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ABSTRACT OF THE PhD THESIS

AUTHENTICITY AND QUALITY MARKERS OF WINES EVALUATED BY ADVANCED ANALYTICAL TECHNIQUES

PhD Student,
Birăruți Elisabeta-Irina
(căs. Geană)

Scientific supervisor,
Univ.Prof.PhD. Bala Camelia

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Introduction

Wine is a product widely consumed and establishing its authenticity is one of the most important aspects in food quality and safety. Autentication, identifying fraud and determining the specifications of the product with the label are requirements of consumers and the European Community. For optimal solving of this problem, development and harmonization of validated analytical methods at national and European level and also establishing and broadening the database needed to improve the efficiency of wine control represent international priorities [2]. Valorification of the Romanian wine-growing potential, in order to obtain sustainable competitive advantages in foreign markets, requires the development and promotion of the identity of Romanian wines, based on analytical investigations certifying quality and natural chemical composition of the wines.

Wines authentication involves several aspects, such as, geographical origin [11,23], year of production [41], variety [34], producer and quality [154, 155]. It is important that authenticity of wine to rely on those chemical parameters that do not change during vinification or that are difficult to forge. Over the years, numerous methods for verifying the authenticity of wines were developed, from traditional analytical methods, up to methods based on advanced instrumental techniques. On the other hand, development of faster and less expensive methodologies to obtain full compositional profile of wines by advanced techniques it is required, this being possible through the use of multielement techniques that allow to obtain a large amount of data.

Analytical techniques used for wine autentication (called fingerprinting techniques) involve organic compounds (carbogydrates, aminoacids, proteins, enzymes, organic acids, phenolic compounds, vitamins, volatile compounds) [35,40], inorganic (minerals) [22, 23] and isotopes [9, 143, 144] analysis from the studied matrix using a wide range of advanced analytical equipment. The obtained analytical data are statistically interpreted to provide a fingerprint of the investigated product. It should be mentioned that wine is a complex mixture of organic and inorganic compounds and its composition is influenced by various factors, including: soil and climate conditions for each region (determining the quality of grapes), variety of vine from which the wine is made, and also the human factor represented by the agricultural practices used, the process of winemaking, maturation and storage conditions. Specifically, the “terroir” (climate and soil) represents the decisive factor in the formation of wine, thus, the same species of vine processed in the same way, but in different regions, will present a different chemical composition [43].

On the other hand, wines adulteration represent a concern for a long time, because such fraudulent practices endanger the consumer health, being in to the detriment of original quality wines, produced in specific regions. For this reason, there is a growing interest for wines authentication and identification of analytical methods capable to certify the origin of wines and accurately detect the frauds [148].

Thus, defining authencicity and classification of Romanian wines produced in nationally and internationally recognized vineyards and creation of data banks is now an important subject and necessity at the national level. For this reason, in this thesis there have been developed and implemented new methods for multi-element and isotopic analysis that allow establishing the origin and quality of Romanian wines.

The thesis is divided into two main parts, namely: CURRENT STATE OF KNOWLEDGE which refers to the literature data – chapter 1 and PERSONAL CONTRIBUTIONS which refers to the original results presented during ~~the~~ six chapters.

The study of literature includes a review of current methodologies used for authentication and quality control of wines, at national and international level, focusing on the main authentication markers and also the analytical techniques used for authentication and classification of wines according to the geographical origin, grape variety, year of production and quality. Thus, emphasis was placed on evaluating the authenticity of wines based on the elemental profile, organic compounds (phenolic compounds – phenolic acids, flavan-3-ols, rutin, *trans*-resveratrol, anthocyanins, organic acids, aminoacids, sugars) and isotopic fingerprints, investigations conducted using modern analytical techniques such as: inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrophotometry (AAS) for

elemental profile analysis; high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR) for organic compounds investigation; ICP-QMS, isotope ratio mass spectrometry (IRMS) and SNIF-NMR for isotopic fingerprints; and also the main statistical methods used for statistical interpretation of analytical data (LDA, PCA, ANOVA, AHC, etc.).

PERSONAL CONTRIBUTIONS

This thesis had the goal to obtain a complex compositional profile of wines, by using advanced analytical techniques, with particular emphasis on techniques such as HPLC, ICP-MS, AAS, NMR, IRMS and SNIF-NMR, and also statistical tools, with the aim of developing wine authentication methodologies which will be the basis for building databases with reference samples by which origin of unknown wine samples will be evaluated.

The main objectives of the thesis were:

- The determination of the elemental composition of wines from the most important wine regions in Romania and to use analytical data and multivariate statistical analysis for the differentiation of wines based on the geographical origin.
- Develop and implement a method for determining the $^{87}Sr/^{86}Sr$ isotopic ratio from wines and use this ratio for geographical traceability of wines.
- Investigate the profile of phenolic compounds in wines made from different varieties of vines and production years, coming from representative wine regions and the identification of specific markers used for wines classification by variety, year of production and region of origin.
- Characterization of wines based on *trans*-resveratrol content and assessing the possibility of differentiating the wines.
- Investigating the influence of grape variety, harvest year and grape harvest time on *trans*-resveratrol content from grape skins, for red grape variety.
- Wines discrimination by cumulating the data related to the elemental profile and phenolic compounds from the natural chemical composition of wines.
- Red wines classification according to variety and harvest year, based on anthocyanins profile, anthocyanins ratios, organic acids profile, ^{1}H NMR and ^{13}C NMR fingerprints and also isotopic fingerprint ($^{13}C/^{12}C$, $^{18}O/^{16}O$ and D/H), and cummulation of significant variables for each compounds class, thus providing a complex fingerprint of the wines, on which an accurate interpretation of unkmown wines could be realised.
- Autenticate red table wines, available on the market, based on the stable isotope method ($\delta^{13}C$ and $\delta^{18}O$) and evaluating the possibility of using alternative analytical investigations for red wines authentication by monitoring the alcoholic strength of wine (% vol.), adulterants (sweeteners and synthetic red dyes), HMF or by anthocyanins profile, as indicator of red colour of wines.
- Statistical evaluation of each data set using multivariate statistical analysis through techniques like PCA, LDA, ANOVA, HCA.

CHAPTER 2
INVESTIGATION OF THE ELEMENTAL PROFILE OF WINES TO CLASIFY THEM ACCORDING TO THE GEOGRAPHICAL ORIGIN

Establishing the geographical origin of wines is an issue of major concern for countries around the world, and theat is due to consumer concerns about the authenticity of the products they consume and to protect quality products against false declarations on the geographical origin. The starting premise in the authentication process of the geographical origin of wines is that the elemental profile of vegetation reflects the elemental profile of soil and depends on topography, geology and soil characteristics [43]. Thus, two regions will not have an identical soils map, and for that, the elemental profile of foods could be related with their geographical origin.

The aim of this study was the investigation of the elemental profile (considering elements like Cr, Ni, Rb, Sr, Ag, Zn, Mn, Cu, Co, V, Pb and Be) of authentic wines from famous Romanian vineyards, from different regoins, namely: Muntenia (Valea Calugareasca vineyard), Dobrogea (Murfatlar vineyard) and Moldova (Iasi, Cotnari, Bujoru, Panciu, Odobesti and Nicoresti vineyards), in an attempt to differentiate the winesaccording to the geographic region. A total of 60 wine samples, that included 26 red wines and 34 white wines, were analysed by ICP-MS, after a previous stage of sample preparation by microwave acid digestion. Also, the correlation between elemental profile of wines and provenance soils was investigated.

Comparing the average values of elemental concentration in wines coming from the three wine regions, for most of the elements was observed an overlap of concentration range. However, elements less influenced by environmental and technological factors, allow the development of a discrimination model, based on wine region. The results show that a good differentiation of Romanian wines can be achieved based on elements like: Ni, Ag, Cr, Sr, Zn and Cu for Valea Calugareasca region, Rb, Zn and Mn for wines from Moldova region. The correlation between Mn, Cr, Rb, Sr, Ag and Co allows a good discrimination of wines according to the geographical origin.

Using principal component analysis of the data set, it was observed that the distribution of the variability was expressed based on three principal components, which summarizes approximatively 82% of the variability of the data. Elements like Mn, Cr, Sr, Rb, Ag and Co were identified as important markers for discrimination of wine and soil coming from three important wine producing regions in Romania.

It was shown the correlation between the elemental composition of wines and soils, for the elements: Ni, Ag, Be, Cr, Zn, Pb, Co and Cu. This premise is important for application of fingerprinting methodology based on multielement composition and by using statistical analysis for wine classification according to the geographical origin.

CHAPTER 3
INVESTIGATION OF STRONTIUM ISOTOPIC RATIO $^{87}Sr/^{86}Sr$ AND ELEMENTAL PROFILE OF WINES TO CLASIFY THEM ACCORDING TO THEIR GEOGRAPHICAL ORIGIN

Prestigious wines, with denomination of origin or geographical indication were mostly subject to fraud by false statements on their origin and therefore it is necessary to develop analytical methods, which using specific markers, allow certification of this products [43,128]. The most used variables for wines geographical origin discrimination were: stable isotope ratios of H, C and O [9], and also the elemental profile [22, 23] and isotopic ratio $^{87}Sr/^{86}Sr$ [143, 144] or a combination between them. Numerous studies showed that there is a significant correlation between the $^{87}Sr/^{86}Sr$ isotopic ratio in wines and the soils of origin [11]. Additionally, the strontium isotopic ratio $^{87}Sr/^{86}Sr$ is not affected by the technological process [141], which is the prerequisite for geographical origin authentication. Also, the metabolic processes in plants do not

affect the Sr isotopic fractionation [174], so that the $^{87}Sr/^{86}Sr$ isotope ratio from plants and the resulted products reflect the isotope ratio of soils from the region of origin.

The objective of this study was to develop the method for $^{87}Sr/^{86}Sr$ isotope ratio analysis in wines, by ICP-QMS, after the prior separation of Sr and Rb from the sample matrix, separation which was made on a Dowex 50W-X8 resin and the elution was done with EDTA solution with different concentrations. The method was used for determining the strontium isotope ratio for 21 red wines with denomination of origin (DOC) or geographical indication (IG), coming from three geographical region in Romania, located relatively close one to another (Dealurile Vrancei, Terasele Dunării – Înșurăței and Cuza Vodă), followed by the evaluation of $^{87}Sr/^{86}Sr$ isotopic ratio and the elemental profile (measured with ICP-MS, F-AAS and GF-AAS techniques) to determine the geographical traceability of the analysed wines.

The precision and accuracy of the method were determined using NIST SRM 987 certified reference material, with a certified value for $^{87}Sr/^{86}Sr$ isotopic ratio– 0.71034. The obtained analytical results for NIST SRM 987 were between 0,70652 – 0,71169, with an average value of 0,70971, being in agreement with the accepted value. RSD (%) ranged between 0,41 – 0,68%, with a average value of 0,53%.

The analytical results obtained for the $^{87}Sr/^{86}Sr$ isotopic ratio in the investigated red wines ranged between 0,71015–0,72311. Differences between the isotopic ratios $^{87}Sr/^{86}Sr$ were observed, even in the case of wins coming from Înșurăței and Cuza Vodă, located relatively close one to another. The obtained results illustrate that the $^{87}Sr/^{86}Sr$ isotopic ratio represents an important parameter for differentiation of wines by geographic origin.

Analytical results for those elements whose content in wines is not affected by external factors were chemometrically evaluated using LDA analysis, to classify wines according to the geographical origin. Based on the elemental profile coupled with the K/Rb and Ca/Sr ratios it was obtained a good discrimination of wines according to the geographic origin, which demonstrates the importance of the elemental profile for the geographical traceability of wines. Coupling elemental profile, K/Rb and Ca/Sr ratios, with strontium isotopic ratio $^{87}Sr/^{86}Sr$ it was obtained a good differentiation of wines according to geographical origin. Amongst the investigated variables, Ga, Al and strontium isotopic ratio $^{87}Sr/^{86}Sr$ were significant for geographical differentiation of wines.

Wines from Dealurile Vrancei region can be differentiated especially based on Ba, Pb, Ag, Mg, Zn, Cr, Ni, Se, Fe and strontium isotopic ratio $^{87}Sr/^{86}Sr$, while wines from Terasele Dunării-Înșurăței can be discriminated based on Al, Ga and Rb and wines from Terasele Dunării-Cuza Vodă can be discriminated based on Li, Na and K/Rb ratio.

The variation of strontium isotopic ratio $^{87}Sr/^{86}Sr$ in wines from different geographic regions, reinforce the relationship with the geological substrate, thus, representing a robust geological marker for determining the geographical origin of wines because it is not influenced by agricultural practices and the wine-making process. However, the elemental profile represents a valuable tool for distinguishing the geographical origin of wines, because the analytical determinations were made easier and at a lower cost.

CHAPTER 4
CHARACTERIZATION AND CLASSIFICATION OF WINES BASED ON PHENOLIC COMPOUNDS INVESTIGATION

Wine phenolic compounds are substances which play a significant role in many of its sensory properties, such as color, flavor, body and astringency [56]. Moreover, the type and concentration of phenolic compounds in wine depend on the grape variety, ripening climate and winemaking techniques [61, 62].

Consumption of foods rich in antioxidant content has many benefits on human health [82]. Wines, especially red wines, contain a variety of polyphenolic antioxidants, of which ~~the~~ *trans*-resveratrol is considered with the greatest significance. [84].

Trans-resveratrol is a particularly significant biologically active ingredient for humans, which is why numerous studies have been conducted in order to identify natural sources rich in trans-resveratrol, including red wines and the grapes skins [187]. Also, several authors have studied the transfer of *trans*-resvratrol from grape to wine [185]. To obtain red wines with a high content of trans-resveratrol is very important to know the optimum time of the grapes harvesting, so they can achieve a maximum accumulation of trans-resveratrol.

Chapter 4 assesses the possibility to identify wines based on various phenolic compounds such as phenolic acids, flavan-3-ols, rutin, trans-resveratrol and anthocyanins. Measurements were performed by HPLC on wine samples selected from representative wine growing regions of Romania and the analytical results were statistically processed (LDA, ANOVA) to identify key markers for establishing the geographical origin, the grape variety from which the wine were obtained and possibly, the vintage.

Classification of wines based on the phenolic acids, flavan-3-ols, rutin and trans-resveratrol

This study investigated the polyphenolic profile of authentic wines made from representative grape varieties (red varieties - Cabernet Sauvignon, Feteasca Neagra , Pinot Noir, Merlot and Burgund Mare; white varieties - White Fetească and Muscat Ottonel) grown in different regions of Romania (Valea Călugărească, Murfatlar, Moldova (Bujoru and Iasi) and Minis) with the aim to classify them according to the geographical origin.

The results showed that the phenolic profile of red wines is better defined than that of white wines. This is due to the specific fermentation procedure for obtaining red wines [179]. The phenolic compounds mainly encountered in the studied wine were the (+)-catechin and (-)-epicatechin from the group of flavonoids and the gallic acid from the class of phenolic acids.

The profile of phenolic compounds (gallic acid, (+)- catechin, (-)-epicatechin, p-coumaric, resveratrol and rutin) allowed the differentiation of red wine (Fetească Neagră, Cabernet Sauvignon, Pinot Noir and Merlot) and white (Muscat Ottonel, White Fetească) by their region of origin. It has been found that for wines from Murfatlar region parameters such as resveratrol, gallic acid and p-coumaric are important for differentiation, while for wines from Valea Calugareasca the content of (+)-catechin and (-)-epicatechin is sensitive. For wines originated from Moldova region, the content in rutin is a discriminant factor.

Characterization of wines based on the content of trans-resveratrol

Given that *trans*-resveratrol is a biologically active ingredient found in the composition of wines, its content was investigated in a total of 70 red and white wines belonging to the following varieties: Fetească Neagră, Cabernet Sauvignon, Merlot, Pinot Noir, Mamaia, Burgund Mare, Fetească Regală, Tămâioasă Românească, Sauvignon Blanc, Fetească Albă, Riesling Italian, Muscat Ottonel and Chardonnay, originated from four wine regions in Romania (Muntenia, Dobrogea, Oltenia and Transylvania), years of production between 2008 and 2011. The obtained analytical data were statistically processed to discriminate the wine samples by their region of origin, variety and possibly, vintage, considering the content of *trans*-resveratrol as marker.

The concentration of *trans*-resveratrol in the investigated wines ranged from 0.03 mg/L to 10.23 mg/L for red wines and between 0.07 mg/L to 2.57 mg/L for white wines, depending on the region of origin, variety and year of production. An overlapping was noted between the ranges of trans-resveratrol concentration in the studied red and white wines, which makes the differentiation by variety to be inconclusive. It was observed that red wines produced from grape varieties grown in the Murfatlar vineyard (Dobrogea region), have a higher content of *trans*-resveratrol, possible due to a longer exposure to the sun [78] or in the special case of the grape

variety Mamaia due to the genetic potential. An accurate differentiation of red wines depending on the vintage year was difficult to assess based on the trans-resveratrol content since the obtained values were very close.

Monitoring the content of ~~the~~ *trans*-resveratrol in a total of 70 red and white wines showed that red wines belonging to the varieties Mamaia, Pinot Noir and Fetească Neagră prove to be a good source of introducing ~~the~~ trans-resveratrol in food. Significant amounts of *trans*-resveratrol were also reported in wines from Merlot and Cabernet Sauvignon varieties. White wines showed a significantly lower content of trans-resveratrol compared to the red ones. Apparently, a differentiation of wines from Dobrogea region can be achieved, since they are wines with a high content of *trans*-resveratrol.

Monitoring of trans-resveratrol from grape skins during the ripening of grapes and determination of trans-resveratrol content in the resulted wines

In this study, an investigation regarding the influence that grape variety, vintage and the harvesting date may have on the content of *trans*-resveratrol in the skin of the grapes for a number of red varieties (Pinot Noir, Merlot, Cabernet Sauvignon, Feteasca Neagra, Mamaia) grown in the Murfatlar vineyard, harvest years 2012 and 2013 was performed. The correlation between the trans-resveratrol content measured in the grape skins and the resulted wines was also observed during the two years of harvest.

The results of monitoring the content of trans-resveratrol in grape skins showed that it is significantly influenced by the grape variety, the vintage year, each variety presenting a different aging trend. The maximum level of trans-resveratrol has been reached towards the end of the ripening period, for all the studied varieties. This finding is important for managing the wine making process in order to obtain wines with a higher content of trans-resveratrol.

The *trans*-resveratrol was significantly higher in the harvest of 2012 compared to 2013, both in the skin of grapes and wines, confirming thus the importance of the climatic conditions in the accumulation of *trans*-resveratrol in grapes and also the possibility to differentiate wines according to the year of production.

Furthermore, there was a good relationship between the amount of *trans*-resveratrol in the grape skin and that of wines, showing that the level of *trans*-resveratrol in the grape skin can be used as a marker to predict the level of *trans*-resveratrol in the resulted red wines.

CHAPTER 5
INVESTIGATION OF THE ELEMENTAL PROFILE AND PHENOLIC COMPOUNDS IN WINES TO DIFFERENTIATE THEM ACCORDING TO THE VARIETY AND GEOGRAPHICAL ORIGIN

Monitoring the level of micro- and macro-elements in wines is of particular importance due to their ability to differentiate wines according with the area in which grapes have been grown, making it a valuable tool for the geographical traceability of wines [43]. A fundamental role is given to the phenolic compounds, those greatly contributing to the formation of wine specific characteristics, such as color, aroma, flavor, valuable parameters for differentiating between varieties [56]. Also, there is a growing tendency to study the composition of wines based on its minor constituents and to obtain a more complex characterization, underlying the wines authentication process [81].

The present study examined the possibility of using the elemental profile (21 elements - Li, Be, Co, Ni, Cs, U, Pb, V, As, Ba, Cr, Cu, Zn, Al, Mn, Rb, Sr, Fe, Ca , Mg, Na, K) and the phenolic compounds ((+)-catechin, (-)-epicatechin, gallic acid and resveratrol, p-coumaric acid, ferulic acid and trans-cinnamic acid) to differentiate the wines from the vineyards of Recas and Dragasani according to their geographical origin and variety.

The analytical determination has been carried out using the ICP-MS and F-AAS techniques to determine the elemental profile, and by HPLC for profiling the phenolic compounds; the obtained analytical data were statistically processed using PCA analysis, highlighting the main authentication markers.

Wines investigated in this study are authentic wines, sweet and dry, obtained in the Drăgăşani vineyard (wine region Oltenia) and Recaş vineyard (wine region Banat). The investigated varieties were: Tamâioasă Românească, Crampoşie Selecţionată, Fetească Regală, Sauvignon Blanc, Chardonnay and Muscat Ottonel for white wines and Negru de Drăgăşani, Pinot Noir, Cabernet Sauvignon and Merlot for red wines. By the multivariate statistical analysis of data regarding the elemental profile and phenolic compounds a good differentiation of wines was achieved considering their geographical origin and variety, and even a differentiation of wines from the viticultural areals, situated at relatively small distances one from another (the North and South of the Drăgăşani vineyard). Thus, elements such as Ba, Be, Cr, Cs Li, Mg, Na, Ni, Sr, U and Zn, and phenolic compounds, such as (+)-catechin, (-)-epicatecina, resveratrol, ferulic acid and p-coumaric acid were identified as important markers to differentiate wines on the basis of the geographical origin.

For wines from the Drăgăşani vineyard, it was identified the possibility of differentiating the variety on the basis of elemental composition and profile of the phenolic compounds, highlighting some markers to classify wines according to the grape variety. Native varieties from Drăgăşani vineyard (Crâmpoşie Selectionata and Negru de Drăgăşani) had significant amounts of Zn and Ba and low amounts of Na and Cs compared to other investigated varieties (Tămâioasă Românească, Sauvignon Blanc and Feteasca Regala). With regard to the organic markers, depending on the variety of wines, differentiation can be achieved based on the content of (+)-catechin, ferulic acid and *trans*-resveratrol. The variety Fetească Regala presented a high content of (+)-catechin and ferulic acid and a low content of *trans*-resveratrol, compared to other varieties. Tămâioasa Românească variety is characterized by large quantities of *trans*-resveratrol and ferulic acid.

Based on both the inorganic markers (Cs, Na, Zn, Ni, U and Ba) and organic ((+)-catechin, ferulic acid and *trans*-resveratrol) was achieved a differentiation of the specific varieties from Drăgăşani vineyard compared to the international ones, with a percentage of 74%. The proposed methodology can be applied to determine the geographical origin and the variety of the commercially available wines.

CHAPTER 6
INVESTIGATING THE PROFILE OF ANTHOCYANINS AND ORGANIC ACIDS, OF NMR AND ISOTOPIC SIGNATURE TO DIFFERENTIATE THE WINES ACCORDING TO THE VARIETY AND VINTAGE

In recent years, assessing traceability and authenticity of wines has become a prerequisite in many more countries around the world [2]. In the wine industry, developing chemometric applications which take into account a large amount of analytical information concerning the composition of wines proved to be a versatile and valuable tool for evaluating geographical origin [9], the variety [41, 206], year of production [41, 207] and technological characteristics [208].

In this study we aimed to develop methodologies for classifying authentic wines by variety and year of production and to identify specific markers for each type of classification. There were investigated five varieties of authentic red wines (Sauvignon, Merlot, Fetească Neagră, Pinot Noir şi Mamaia) originated from Murfatlar vineyard which is characterized by a climate that favors the production of top quality wines. Both young and old wines, ~~by~~ vintages 2009 to 2014, were considered for the study. The analytical data on the composition of anthocyanins, organic acids, isotopic parameters ($^{13}C/^{12}C$, $^{18}O/^{16}O$ and D/H), and the information obtained from the ^{1}H NMR and ^{13}C NMR spectra were statistically processed using LDA

analysis. The analytical techniques used were the HPLC and NMR to obtain fingerprints of different chemical classes of the organic composition of wines and the SNIF-NMR and IRMS to obtain the isotopic fingerprint.

Overall, based on the used markers was succesed a differentiation of wines in 5 groups, clearly separated, corresponding to the five wine varieties investigated in this regard. Important markers were the malvidin-3-O-glucoside (Mv), the ratios between anthocyanins, namely, the ratio of malvidin-3-O-acetilglucozide and malvidin-3-O-glucoside (Mva/Mw) and the ratio of acetylated anthocyanins and the coumaryl of peonidinei and malvidine (R/C); the oxalic acid, shikimic acid, lactic acid, citric and succinic acids; ratio of $(D/H)_I$ and $\delta^{13}C$; 2,3 butanediol, methanol, glucose, and amino acids such as alanine, histidine and leucine - variables identified in the NMR spectra.

To differentiate wines by vintage, the concentrations of wine analytes were set as independent variables and the production years (between 2009 and 2014) were set as classification variables. Thus, the differentiation between vintages was based on the following variables: delphinidin-3-O-glucoside (De), peonidin-3-O-glucoside (On), malvidin-3-O-acetilglucozide (MVA), malvidin-3 -O-cumarilglucozida (MVC) peonidin-3-O-cumarilglucozide (Pec), but also on different ratios of anthocyanins, including the relationship between cyanidin-3-O-glucoside (Cy), petunidin-3-O-glucoside, malvidin-3-O-cumarilglucozida and malvidin-3-O-glucoside (Cy/Mv, Pt/Mv, Mvc/Mv) and the ratio between the amount of anthocyanins and malvidin 3-O glucoside (S_{ant}/Mv) as anthocyanin parameters; isotopic parameters ($\delta^{18}O$, $\delta^{13}C$ $(D/H)_I$ and R - the relative distribution of deuterium in methyl and methylene groups of the ethanol molecule; glucose, amino acids (alanine and isoleucine), glycerol - variables identified from the NMR spectra.

Thus, it was demonstrated that using multivariate statistical analysis for a complex set of analytical data obtained by various instrumental techniques, a correct classification of wines by variety and year of production can be achieved. Validation of the proposed statistical models for red wines classification was based on a set of control wines and by using the cross-validation technique. For each variety and year of production we obtained a correct classification of the control wine samples, which confirms the validation of the statistical models and suggests the possibility of their use to authenticate unknown wine samples.

CHAPTER 7

RESEARCH ON THE QUALITY OF RED WINES USING MULTIPARAMETER INVESTIGATIONS FOR FRAUDULENT PRACTICES IDENTIFICATION

The matrix “wine” presents a particular importance from the point of view of authenticity testing, because it has always been subjected to various malpractices. The production and marketing of wine have always been associated with high costs and, for that, their counterfeiting and false statements about their origin are increasingly found [128]. The wines that are commonly adulterated are table wines, which are packed in PET bottles and marketed under different names, and less with quality wines.

In this study we proposed the use of advanced analytical techniques for certifying the authenticity and traceability of wines, in order to detect any fraudulent practices in the wine industry. For that, 29 table wines, packaged in PET bottles, purchased on the market, were investigated to detect fraudulent additions of sugar, water, synthetic sweeteners and synthetic coloring agents. Also, in this study were investigated 23 authentic red wines obtained by microvinification in the Murfatlar vineyard (Romania), these samples being considered as reference samples.

The addition of exogenous sugar and water in counterfeit red table wines was detected by measuring the content of stable isotopes ($\delta^{13}C$ and $\delta^{18}O$), and additionally, wines adulteration

was confirmed based on classical parameters, such as alcoholic strength of wines (% vol.), the presence of 5-hydroxymethylfurfural (HMF), and also the presence of synthetic sweeteners and syntetic red dyes used to correct deficiencies of taste and color. Also, in order to establish the authenticity of table wines, we have investigated the anthocyanins profile as indicator of red wine color change, after the adulteration practices.

In the first part of this study, for the assessment of the authenticity of the investigated wine samples (commercial and authentic), the stable isotopes method (SIRA) was used, consisting in comparing the stable isotope values of the investigated wines ($\delta^{13}C$ and $\delta^{18}O$) with isotopic databases for authentic wines from the same geographical region and years of production. This procedure allowed detection of wines whose isotopic data is outside the range of variation, suggesting the adulteration by adding sugar, alcohol or water. Thus, the investigated wines (23 authentic wines and 29 commercial table wines) were grouped into four categories: authentic wines (23 samples), good table wines (16 samples), table wines suspected of adulteration (5 samples) and adulterated table wines (8 samples).

At the same time, for wines which show modified organoleptic properties, for assessing their authenticity, other analytical investigations could be realised, investigations which are accesible in many laboratories, such as, chromatographic investigations enabling the identification of unauthorized food additives in wine industry (synthetic sweeteners and synthetic red dyes), as well as the presence of 5-hydroxymethylfurfural (HMF), on which certain prohibited oenological practices can be detected. Thus, table wines classified as adulterated were sweetened by the prohibited addition of synthetic sweeteners (K acesulfame, saccharine and aspartame) or artificially colored by illegal use of synthetic red dye –azorubine, confirming the falsification of those wines in order to correct the taste and color deficiencies after exogenous water addition. HMF was detected in one sample of table wine, indicating a possible addition of fructose corn syrup (HFCS) for chaptalisation. Even if the anthocyanins represent patterns for wine variety differentiation [95, 227], we investigated this compounds closely related to the modification of the red colour of wines due to adulteration practices.

For the commercial table wines, differences in the anthocyanins profile for the different categories (good, suspect and adulterated wines) were observed, where the amounts expressed as mg/L Mv are higher in good table wines compared to those suspect or adulterated. Adulteration of table wines by adding exogenous water led to lower anthocyanins content, which is reflected in the colour of the red wines, in this regard, delphinidin 3-O-glucozide (De), petunidin-3-O-glucozide (Pt) and malvidin-3-O-glucozide (Mv) represent valuable markers.

Particularly, anthocyanin ratios represent valuable tools for differentiation of adulterated table wines which show highly modified isotopic fingerprints, which was the case of T23, T24 and T25 wine samples, characterized by excessive adulteration with C4 plants and water addition. Also, the dilution of wines with water followed by color correction by the addition of synthetic dyes such as azorubine, lead to changes in the anthocyanin profile.

The results clearly indicate that anthocyanins profile and proposed anthocyanins ratios, coupled with statistical interpretation of the data, provide reliable information to differentiate the studied table wines in classes corresponding to wine quality. Thus, the anthocyanins profile and proposed anthocyanins ratios reinforce the results obtained through isotopic analysis.

Investigating a large number of chemical parameters, such as isotopic parameters, anthocyanins and also additives (sweeteners and dyes), we can obtain a reliable assessment of the commercial red table wine authenticity. By creating a database with anthocyanins profiles of natural red wines, we also can identify adulterated red wines on the market, this being achieved by using analytical techniques available in many laboratories.

The results of this study show that amongst the investigated table wines, 28% were adulterated and 17% were suspect of adulteration, indicating an alarming increase of fraudulent practices in the wine industry like, addition of natural or artificial sugars, water and colouring agents.

GENERAL CONCLUSIONS

In this thesis, different wine authentication and control methodologies were achieved, focusing on the main authentication markers and analytical techniques used for wines classification depending on the geographical origin, grape variety, year of production and quality. Thus, investigations related to the compositional profile of authentic wines from representative viticultural areas from Romania (Valea Călugărească, Murfatlar, Moldova, Drăgășani, Recaș, Miniș, Dealurile Vrancei, Terasele Dunării – Însurăței and Cuza Vodă), both red wines (Fetească Neagră, Cabernet Sauvignon, Merlot, Pinot Noir, Mamaia, Burgund Mare) and white wines (Fetească Regală, Tămâioasă Românească, Sauvignon Blanc, Fetească Albă, Riesling Italian, Muscat Ottonel and Chardonnay) and different years of production (2009-2014) were realised. The classification of wines according to the geographical origin, grape variety and year of production was performed, based on the analytical data on the elemental composition, different organic compounds (phenolic compounds – phenolic acids, flavan-3-ols, rutin, *trans*-resveratrol, anthocyanins; organic acids; aminoacids, sugars) and isotopic fingerprint, using advanced instrumental techniques (ICP-MS, AAS, HPLC, NMR, IRMS, SNIF-NMR) and multivariate statistical analysis (PCA, LDA, ANOVA, AHC) for the interpretation of the analytical data.

Also, the authenticity of some red table wines was verified based on isotopic (IRMS) and chromatographic (HPLC) investigations, in order to identify fraudulent practices (addition of sugar and water in wine, addition of synthetic sweeteners and synthetic red dyes) used in the winemaking process.

The main markers, analytical techniques and chemometric methods used in this thesis to authenticate wines were:

Markers	Instrumental techniques/statistical methods	Wines authentication
Elemental profile	ICP-MS, AAS / PCA, LDA, ANOVA	Geographical origin
Strontium isotopic ratio $^{87}Sr/^{86}Sr$	ICP-QMS / LDA	
Phenolic compounds: *fenolic acids, flavan-3-ols, flavonols, stilbens, anthocyanins*	HPLC-PDA / ANOVA	Variety Geographical origin Year of production
Organic acids	HPLC-PDA / LDA	Variety Year of production
NMR fingerprint	NMR / LDA	
Isotopic fingerprint ($\delta^{13}C$, $\delta^{18}O$ and D/H)	IRMS, SNIF-NMR / LDA	Year of production Variety
Isotopic fingerprint ($\delta^{13}C$ and $\delta^{18}O$)	IRMS / LDA, HCA	Identification of fraudulent practices
Food additives (sweeteners, dyes), HMF, anthocyanins profile	HPLC-PDA / LDA, ANOVA	

The results presented in this thesis represent the starting point for the construction of databases with the main classes of chemical compounds from natural chemical composition of wines (elements, phenolic compounds, sugars, aminoacids, volatile compounds), supplementary to the isotopic wine database, on which the autentication of unknown wines can be done.

Wines classification methodologies proposed in this thesis can be applied also to the wines coming form other wine regions, different varieties and years of production, thus being useful for prevention of fraudulent practices in the wine industry.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The originality of the thesis consist in the investigation of the natural chemical composition of wines from Romania, highlighting the analytical and statistical methods used for discrimination of wine according to the geographical origin, variety, year of production and quality.

In this thesis was investigated for the first time the possibility of using the elemental profile and the strontium isotopic ratio $^{87}Sr/^{86}Sr$ to distinguishing the geographical origin of wines from representative Romanin wine regions, the method of determining the isotopic ratio $^{87}Sr/^{86}Sr$ in wines being implemented for the first time in Romania.

Also, it was studied the potential of phenolic compounds from wine, highlighting the specific markers for wine differentiation according to variety, year of production and geographical region of origin. For the first time in Romania, it was carried out a study on monitoring *trans*-resveratrol content in the skin of red grapes, establishing the optimal harvest time, in order to obtain wines with high content of *trans*-resveratrol, biologically active principle with numerous health benefits. Investigation of *trans*-resveratrol content in a number of 70 red and white wines from Romania demonstrated that wine belonging to red varieties Mamaia, Pinot Noir and Fetească Neagră prove to be a good source of *trans*-resveratrol in the human diet.

For the first time, it was investigated the possibility of differentiation of some red wine according to variety and year of production, based on multiparameter analytic investigations made using advanced analytical techniques such as HPLC, NMR, IRMS and SNIF-NMR, establishing specific markers for every type of classification.

In the end, this thesis provides valuable information on the possibility of identifying the commercial adulterate red table wines, based on stable isotope method (carbon and oxygen) and the use of isotopic wine database, in order to detect water and sugar addition, and also, the use of alternative analytical investigations for the confirmation of detected fraudulent practices. The anthocyanins profile of red wines was used, for the first time, to assess the quality of commercial red table wine.

The results of the research conducted in this thesis were disseminated by developing and publishing, as first author, of 8 scientific papers in ISI journals; publishing 5 original papers in journals indexed in national and international databases; participation at 5 international scientific conferences; participation at 8 national scientific conferences.

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LIST OF PUBLICATION IN THE FIELD OF THE DOCTORAL THESIS

A) ISI journals:

1. Verifying the red wines adulteration throught isotopic and chromatographyc investigations coupled with multivariate statistic interpretation of the data, **Geană, E.I.**, Popescu, R., Costinel, D., Dincă, O.R., Ştefănescu, I., Ionete, R.E., Bala, C., Food Control, 62, 2016, 1-9, (IF = 2,806).
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