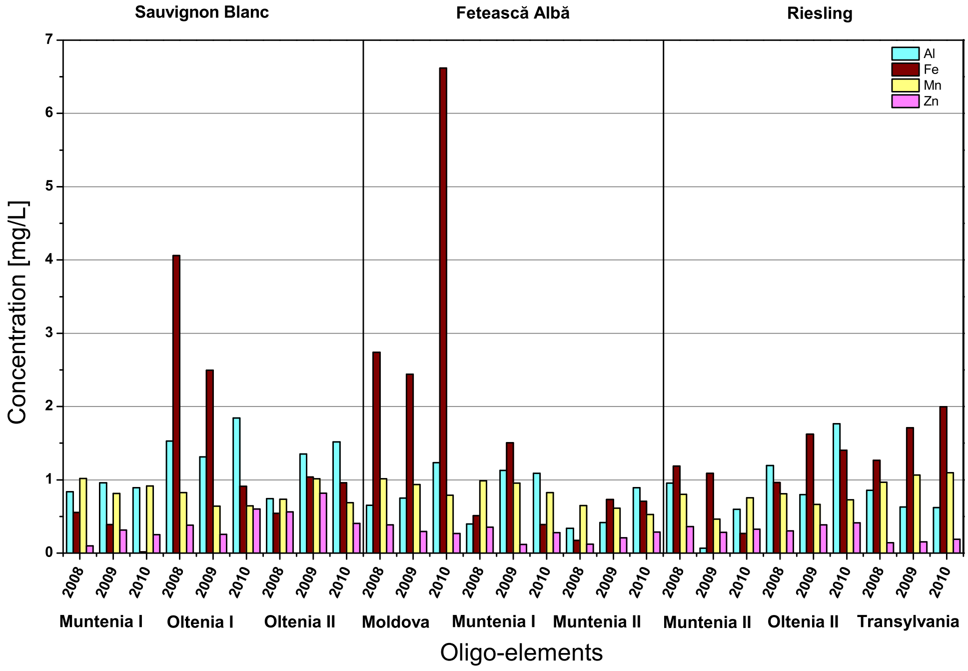

Figure 3.3 *Distribution of the main trace elements in samples of white wine.*

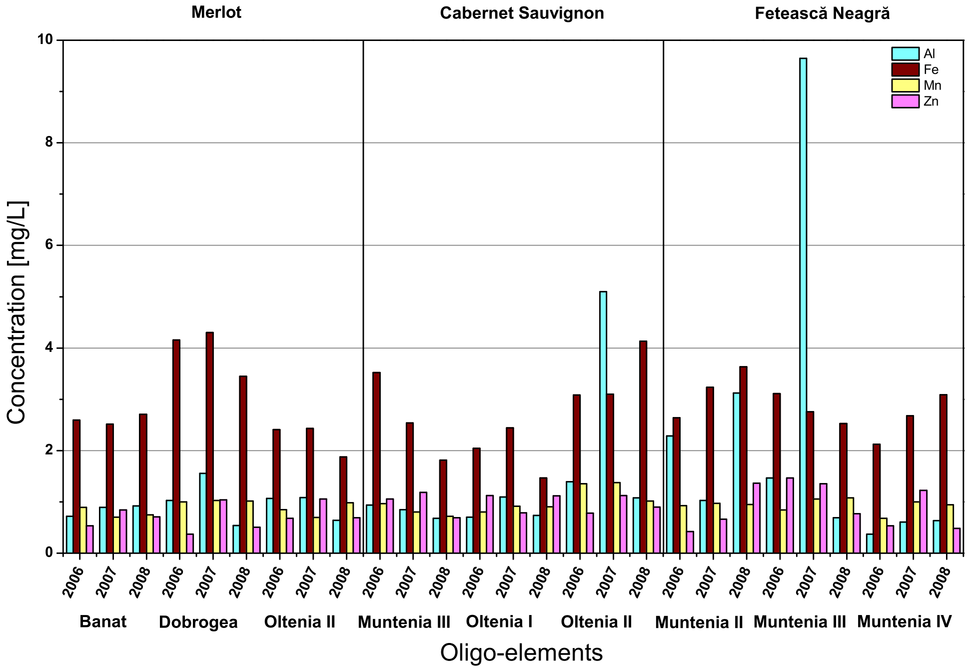

Figure 3.4 *Distribution of the main trace elements in samples of red wine.*

Considering on the following the elements of special interest, *Zn*, *Ni*, *Cr*, *Cd*, *Pb*, *As*, mainly due to their toxicity in case of excess [47], in all our samples, we measured concentrations much lower than the limit. The relative level of these elements in white

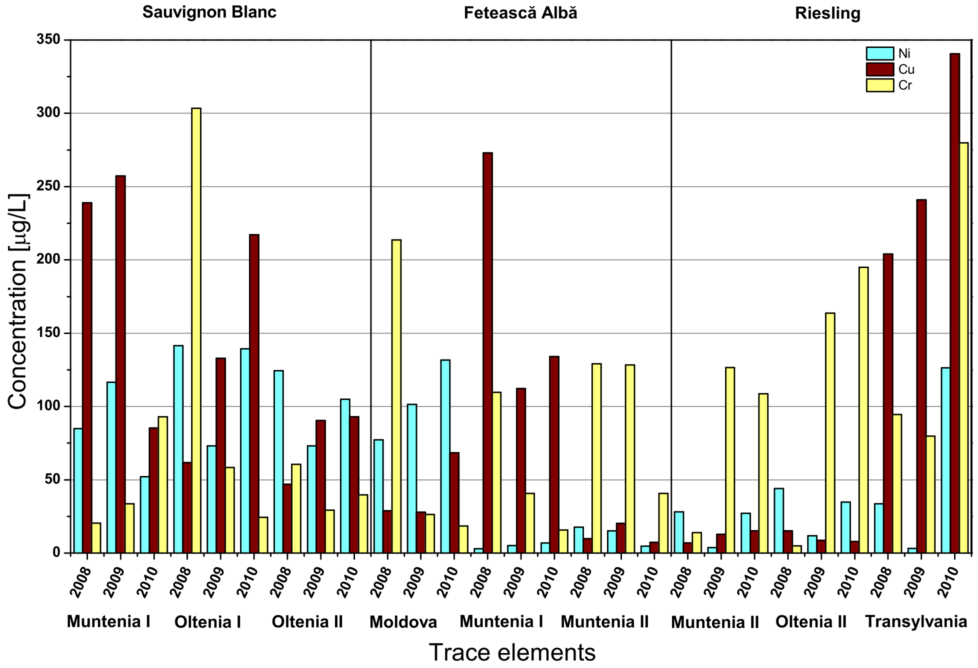

Figure 3.5 *Distribution of the elements Cu, Cr and Ni in samples of white wine.*

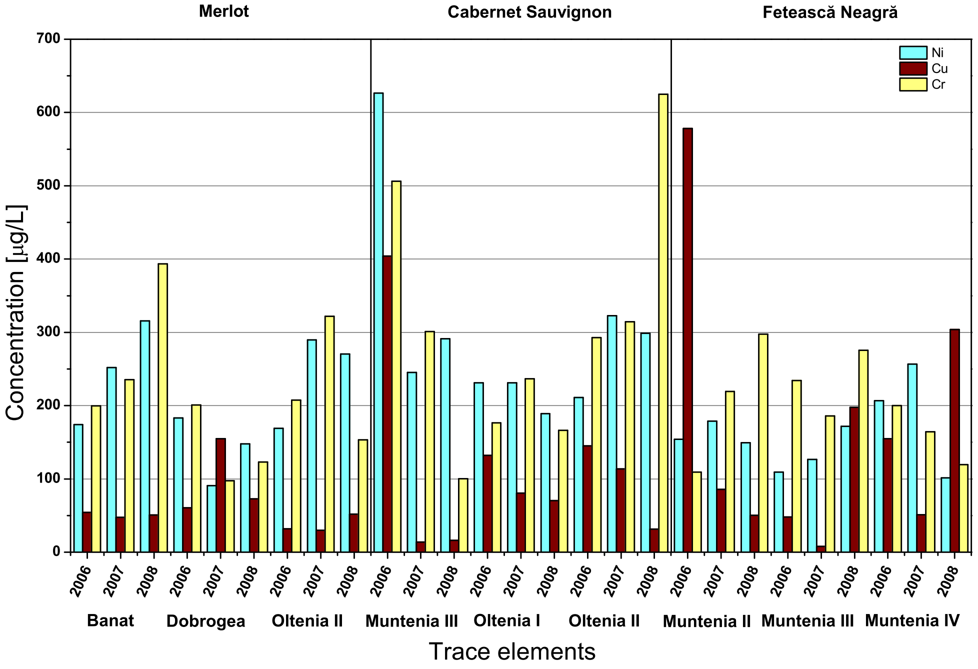

Figure 3.6 *Distribution of the elements Cu, Cr and Ni in samples of red wine.*

and red wines are depicted in the Figure 3.5, 3.6, and, respectively, in 3.7 and 3.8, except for *zinc* presented in previous graphs.

The presence of **arsenic** and **cadmium** was detected in all analyzed wines. **As** concentrations were between *<0.001-9.78* µg/L, except for a few samples, where higher concentrations were found. A possible explanation could reside in the fact that, *As* concentration depends both on the geochemistry of the soil as well as on the use of pesticides, fungicides and fertilizers, containing compounds with *As*, which may lead to an increase of its amount in soil and grapes and therefore in wine [44,48].

Cd concentrations varied between *<0.001-0.72* μg/L, in *white wines* and between *<0.001-1.06* μg/L, in *red wines*. For only two samples a similar higher concentration of 2.78 μg/L was found, one of white wine and the other of red wine. *Cd* present in wines may be due to agrochemicals residues used as insecticides and fungicides which contain cadmium or to phosphate fertilizer which has higher concentrations of *Cd* [48–51]. Another cause for higher concentrations of *Cd* in wines may be due to the contact with equipment used in manufacturing and packaging process [51].

In all wines analyzed *Pb* content was below the limit set by Law 244/2002 [45], being within the range *2.26-48.3* μg/L in *white wines* and in the range *4.34-34.16* μg/L for *red* ones, with only one exception where the measured concentration was of 161 μg/L, value close to the admitted maximum of 200 μg/L. *Pb* content in wine can increase as a result of corrosion of metals or alloys used in wine cellars [47], and due to the use of pesticides, fungicides and fertilizers during grapes development [43].

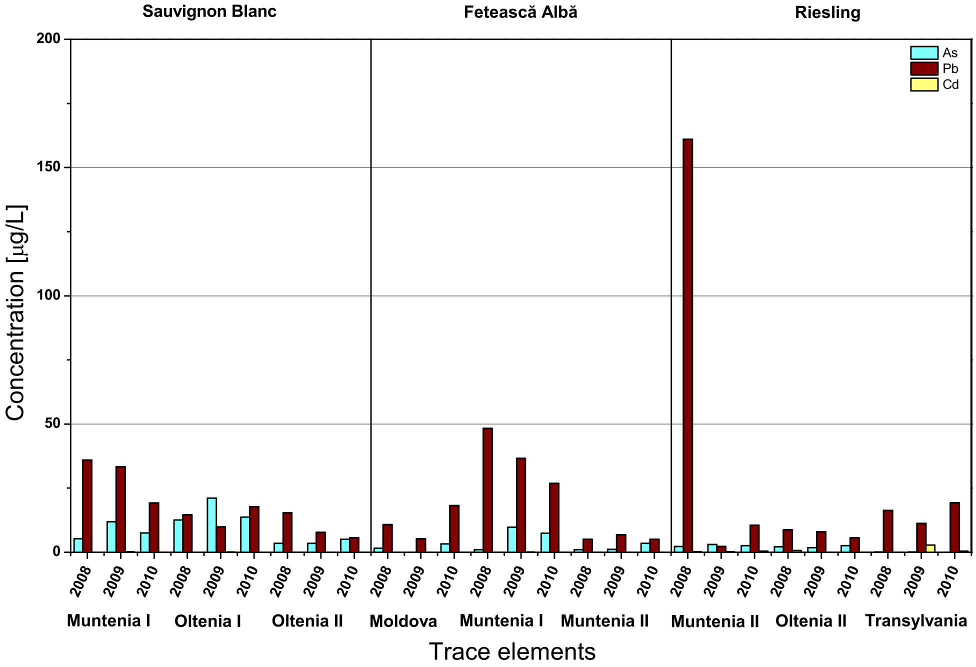

Figure 3.7 *Distribution of trace metals in samples of white wines.*

In Table 3.2 we present a comparative study in terms of the metal content between *white* and *red* wines. The the analysis of measured data proved that, in general, the samples of *red wine* had a significantly different metal content than those of *white wine*.

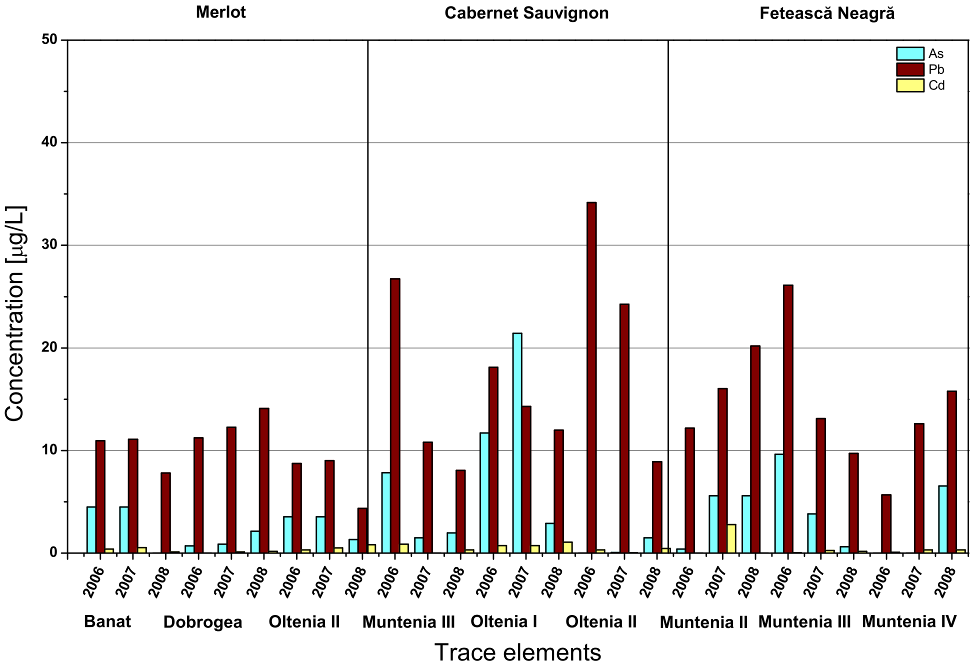

Figure 3.8 *Distribution of trace metals in samples of red wine.*

Thus, concentrations of *Ba*, *Mn*, *Fe*, *Al*, *Zn*, *Ni*, *Cr*, *Sr* and *Rb* are generally higher in *red wines* and those of *V* and *Ti* are usually higher in samples of *white wine*, results that are consistent with those from literature [47, 52]. Also, we can observe that, *red wines* have higher average concentrations of trace elements than white. Therefore, the presupposition that *red wines* has higher concentrations of trace elements than *white wines*, due to a longer contact with grape skin during the winemaking process, is confirmed also by our data.

3.4 Conclusions

Using ICP-MS technique we analyzed 54 samples of commercial wine, 27 of white wine and 27 of red wine. In all wine samples measured, the toxic metals content was found in quatities below the limits set by the legislation, namely by the *International Organization of Vine and Wine* (O.I.V.).

ICP-MS measurements have shown an important content of metals in wines studied. Relatively large ranges of variation in the analyzed samples of wine were due to the diversity of regions from which they are originating, from different areas of Romania, with diverse quality of the soil, but also as a result of anthropical impact. As detailed within the analysis of measured data, higher content of certain metals due to anthropic impact may be due to:

Table 3.2 Average concentrations for major metals.

Element	Minimal value [μg/L]	Maximal value [μg/L]	Mean value [μg/L]
		WHITE WINES	
Ba	76.58	311.46	141.81
Mn	465.38	1095.48	812.99
Fe	14.94	6620.1	1418.02
Ni	3.02	139.3	58.72
Cr	4.88	303.4	90.8
Al	67.14	1843.3	939.35
Zn	99.48	818.56	316.62
Sr	134.98	588.4	222.12
Rb	392.8	876.96	543.57
Ti	1.34	339.32	122.99
V	13.72	942.82	181.46
		RED WINES	
Ba	92.54	639.04	295.73
Mn	681.28	1377.22	933.74
Fe	1467.4	4298.9	2827.79
Ni	90.6	626.32	221.95
Cr	97.38	624.78	239.15
Al	371.44	9648.8	1513.04
Zn	371.38	1.464.80	868.87
Sr	258.2	1179.5	664.62
Rb	713.78	2226.8	1395.41
Ti	2.8	178.28	88.66
V	0.06	368.22	62.72

- viticulture practices, through the use of fertilizers for cultivation (K, Ca, Cu) or as a consequence of the use of pesticides, fungicides, during the development of grapes (Cd, Cu, Mn, Pb or Zn),
- winemaking process, through longer contact with processing equipment (Al, Cd, Cr, Cu, Fe and Zn) or through addition of substances at different stages of wine production. Thus, contamination with Na, Ca or Al may be associated with the utilization of substances for wine clearing as bentonite.

A long-term detailed analysis of wines originating from the same vineyard, in parallel with an analysis of the soil and an oversight to the extent of oenological practices used, could contribute to an identification with good accuracy of the parameters involved. Establishing a national database and its alignment with those already existing in other countries would contribute significantly to a refinement of the analysis, a better identification of fraud and, consequently, to the improvement in the quality of wines.

CONCLUSIONS

The main goal of this thesis was to perform a systematic investigation of the chemical composition of wines from the major wine regions of Romania (Banat, Dobrogea, Moldova, Muntenia, Oltenia and Transylvania). The analyzed parameters were: volatile content, the oxygen isotope ratio ($^{18}O/^{16}O$) and metal content. We used the following analysis methods: *gas chromatography coupled with mass spectrometry* (GC-MS), *isotope ratio mass spectrometry* (IRMS) and *inductively coupled plasma mass spectrometry* (ICP-MS). 27 samples of white wine were investigated produced in the years 2008, 2009, 2010 and 27 samples of red wine produced in the years 2006, 2007, 2008. Using the GC-MS method the white wines were analyzed and by IRMS and ICP-MS, we analyzed both types of wines. This study was performed in the *Department of Mass Spectrometry, Chromatography and Applied Physics* at the *National Institute for Research and Development of Isotopic and Molecular Technologies* (INCDTIM), Cluj-Napoca.

In the white wines investigated using GC-MS, 48 volatile compounds were identified belonging to 8 different classes, such as: alcohols, ethyl esters, fatty acids, phenolic compounds, aldehydes, ketones, terpenes and lactones. Their contribution was reported as a percentage of the total area of volatile compounds identified. This type of analysis proves to be useful offering a simple way to make connections between the values with a sufficient approximation. Thus, for *alcohols* and *esters*, which are major components of wine, representing more than 90% of the volatile fraction identified, there is a clear correlation of the evolution of their values, which is certainly due to the chemical esterification of alcohols with acids during the process of aging. Also, their level depends substantially on grape variety and fermentation conditions. Minor compounds, mostly produced by yeast during fermentation, although present in much lower quantities plays a key role, having a major influence onto the aroma. *Fatty acids* and *phenolic compounds* content increases if maceration during must elaboration take place for a longer time in contact with the skin, and does not seem to depend on geo-climatic conditions. The aldehyde levels are not dependent on wine variety, higher content of *phenylacetaldehyde* in some wines may be an indication of their grapes botrytization. In contrast, the level of *ketones* seems to be a good indicator of wines aging in oak barrels. The values obtained for single terpene identified, 2-acetyl-carene seem to depend on geo-climatic conditions.

Dependence geo-climatic conditions, however, is more pronounced in the case of oxygen isotopic ratios, $\delta^{18}O$, measured by IRMS. The years with heavy rainfall and temperatures near normal values were reflected in the minimum values of $\delta^{18}O$, and in the driest years the values $\delta^{18}O$ were very high. Oxygen isotopic composition is mainly influenced by geo-climate and in a lower extent by grape variety.

Using the ICP-MS method we measured 27 metal concentrations in 54 samples of wine of which, 27 white wine and 27 red. In terms of major elements content higher concentrations of *Na* and *Ca* have been found in white wines, while *K* was more abundant in red wines. These values are due to the use of fining agents in the case of white and fertilizers (associated with a longer contact with the skin) for the red ones. *Mg*, the central ion of the porphyrin ring of chlorophyll structure, occurs at similar concentrations in both types of wines. *Oligo-elements* occur in higher average concentrations in *red wines* due to a longer contact with the grape solid parts (skin and seeds) during the fermentation process. Noticeably higher values in the content of *Fe* observed for *red wines* are generally associated with natural causes, iron uptake from the soil being more important for this kind of wine, but are also due to longer contact with the grape skin. Isolated values much above average observed for *Fe* and *Al* is certainly due to winemaking process and oenological practices used. However a detailed analysis of the *Mn*, a natural constituent in wine, less affected by the oenological practices, could lead to interesting results regarding geographic locatization. Values *Sr* also seem to contain a local touch but a broader analysis is need. On the other side *Cu* seems to be the element most affected by oenological practices used. The levels of *Zn* and *Ni*, although they are essential for plant development, are influenced by pesticides and plant protection products applied. Concentrations of all other elements of interest because of their toxicity, *Zn*, *Ni*, *Cr*, *Cd*, *Pb*, *As*, depend on the soil geochemistry, but are also, with great probability, a consequence of the application of pesticides, fungicides and fertilizers, as well as a result of a longer contact with the tools used in the production, storage or packaging.

Due to the many factors involved, wine variety, region, climate, agricultural practices and winemaking techniques, maturation, aging, acting in diverse and interdependent ways, the analysis of these parameters is not easy an task. Despite the inherent difficulty this research field is in a continuous development and refinement, mainly due to the current need for characterization and differentiation of wines with different geographical origins (and, from a broader perspective, for the majority of food) to certify their quality and prevent fraud. The trend worldwide is to develop data bases as complete as possible, which contains as many of these factors monitored longer periods of time. Subsequent statistical analysis, then possible, have already proved to be useful in identifying certain defining characteristics and to isolate outliers.

Bibliography

[1] J. B. West, J. R. Ehleringer, and T. E. Cerling. Geography and vintage predicted by a nnovel GIS model of wine $\delta^{18}O$. *Journal of Agricultural and Food Chemistry*, 55(17):7075–7083, 2007.

[2] L. Vaclavik, O. Lacina, J. Hajslova, and J. Zweigenbaum. The use of high performance liquid chromatography - quadrupole time-of-flight mass spectrometry coupled to advanced data mining and chemometric tools for discrimination and classification of red wines according to their variety. *Analytica Chimica Acta*, 685:45–51, 2011.

[3] Liang Liu. *Geographic classification of wines using Vis-NIR spectroscopy.* PhD thesis, Faculty of Engineering, Computer and Mathematical Sciences, The University Adelaide, 2006.

[4] M. Gil, J. M. Cabellos, T. Arroyo, and M. Prodanov. Characterization of the volatile fraction of young wines from the Denomination of Origin "Vinos de Madrid" (Spain). *Analytica Chimica Acta*, 563:145–153, 2006.

[5] S. Frías, J. E. Conde, M. A. Rodríguez, V. Dohnal, and J. P. Pérez-Trujillo. Metallic content of wines from the Canary Islands (Spain). Application of artificial neural networks to the data analysis. *Food/Nahrung*, 46(5):370–375, 2002.

[6] I. Andujar-Ortiz, M. V. Moreno-Arribas, P. J. Martín-Álvarez, and M. A. Pozo-Bayón. Analytical performance of three commonly used extraction methods for the gas chromatography-mass spectrometry analysis of wine volatile compounds. *Journal of Chromatography A*, 1216:7351–7357, 2009.

[7] S. Vichi, C. Santini, N. Natali, C. Riponi, E. López-Tamames, and S. Buxaderas. Volatile and semi-volatile components of oak wood chips analysed by accelerated solvent extraction (ASE) coupled to gas chromatography-mass spectrometry (GC-MS). *Food Chemistry*, 102:1260–1269, 2007.

[8] J. S. Câmara, M. A. Alves, and J. C. Marques. Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties. *Talanta*, 68:1512–1521, 2006.

[9] M. M. Losada, J. F. López, A. Añón, J. Andrés, and E. Revilla. Influence of some oenological practices on the aromatic and sensorial characteristics of white Verdejo wines. *International Journal of Food Science and Technology*, 47:1826–1834, 2012.

[10] E. Gómez García-Carpintero, M. A. Gómez Gallego, E. Sánchez-Palomo, and M.A. González Viñas. Impact of alternative technique to ageing using oak chips in alcoholic or in malolactic fermentation on volatile and sensory composition of red wines. *Food Chemistry*, 134:851–863, 2012.

[11] J. E. Welke, V. Manfroi, M. Zanus, M. Lazarotto, and C. Alcaraz Zini. Characterization of the volatile profile of brazilian merlot wines through comprehensive two dimensional gas chromatography time-of-flight mass spectrometric detection. *Journal of Chromatography A*, 1226:124–139, 2012.

[12] S. M. Rocha, F. Rodrigues, P. Coutinho, I. Delgadillo, and M. A. Coimbra. Volatile composition of Baga red wine assessment of the identification of the would-be impact odourants. *Analytica Chimica Acta*, 513:257–262, 2004.

[13] J. M. Oliveira, P. Oliveira, R. L. Baumes, and O. Maia. Changes in aromatic characteristics of Loureiro and Alvarinho wines during maturation. *Journal of Food Composition and Analysis*, 21:695–707, 2008.

[14] M.-P. Sáenz-Navajas, E. Campo, P. Fernández-Zurbano, D. Valentin, and V. Ferreira. An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry*, 121:1139–1149, 2010.

[15] L. Robinson, P. K. Boss, H. Heymann, P. S. Solomon, and R. D. Trengove. Development of a sensitive non-targeted method for characterizing the wine volatile profile using headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. *Journal of Chromatography A*, 1218:504–517, 2011.

[16] A. González-Marco, N. Jiménez-Moreno, and C. Ancín-Azpilicueta. Concentration of volatile compounds in Chardonnay wine fermented in stainless steel tanks and oak barrels. *Food Chemistry*, 108:213–219, 2008.

[17] S. Zamúz and M. Vilanova. Volatile compounds after spontaneous fermentation of musts from Vitis vinifera cv. Albariño grapes cultivated in different origins from Rías Baixas AOC, Spain. *Flavour and Fragrance Journal*, 21:743–748, 2006.

[18] E. Gómez García-Carpintero, E. Sánchez-Palomo, M.A. Gómez Gallego, and M.A. González-Viñas. Free and bound volatile compounds as markers of aromatic typicalness of Moravia Dulce, Rojal and Tortosí red wines. *Food Chemistry*, 131:90–98, 2012.

[19] M. Vilanova, Z. Genisheva, L. Bescansa, A. Masa, and J. M. Oliveira. Volatile composition of wines from cvs. Blanco lexítimo, Agudelo and Serradelo (Vitis vinifera) grown in Betanzos (NW Spain). *Journal of the Institute of Brewing*, 115:35–40, 2009.

[20] J. L. Hixson, N. R. Sleep, D. L. Capone, G. M. Elsey, C. D. Curtin, M. A. Sefton, and D. K. Taylor. Hydroxycinnamic acid ethyl esters as precursors to ethylphenols in wine. *Journal of Agricultural and Food Chemistry*, 60:2293–2298, 2012.

[21] M. T. Liberatore, S. Pati, M. A. Del Nobile, and E. La Notte. Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees. *Food Research International*, 43:996–1002, 2010.

[22] J. Bakker and R. J. Clarke. In *Wine: Flavour Chemistry, 2nd Edition*, pages 142–148. Wiley-Blackwell, Oxford, UK, 2010.

[23] F. L. Sterckx, D. Saison, and F. R. Delvau. Wood aging of beer. part I: Influence on beer flavor and monophenol concentrations. *Journal of the American Society of Brewing Chemists*, 70:55–61, 2012.

[24] M. Ortega-Heras, M. L. González-Sanjosé, and C. González-Huerta. Consideration of the influence of aging process, type of wine and oenological classic parameters on the levels of wood volatile compounds present in red wines. *Food Chemistry*, 103:1434–1448, 2007.

[25] R. Perestrelo, A. Fernandes, F. F. Albuquerque, J. C. Marques, and J. S. Câmara. Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. *Analytica Chimica Acta*, 563:154–164, 2006.

[26] J. S. Câmara, M. A. Alves, and J. C. Marques. Changes in volatile composition of madeira wines during their oxidative ageing. *Analytica Chimica Acta*, 563:188–197, 2006.

[27] M. M. Losada, J. Andrés, J. Cacho, E. Revilla, and J. F. López. Influence of some prefermentative treatments on aroma composition and sensory evaluation of white Godello wines. *Food Chemistry*, 125:884–891, 2011.

[28] J. S. Câmara, M. Arminda Alves, and J. C. Marques. Development of headspace solid-phase microextraction-gas chromatography-mass spectrometry methodology for analysis of terpenoids in Madeira wines. *Analytica Chimica Acta*, 555:191–200, 2006.

[29] C. Coetzee and W. J. du Toit. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International*, 45:287–298, 2012.

[30] S. J. Pérez Olivero and J. P. Pérez Trujillo. A new method for the determination of short-chain fatty acids from the aliphatic series in wines by headspace solid-phase microextraction-gas chromatography-ion trap mass spectrometry. *Analytica Chimica Acta*, 696:59–66, 2011.

[31] S. Selli, A. Canbas, T. Cabaroglu, H. Erten, J.-P. Lepoutre, and Z. Gunata. Effect of skin contact on the free and bound aroma compounds of the white wine of Vitis vinifera L. cv Narince. *Food Control*, 17:75–82, 2006.

[32] T. Cabaroglu, A. Canbas, R. Baumes, C. Bayonove, J. P. Lepoutre, and Z. Günata. Aroma composition of a white wine of Vitis Vinifera L. Cv. Emir as affected by skin contact. *Journal of Food Science*, 62:680–683, 1997.

[33] M. J. Gómez-Míguez, M. Gómez-Míguez, I. M. Vicario, and F. J. Heredia. Assessment of colour and aroma in white wines vinifications. Effects of grape maturity and soil type. *Journal of Food Engineering*, 79:758–764, 2007.

[34] E. Sarrazin, D. Dubourdieu, and P. Darriet. Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. *Food Chemistry*, 103:536–545, 2007.

[35] T. B. Coplen. Guidelines and recommended terms for expression of stable isotopes ratio and gas ratio measurements. *Rapid Communications in Mass Spectrometry*, 25:2538–2560, 2011.

[36] Florinela Georgescu and Gabriela Bancila. Temperaturi extreme in romania in 2007 - Meteo România. `http://vremea.meteoromania.ro/node/82`, 2007.

[37] I. Sandu. Evoluţia regimului climatic în România, Ministerul Mediului Administraţia Naţională de Meteorologie, 2009.

[38] Ministerul Mediului şi Schimbărilor Climatice Agenţia Naţională pentru Protecţia Mediului (ANPM). Raport privind starea mediului in românia. `http://www.anpm.ro/Mediu/raport_privind_starea_mediului_in_romania-15`, 2009.

[39] J. Dunbar. A study of the factors affecting the $^{18}O/^{16}O$ ratio of the water of wine. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 174:355–359, 1982.

[40] L. M. Reid, C. P. O'Donnell, and G. Downey. Recent technological advances for the determination of food authenticity. *Trends in Food Science & Technology*, 17:344–353, 2006.

[41] A. Rădulescu. *Cercetări privind optimizarea tehnologiei de obţinere a vinurilor roşii în podgoria Dragăşani.* PhD thesis, Sibiu, Romania, 2012.

[42] W. Diegor, H. Longerich, T. Abrajano, and I. Hornc. Applicability of a high pressure digestion technique to the analysis of sediment and soil samples by inductively coupled plasma-mass spectrometry. *Analytica Chimica Acta*, 431:195–207, 2001.

[43] G. Grindlay, J. Mora, L.Gras, and M. T. C. de Loos-Vollebregt. Atomic spectrometry methods for wine analysis: A critical evaluation and discussion of recent applications. *Analytica Chimica Acta*, 691:18–32, 2011.

[44] G. C. Tenore, J. Troisi, R. Di Fiore, M. Manfra, and E. Novellino. Nutraceutical value and toxicological profile of selected red wines from Morocco. *Food Chemistry*, 129:792–798, 2011.

[45] ***. Hotărârea nr. 1134 din 10 octombrie 2002 pentru aprobarea Normelor metodologice de aplicare a Legii viei şi vinului în sistemul organizării comune a pieţei vitivinicole, nr. 244/2002.

[46] C. E. I. dos Santos, L. R. Manfredi da Silva, L. A. Boufleur, R. Debastiani, C. A. Stefenon, L. Amaral, M. L. Yoneama, and J. F. Dias. Elemental characterisation of Cabernet Sauvignon wines using Particle-Induced X-ray Emision (PIXE). *Food Chemistry*, 121:244–250, 2010.

[47] I. V. Vrček, M. Bojić, I. Žuntar, G. Mendaš, and M. MedićŠarić. Phenol content, antioxidant activity and metal composition of Croatian wines deriving from organically and conventionally grown grapes. *Food Chemistry*, 124:354–361, 2011.

[48] P. Kment, M. Mihaljevič, V. Ettler, O. Šebek, L. Strnad, and L. Rohlová. Differentiation of Czech wines using multielement composition: A comparison with vineyard soil. *Food Chemistry*, 91(1):157–165, 2005.

[49] I. Geana, A. Iordache, R. Ionete, A. Marinescu, A. Ranca, and M. Culea. Geographical origin identification of romanian wines by icp-ms elemental analysis. *Food Chemistry*, 110:1125–1134, 2013.

[50] A. Sass-Kiss, J. Kiss, B. Harvadi, and N. Adányi. Multivariate statistical analysis of botrytised wines of different origin. *Food Chemistry*, 110(3):742–750, 2008.

[51] R. Lara, S. Cerutti, J. A. Salonia, R. A. Olsina, and L. D. Martinez. Trace element determination of Argentine wines using ETAAS and USN-ICO-OES. *Food and Chemical Toxicology*, 45:293–297, 2005.

[52] S. M. Rodrigues, M. Otero, A. A. Alves, J. Coimbra, M. A. Coimbra, E. Pereira, and A. C. Duarte. Elemental analysis for categorization of wines and authentication of their certified brand of origin. *Journal of Food Composition and Analysis*, 24:548–562, 2011.