The Doctoral School of Engineering Sciences
Mathematics

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DOCTORAL THESIS

DEVELOPING THE QUALITY CERTIFICATION CAPACITY OF WINES USING ADVANCED FINGERPRINTING TECHNIQUES ON THEIR AUTHENTICITY

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ABSTRACT

The numbering of tables, figures and bibliographic references is the one in the doctoral thesis.

SUMMARY

The research undertaken in this study connects the scientific support publications consulted and my own research within the National Institute for Research and Development for Biotechnologies in Horticulture – Ștefănești, Argeș.

The present thesis includes the pedological and climatic description of eight geographical areas: the hills of Moldova, with Cotnari vineyard and the wine-growing center of Iasi, the hills of Târnavelor with the wine-growing center of Blaj, the hills of Muntenia with Ștefănești wine-growing center, the hills of Vrancea with Focșani vineyard, the hills of Banat with Silagiu vineyard, the hills of Oltenia with Drăgășani vineyard and the hills of Dobrogea with Babadag vineyard.

The doctoral thesis research started with a mineral and heavy metals soil study in four major wine-growing centers and vineyards (wine-growing center Iasi, Ștefănești wine center, Blaj wine center and Babadag vineyard). With the help of the data obtained, correlations were also made between the minerals existing in the soil and the minerals in the wines of each geographical area studied.

The thesis research, undertaken between 2019-2021, aimed to certify the quality of wine by determining the elemental composition of wine, their mineral and metallic profile, but also from the point of view of the polyphenolic profile. Research has been carried out for both white wines and red wines. These determinations were made by advanced analytical methods (Midray BA200 Analyzer, ICP-MS Varian MS820, atomic absorption spectrometry, FAAS flame technique, UHPLC-ESI/HRMS, Real Time PCR and and statistical interpretation of the data obtained by PCA, ANOVA, AHC, DUNCAN.

In order to authenticate the wines, the molecular determinations, of fingerprinting and quantification of DNA from its must and wine, on 11 varieties from Ștefănești, were also used to highlight reliable markers that can certify the authenticity and typicality of the wine.

The main objectives pursued in this study were:

- The pedological, climatic and oenological assessment of eight geographical areas in Romania under study.
- Certification of the typicality and authenticity of wines from the eight geographical areas of Romania studied on the basis of qualitative markers.
- Determination of micro-, macro-minerals and heavy metals in wine.
- Determination of micro-, macro-minerals and heavy metals in soil samples and their interpretation in relation to the values obtained in wine.
- Determination of the authenticity of wines from the eight geographical areas of Romania using the polyphenolic profile.
- Use of molecular techniques of "fingerprinting of wines" in order to certify their typicality and authenticity.
- Achievement of the correlation between the above mentioned activities and the use of the compounds identified by statistical analysis: PCA, ANOVA, AHC, DUNCAN.

Doctoral thesis with the title: "Developing the quality certification capacity of wines by using advanced fingerprinting techniques on their authenticity" is structured in five chapters.

In Part I "The current state of knowledge " is included the summary of the thesis and chapter I. In the second part, with the name "Own contributions and subsequent directions to be followed in research" are included chapters II, III, IV, V, Annexes, "List

of publications", "List of conferences concluded, " List of research contracts"and Bibliography.

In chapter I entitled "The current state of research on the methods of qualitative certification of wines" includes 2 subchapters called "Wine quality" and "Modern techniques and methodologies for certification of authenticity and typicality of wine". The second subchapter consists of four parts and presents advanced techniques for certifying the quality of wine existing in the literature.

One of the important chapters of the doctoral thesis begins with chapter 2, entitled " The purpose and objectives of the research. Research material and methods". As the chapter is entitled, it is divided into three subchapters, where they are described: the purpose and objectives of the study, the research material and the working method. Under sub-chapter "research material" includes the study of monovarietal wines from eight geographical areas, of the most important wine centres and vineyards in Romania. Within this sub-chapter, studies were carried out on the pedo-climatic conditions in each vineyard and wine-growing center studied. Also in chapter two, the advanced methods of certification of wine quality used in our research and the equipment used in determinations were presented.

In Part II, Chapter 3 entitled " Own results on the authenticity and typicality of wines by analysing their qualitative markers, depending on their geographical origin". This chapter is divided into four subchapters, which present the research, on four major directions of certification of wine quality.

In sub-chapter 4.1, named "Own results regarding the investigation of the elemental profile of wines, in order to determine their authenticity and typicality depending on the variety and the region" monovarietal wine samples from the production of 2019 and 2021 were taken as research material. The wine samples were in number: 35 varieties of white wine and 35 varieties of red wine, from 11 vineyards and wine centers. The wine samples were collected from: the geographical area of Transilvania, from the wine-growing research resort Blaj and the vineyard of Târnave; the geographical area Dobrogea from the Babadag vineyard; the geographical area of Banat from Silagiu vineyard; the geographical area Moldova from the Cotnari vineyard, from the wine-growing center Iași and the Focșani vineyard from the Gârboiu wine-growing area; the geographical area of the Dragasani vineyard; the Muntenia area from the Ștefănești wine-growing center and the Dealul Mare vineyard. Within this sub-chapter after a statistical investigation of the data presented, conclusions were drawn regarding the wine varieties belonging to the studied geographical areas.

In chapter 4.3 named "Own results regarding the investigation of the polyphenolic profile of wines and bioactive compounds, in order to determine their authenticity and typicality depending on the variety and the geographical region" was taken as research material monovarietal wines: 25 white varieties (Fetească Regală, Riesling Italian, Riesling de Rihn, Sauvignon Blanc, Muscat Ottonel, Tămâioasă Românească, Chardonnay, Aligote, Fetească Albă, Crâmpoșie Selecționată, Grasă de Cotnari, Traminer Roz, Pinot Gris, Muscat de Măderat, Busuioacă de Bohotin) and 25 red varieties (Merlot, Burgund Mare, Cabernet Sauvignon, Fetească Neagră, Pinot Noir, Petit Verdot, Cabernet Franc, Baluerzweigelt, Syrah, Negru de Drăgășani), from two years of production 2019 and 2021. Also within this sub-chapter, statistical correlations were made between polyphenolic parameters, wine varieties, studied geographical areas and production years, resulting in some conclusions.

In sub-chapter 4.4. entitled "Own results on the investigation of DNA from vin" was used as research material must and monovarietal wine one year old. The wines were from the 2020 production year. There were selected to be analyzed samples of must and wine obtained from five varieties of white wine (Sauvignon Blanc, Riesling

Italian, Fetească Regală, Chardonnay și Muscat Ottonel) and six varieties of red wine (Blauer Zweigelt, Burgund, Fetească Neagră, Merlot, Cabernet Sauvignon and Pinot Noir) from Stefanesti vineyard. A DNA identification and quantification was made, after which a statistical interpretation of the methods of analysis was made, depending on the variety and the amount of DNA produced.

In chapter 5 ”Originality and innovative contributions of the doctoral thesis, further directions to be followed in research ”, are presented the general conclusions, together with own contributions, resulting from the research made in this doctoral thesis, as well as further directions to be followed.

The thesis contains 172 pages, 39 tables and 105 figures. The thesis has attached 15 annexes, contains 393 bibliographic references, has attached a list of publications in the field of doctoral thesis, a list of participation in conferences in the field of doctoral thesis and a list of research contracts concluded during the doctoral thesis.

Through this thesis I address my gratitude to Univ. Prof. Phd. Eng. Ovidiu Tița, for his contribution in the elaboration of the plan, the documentary materials provided, the guidance and the coordination of the doctoral thesis, the multiple suggestions and the aid granted throughout the doctoral thesis.

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I thank from my heart the family who was with me and I dedicate this doctoral thesis to them.

INTRODUCTION

Wine is a highly used but also easily falsified food, because of this the study of mineral, organic elements (Gean, 2014; Huang, 2017), polyphenolic composition and elemental composition (Avar, 2007; Di Paola-Naranjo, 2011; Geana, 2011; Dumitrov, 2018; Jakabova, 2021) are the tools used in authentication and classification of wines. Molecular analysis and DNA determination of –of wine could be a useful tool for authentication, determination of their origin and year of production (This, 2006; Raimondi, 2020; Galstyan, 2021; Onache, 2021). The study of the phenolic composition of wine may allow the establishment of one or more biomarkers specific for a particular type of variety (Garrido, 2013; Gean, 2014).

Wine analysis is laborious and expensive work (Giosanu, 2011). As the analytical results should be useful, they should express the physico-chemical parameters of the wine composition, based on the determination of the wine quality (Cardea, 2007; Giosanu, 2011). The typicality and authenticity of the wine are the basic attributes that generate the quality of the wine (Lopez, 2018; Palade, 2018). The criteria for verifying the authenticity of red wines are: anthocyanin profile, amino acid footprint, wine alcohol and isotopic composition of water (Giosanu, 2011). The control and proof of authenticity is generally carried out using various analytical (Schlesier, 2009) and statistical (Smeyers-Verbeke, 2009; Romisch, 2009) methods, which are based on the differentiation of geographical origin, grape variety, wine age and production technology (Palade, 2014).

CHAPTER I

The thesis is divided into two main components, namely: Chapter I – The current state of knowledge, in which the literature data on wine quality in the current climatic conditions are updated, on global production and last but not least the current methodology used in certification of wine quality.

The chemical composition and sensory characteristics of wine are strongly influenced by geographical origin and climatic conditions (Mira de Orduna, 2010; Dal Santo, 2013; Bora, 2016), soil, production area, sun exposure, the degree of maturation of grapes (Robinso, 2014), the impact of agrotechnical measures (Bureau, 2000; Dumitrov, 2018), winemaking technology (Marini, 2006; Sen, 2014) grape variety and genetic contribution of vine variety (Prins, 2009; Isci, 2009 and 2014; Catalano, 2016; Pereira, 2018; Raimondi, 2020; Purwidyantri, 2023), its ability to accumulate compounds with a strong aromatic effect in the grape bean and transfer it to wine (Abrasheva, 2008; Gonzalez-Barrei, 2018).

Minerals in wine come from the production capacity of the vine to take elements from the soil (geographic region), climatic factors such as heavy rains, environmental conditions such as pollution and agricultural applications such as fertilizers and pesticides. The mineral content of red and white wines in the same region may differ due to the winemaking process (such as maceration) and impacting on the elemental composition (Coetzee, 2005; Sen, 2014). Minerals allow differentiation according to the geographical area of origin due to a direct relationship with the composition of the soil.

Wine benefits from rigorous and well-established legislation, both in terms of production technologies and quality standards. All wine-growing countries have national legislation in the field of viticulture and wine production, which is harmonized with the resolutions of the International Organisation of Vine and Wine (OIV) and the regulations of the European Union (no 1493/1990).

To determine the typicality and authenticity of wines, some researchers have combined all the physical-chemical parameters of the wine, such as organic acids (Arvanitoyannis, 1999; Makris, 2006), amino acids (Hernandez-Orte, 2002; Rapeanu, 2009) and volatile compounds (Ivanova, 2013; Gean, 2014). Elementary and polyphenolic profile is another method to authenticate wines by origin (Avar, 2007; Di Paola-Naranjo, 2011; Gean, 2011; Gean, 2013; Gean, 2014).

The composition of the wine was subject to authentication by various methods of analysis (Moret, 1994; Arvanitoyannis, 1999; Onache, 2020), such as high-pressure liquid chromatography – HPLC (Etievant, 1988; Soufleros, 1998; Ranca, 2011; Gean, 2014; Tita, 2025), gas chromatography –GS (Forcen,1992), atomic absorption spectroscopy. B. Tawali, 1997; Muranyi, 1998; Dutre, 2011; Gean, 2014) and capillary gas chromatography – CGC (Garcia-Jares, 1995), and mass spectrometry - MS (Baxter, 1997; Galani-Nikokaki, 2002). Minerals are used to recognize geographical origin (Arhurst, 1996) and can be determined the micro-minerals of wine using an inductively coupled plasma mass spectrometer ICP-MS and optical emission spectrometer ICP-OES inductively coupled plasma (Gean, 2012; Vaudour, 2015; Porezka, 2018; Onache, 2022. Macrominerals in wine can be identified using an atomic absorption spectrophotometer with the flame technique having a graphite furnace, compared to macrominerals in the soil of the same vineyard, by direct analysis of the solid sample using HR-CS-AAS (Stafilov, 2009; Gean, 2014; Onache, 2022).

In *the second part* are presented *the Personal Contributions* brought within the thesis, which refer to the personal and original data, presented during four chapters.

CAPITOLUL II – THE PERSONAL CONTRIBUTIONS

Chapter II outlines the Purpose and Objectives of Research. The purpose of this research is to certify the quality of the wine through determinations of the elementary composition, of the polyphenolic profile, of the mineral and metallic profile for the red and white wines from the eight important Romanian wine regions. The qualitative certification of the wine was well represented, by fingerprinting and quantifying the DNA of the must and monovarietal wine, in order to highlight reliable markers for determining the authenticity and typicality of the wine..

OBJECTIVES*:*

- Pedoclimatic and oenological assessment of the 8 geographical areas in Romania under study
- Certification of the typicality and authenticity of wines from the 8 geographical areas of Romania studied based on qualitative markers
- Determination of micro-, macro-elements and heavy metals in wine (using inductively coupled plasma mass spectrometer ICP-MS.
- Determination of micro-, macro-elements and heavy metals in total forms from soil samples (using atomic absorption spectrometry, FAAS flame technique) and their interpretation in relation to the values obtained in wine.
- Determination of the authenticity of wines from geographical areas of Romania with the help of the polyphenolic profile (using UHPLC-ESI/HRMS - mass spectrometry with ionization in tandem with high performance liquid chromatography)
- Use of macromolecular techniques of “Fingerprinting of wines” to certify their typicality and authenticity (Real Time PCR).

- Achievement of the correlation between the above mentioned activities and the use of the compounds identified by the statistical analysis: PCA, ANOVA, AHC, DUNCAN.

Also in this chapter are presented the material used and the working method.

The research material for establishing the authenticity and typicality of the wine was used 740 varieties of wine, coming from 8 main geographical areas of wine production in Romania: from wine making research center Blaj, Miniș and Iași, Institute for Research Development for Biotechnologies in Horticulture Ștefănești from Argeș, Romanian producers: Cotnari vieyard – Dealurile Moldovei, Gârboiu Winery Focșani, Agricola Stirbey from Drăgășani vineyard, from Tarnavelor vineyards, Babadag – Dobrogea Hills and Banat Hills – Silagiu. The wines were produced in 2019 and 2021.

The wine varieties subject to research were: from Babadag – Aligote, Pinot Gris, Chardonnay, Merlot, Burgund Mare; Pinot Noir; from Silagiu vineyard – Feteasca Albă, Muscat Ottonel, Burgund Mare and Cabernet Franc; from Cotnari vineyard – Grasă de Cotnari and Feteascӑ Neagӑ, Gârboiu vineyard from Focșani – Sauvignon Blanc and Feteasca Neagră, from Miniș: Muscat de Miniș and Cadarcă; from Târnave vineyard: Feteascӑ Regalӑ și Pinot Noir; Drăgășani vineyard: Chardonnay, Crâmpoșie Selecționată, Feteasca Regală, Tămâioasă Românească, Cabernet Sauvignon, Merlot, Negru de Drăgășani; from Stefanesti – Riesling Italian, Feteasca Regală, Feteasca Albă, Sauvignon Blanc, Muscat Ottonel, Tămâioasă Românească, Burgund Mare, Merlot, Cabernet Sauvignon, Pinot Noir, Feteascӑ Neagrӑ.

Regarding the analyzes, chemometric determinations were made, metal and mineral profiles of wines were made, polyphenolic and bioactivity profiles of wines were made, DNA extraction and quantification was made from must and wine, the biological material used in the experience was made up of eleven varieties of musts and monovarietal wines vinified at laboratory scale (after European Regulations 2729/2000, updated European Regulations 2030/2006), from the Research Institute – Development for Biotechnologies in Horticulture-Ștefănești, Argeș.

Pedological and climatic description of the studied geographical areas:

- Iași – Copou vineyard, geographically located at the intersection of the parallel of 47o10′ north latitude, with the meridian of 27º35′ east longitude. Pedological feature of the vineyard are soils chernozems and slang chernozems (Toti, 2017).
- Cotnari Vineyard is located on the hill of Catalina, and 2,500 years ago there was a Thracian-getical fortress on 5 ha, it was concluded that Cotnari vineyard were cultivated on Burebista's will (82-44 BC). Place at the geographical coordinates of 47°21‘25”N north latitude, E 26°55‘10”, with an altitude of 312,2 m and eastern exhibition, with soils proxicalcaric chernozems, typical chernozems, carbonatic and texture being sandy-clay, the texture has a grayish brown color and is a medium, yellowish brown clay (Cotea, 2000; All, 2017).
- The Babadag vineyard is located in the eastern part of Dobrogea, south of the Danube Delta, between the hills of Tulcea and the hills of Niculițel N and NV, the Babadag plateau at S and SE (Cotea, 2000). Soils are 70% dill type, typical chernozem 20%, gleaned 10% (Toti, 2017). The weather station is located at latitude 44°53′36″N and longitude at 28°42′43″E, having an altitude of 36 m (www.freemeteo.ro).
- The Blaj wine centre is 7 km from from Blaj, located in the centre of one of the oldest vineyards "Podgoria Tirnave". Soil type is from clay-covered soil class, brown, brown-mesobatic soils, brune-podzolytic soils and some podzolytic soils (Cotea, 2000; Toti, 2017). The weather station is located at the geographical

coordinates latitude 46◦10‘51,2”N and longitude 23°55‘40,5” with exposure E, having an altitude of 330 m.

- Silagiu vineyard is located to the left of the Danube river and to the south of the Banat mountains (Cotea, 2000). Soil type consists of deluvio-proluvial quaternary deposits of piedmont, sandy-stone glacis (Cotea, 2000; Toti, 2017). The weather station is located at 46◦14‘51”N latitude and longitude at 21◦39‘52″ E exposure, having an altitude of 176 m (www.freemeteo.ro).
- Focsani vineyard located at the curve of the Carpathians near the plateau of Moldova, has an altitude 300-400 m at the contact with the subcarpathians and towards the contact with the Romanian plain (Cotea, 2000). Soil type is typically 35% leached chernozem, gray with 25% alluvial and 30% alluvial (Toti, 2017).
- Drăgășani vineyard has favorable conditions for vine culture, having an exposure of NNV-SSE (Cotea, 2000). Drăgășani vineyard has the weather station located at the geographical coordinates 44°39‘36,81”N and 24°14‘05,86”E, with altitude 201 m. (www.freemeteo.ro). Soil type is eumezobasic brown eroded with eumezobasic brown, heavily eroded, with pseudoglized podzolit brown soil (Toti, 2017).
- Ștefănești wine centre is located on the left terraces of the Argeș river, on the direction of NV-SE, at the junction of the Doamnei River and the Glambocel brook (Cotea, 2000). Soil type is one of pseudogleic podzolite, regosol, aluvial molic soil (Toti, 2017). The weather station is located in the Pilot Station Goleasca, at latitude 44°51' N and longitude 24°57' exposure E, having an altitude of 250 m.

Methods of research:

Physico-chemical analyses of wine were carried out law of Vine and Wine Lg 164/2015 and methodological norms Hg 512/2016 of the law of Vine and Wine. The characteristics of the wine were determined after the Compendium of International Methods of Wine and Must Analysis, OIV – 2022. The alcoholic strength was determined by the hydrostatic balance method (OIV-MA-AS312-02; Resolution Oeno 24/2003 - 377/2009). The determination of the total sugar content, expressed as non-reducing sugar (g/l), was made by defecation using the Schoorl method (Schoorl, 1929; OIV-MA-AS311, Oeno 11/2006 revised by 377/2009). Determination of total acidity, expressed in g/l tartaric acid, was achieved by titrimetric analysis (OIV-MA-AS313-01, revised by 551/2015). Volatile acidity, expressed in g/l acetic acid, was determined by distillation (OIV-MA-AS313-02, revised by 549/2015). Determination of total dry extract (g/l) was made by densimetric method (OIV-MA-AS2-03B, revised 465/2012). Determination of the content of free and total SO2 (mg/l) using the iodometric method (OIV-MA-AS323-04B, Revised A17 377/2009; OIV-MA-AS323-04B, A17 revised 377/2009). The pH determination of wine was made by conductivity, using a pHmeter (OIV-MA-F1-06). Organic acids (malic acid, lactic acid, tartaric acid) in wine were determined by the enzymatic method (OIV-MA-AS313-07, OIV-MA-AS313-09, OIV-MA-AS313-11) using an oenological analyzer.The determination of nitrous substances in wine (ammoniacal nitrogen and aminic nitrogen) was also carried out by the enzymatic method using the automatic oenological analyzer.

1.Apparatus used in the elementary determinations of wines:

- ✓ Alcoholic strength is achieved by distillation, with a super DEE digital oenological distillator according to OIV-AS-312-01-TALVOL.
- ✓ Determination of extract in wine was used hydrostatic balance Densimat&Alcomat 2 (OIV-AS-2-03-EXTSEC).

- ✓ Determination of volatile acidity was achieved by distillation and uptake of volatile acids using oenological distiller (OIV - AS-313-02-ACIVOL).
- ✓ pH analysis with a pH meter Mettler Toledo Seven Easy (OIV - AS-313-15-PH).
- ✓ Determination of total acidity by OIV method - AS-313-01-ACITOT.
- ✓ Determination of organic acids (tartar, malic, lactic, citric and nitrogenous substances – by enzymatic method) using the Shenzhen Midray BS 200 automatic oenological analyzer.

2. Apparatus used in micro- , macro-mineral and heavy metals determinations of wine and soil

- ✓ Micromineral analysis was performed with an inductively coupled plasma mass spectrometer ICP-MS Varian 820 MS (OIV-MA-AS323-01A).
- ✓ Macromineral analysis was performed with an atomic absorption spectrophotometer – flame technique, with graphite furnace and direct analysis for HR-CS-AAS solid samples. Analytical determination K, Na, Ca (OIV-MA-AS322-04), Fe (OIV-MA-AS322-05A), Cu (OIV-MA-AS322-07), Mg (OIV-MA-AS322-07), Zn (OIV-MA-AS322-08), Ag (OIV-MA-AS322-09), Ag (OIV-AS322-09) Cd (OIV-MA-AS322-10), Pb (OIV-MA-AS322-11), As (OIV-MA-AS322-13), Br (OIV-MA-AS323-03), Hg (OIV-MA-AS323-06).
- ✓ The soil samples were prepared by microwave digestion, with a CEM – Mars 6 system and directly analyzed HR-CS-AAS (High Resolution Continuum Source Atomic Absorption Spectrometer)- ContrAA 700

3. Apparatus used in the polyphenolic determinations of wines:

- ✓ Determination of total polyphenolic compounds, total anthocyanins, total catechins, total tannins and antioxidant activity is used UV-VIS spectrophotometer.
- ✓ Determination of the polyphenolic profile for individual phenols (phenolic acids, flavonoids and stilbenes) was carried out by UHPLC-ESI/HRMS (mass spectrometry with tandem ionization with high performance liquid chromatography)

4. Apparatus used in molecular determinations of wine DNA:

- ✓ When DNA extraction, a centrifuge with a rotation speed of 14000 rpm is used.
- ✓ Amplificability of extracted DNA used a Real Time PCR.
- ✓ DNA detection is electrophoretic and was performed using a camera, followed by viewing DNA in a UV transluminator.
- ✓ The purity of DNA, expressed by the ratio of A260/A280 absorbers, is read at the spectrophotometer.

CHAPTER III-OWN RESULTS ON THE AUTHENTICITY AND TYPICALITY OF WINES OBTAINED BY ANALYSING THEIR QUALITATIVE MARKERS ACCORDING TO GEOGRAPHICAL ORIGIN

1.1. Own results regarding the investigation of the elementary profile of wines

The wine samples are monovarietal and were raised directly from the producer, from the 8 important wine regions in Romania, representing 11 vineyards and wine centers. Wine samples are from 2019 and 2021 production, with 35 samples of white wine and 35 samples of red wine.

Table 11. The varietal, geographical area and region in Romanian, and harvest year for white wine samples.

Soiul	Simbol	Regiunea	Centrul viticol/ Podgorie	Anul de producție
Riesling Italian	RI19/21_Șt	Muntenia	Ștefănești	2019, 2021
	RI19_Bj	Transilvania	Blaj	2019
Fetească Regală	FR19/21_Șt	Muntenia	Ștefănești	2019, 2021
	FR19_DM	Muntenia	Dealul Mare	2019
	FR19/21_D	Oltenia	Drăgășani	2019, 2021
Sauvignon Blanc	SB19/21_Șt	Muntenia	Ștefănești	2019, 2021
	SB19/21_D	Oltenia	Drăgășani	2019, 2021
	SB19_Iș	Moldova de Nord	Iași	2019
	SB19_Foc	Moldova de Sud	Focșani	2019
Pinot Gris	PG19/21_Bab	Dobrogea	Babadag	2019, 2021
Muscat Ottonel	MO19/21_Șt	Muntenia	Ștefănești	2019, 2021
	MO19_Bj	Transilvania	Blaj	2019
	MO19_Sil	Banat	Silagiu	2019
Riesling de Rihn	RR19/21_D	Oltenia	Drăgășani	2019, 2021
Crâmpoșie Selecționată	CrS19/21_D	Oltenia	Drăgășani	2019, 2021
Fetească Albă	FA19/21_Șt	Muntenia	Ștefănești	2019, 2021
	FA19_Iș	Moldova de Nord	Iași	2019
	FA19_Sil	Banat	Silagiu	2019
Tămâioasă Românească	TR19/21_Șt	Muntenia	Ștefănești	2019, 2021
	TR19_D	Oltenia	Drăgășani	2019
Aligote	AI19/21_Bab	Dobrogea	Babadag	2019, 2021
Chardonnay	CH19/21_Bab	Dobrogea	Babadag	2019, 2021
	CH19_Șt	Muntenia	Ștefănești	2019
Traminer Roz	TR R19_Bj	Transilvania	Blaj	2019
Busuioacă de Bohotin	BB19_DM	Muntenia	Dealul Mare	2019
Muscat de Moderat	MM19_M	Banat	Miniș	2019
Grasă de Cotnari	GC19_Cot	Moldova de Nord	Cotnari	2019

The table below includes red wine varieties with production years and geographical areas.

Table 12. The varietal, geographical area and region in Romanian, and harvest year for red wine samples

Soiul	Simbol	Regiunea	Centrul viticol/ Podgorie	Anul de producție
Burgund Mare	BM19/21_Șt	Muntenia	Ștefănești	2019, 2021
	BM19_Bj	Transilvania	Blaj	2019
	BM19_Sil	Banat	Silagiu	2019
Cabernet Sauvignon	CS19/21_Șt	Muntenia	Ștefănești	2019, 2021
	CS19_D	Oltenia	Drăgășani	2019, 2021
	CS19_Iș	Moldova de Nord	Iași	2019
Fetească Neagră	FN19/21_Șt	Muntenia	Ștefănești	2019, 2021
	FN19_Cot	Moldova de Nord	Cotnari	2019
	FN19_D	Oltenia	Drăgășani	2019
	FN19_Bab	Dobrogea	Babadag	2019

Merlot	M19/21_Șt	Muntenia	Ștefănești	2019, 2021
	M19_Iș	Moldova de Nord	Iași	2019
	M19_Foc	Moldova de Sud	Focșani	2019
	M19/21_Bab	Dobrogea	Babadag	2019, 2021
Pinot Noir	PN19/21_Șt	Muntenia	Ștefănești	2019, 2021
	PN19/21_Bab	Dobrogea	Babadag	2019, 2021
Baluerzweigelt	Bl19_Șt	Muntenia	Ștefănești	2019
Negru de Drăgășani	ND19/21_D	Oltenia	Drăgășani	2019, 2021
Syrah	Sy19/21_D	Oltenia	Drăgășani	2019, 2021
Cabernet Franc	CF19/21_Șt	Oltenia	Drăgășani	2019, 2021
	CF19_Bj	Transilvania	Blaj	2019
	CF19_Sil	Banat	Silagiu	2019
Petit Verdot	PV19/21_D	Oltenia	Drăgășani	2019, 2021
Cadarcă	CD19_M	Banat	Miniș	2019

1.2. Own results regarding the evaluation of wine authenticity based on the elementary composition of wines

The highest alcoholic concentrations in white wines in Silagiu vineyard, Muscat Ottonel (16,33±0,01% vol) and Feteasca Albă (15,11±0,01% vol) in the 2019 production, but also in Ștefănești at the Feteasca Regală (15,35±0,05) and Riesling Italian (14,55±0,03%vol) in the 2019 production. The lowest alcoholic concentrations are observed in Drăgășani vineyard at the Riesling of Rhin which depends on the technology of production of sweet wine (9,68±0,02% vol production 2021 and 11,07±0,02%vol of production 2019) and the wine center Iasi at the Feteasca Albă production 2019 (10,24±0,01%vol). In the other geographical areas studied the alcoholic strength of white wines is maintained between 12,0 and 13,5% vol.

Red wines have high alcoholic concentrations in some varieties of the Ștefănești wine centre (Pinot Noir – 15,7±0,01%vol), Babadag vineyard (Merlot production 2019 – 15,6±0,03%vol, Feteasca Neagră production 2019 (15,5±0,02%vol) and Drăgășani vineyard with Cabernet Franc (15,5±0,01% vol) and Silagiu vineyard with Burgund Mare (16,53±0,01% vol.).

The extracts of wines are given by the techniques of winemaking and the variety from which wine is obtained. The highest extracts are found in red wines. Total acidity is given by the variety from which wine is obtained and the soil in which wine production takes place. The most eloquent example of total acidity is the Riesling of Rhin variety with a total acidity of 9.25 g/l tartaric acid. The highest acidity levels in red wine are found in Merlot from Ștefănești at the 2021 production (8,09±0,02 g/l tartaric acid), Negru de Drăgășani at the 2021 production (7,6±0,02 g/l AT) and Silagiu vineyard year of production 2019 Burgund Mare variety (7,11±0,12). The lowest total acidity is found in red varieties at Pinot Noir in Ștefănești at 2019 production (5,09±0,02g/l AT), Pinot Verdot production 2019 from Drăgășani vineyard (5,07±0,02g/l AT) and Fetească Neagră from Babadag vineyard from 2019 production (4,5±23,15g/l AT).

In red wines, the total acidity depends on the sum of all organic acids (tartaric acid, malic acid, lactic acid, etc.). An example from Table 14, Fetească Neagră Ștefănești production 2019, pH=3,57±0,03, total acidity =5,21±0,02g/l AT, and organic acids have values: malic acid = 0,34±0,01, lactic acid=1,89±0,12g/l and tartaric acid =2,55±0,06g/l.

The content of SO2-free, SO2-total, total nitrogen and ammoniacal nitrogen depends on the vinification technology, while the content of K, Fe, Cu and Ca microminerals depends on the geographical area.

1.3. Correlations, statistical analysis and discussions on the physico-chemical composition of wines from the studied geographical areas

A)

B)

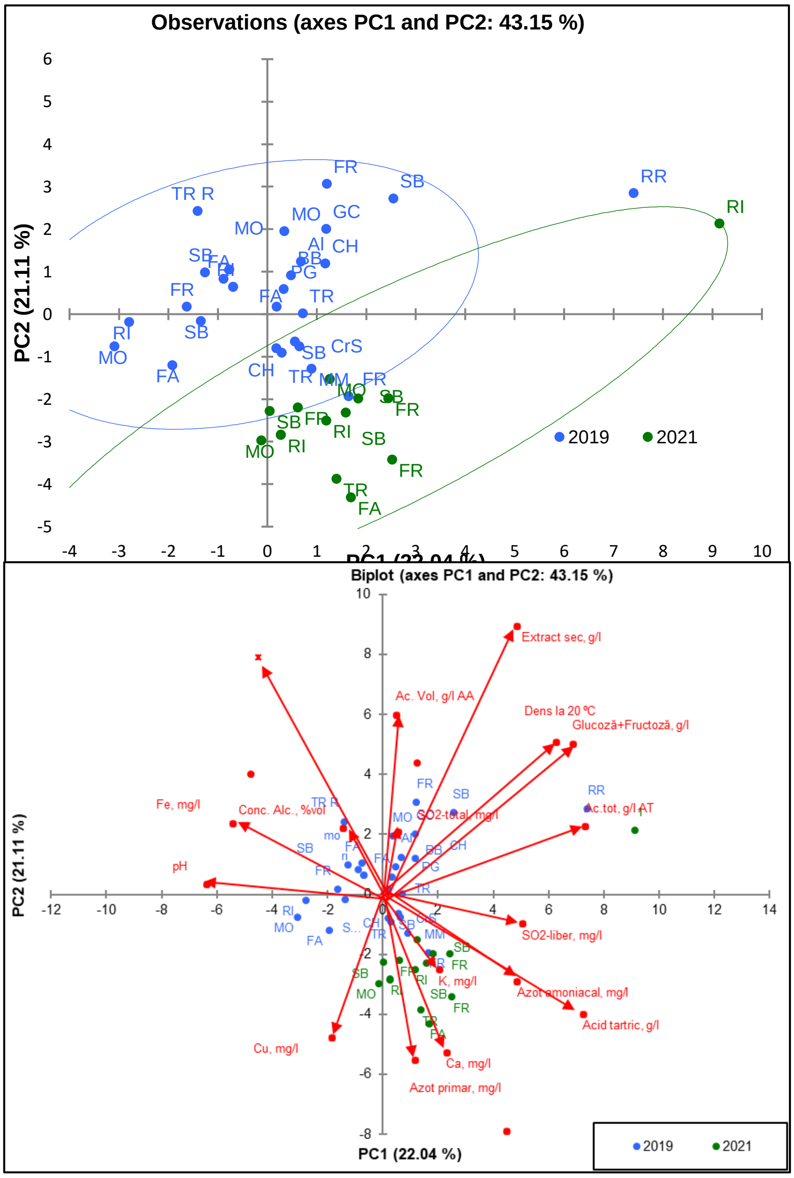


Fig.21 Discriminatory analysis of white wine varieties (A) over the two years (B) of study

In Figure 21 a) white wine varieties are presented for years, with the help of validating the physico-chemical parameters of wines, a clear example is the Risling de Rihn variety that is outside the intersection areas, the cross-validation technique, which demonstrates that the variety is important in distinguishing white wines by physical-chemical parameters. Fig 21 b) shows linear correlations between white wine variety determinations over the two years of study, with an acceptable score of 43.15% (22.04% PC1 and 21.11% PC2).

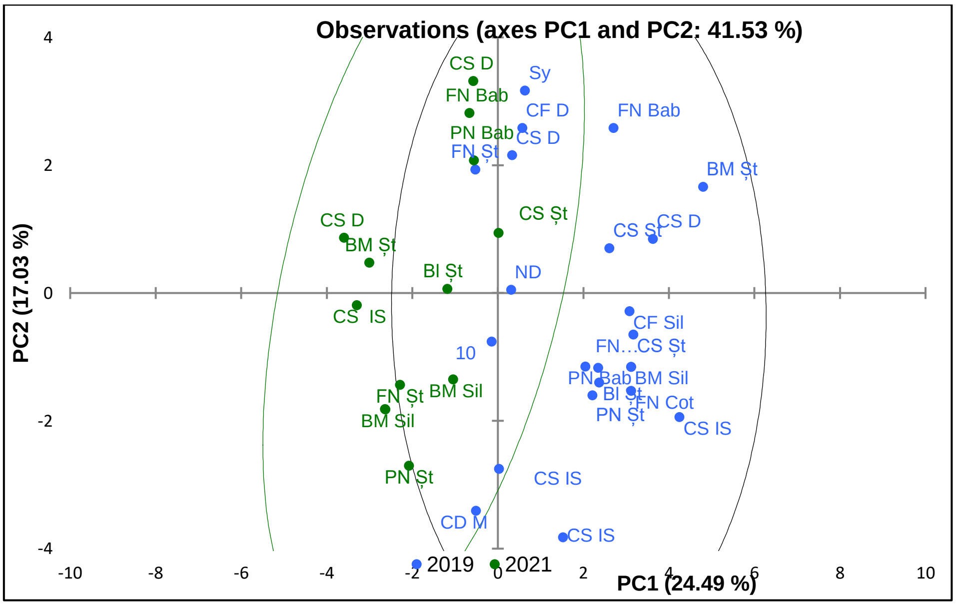


Fig.29 Discriminatory analysis of red wine varieties (A) by (B) years of study

In the discriminatory analysis on the two years of study (figures 29 and 30) the production areas Babadag and Dragasani are interwoven with few varieties from Stefanesti on two areas of discrimination.

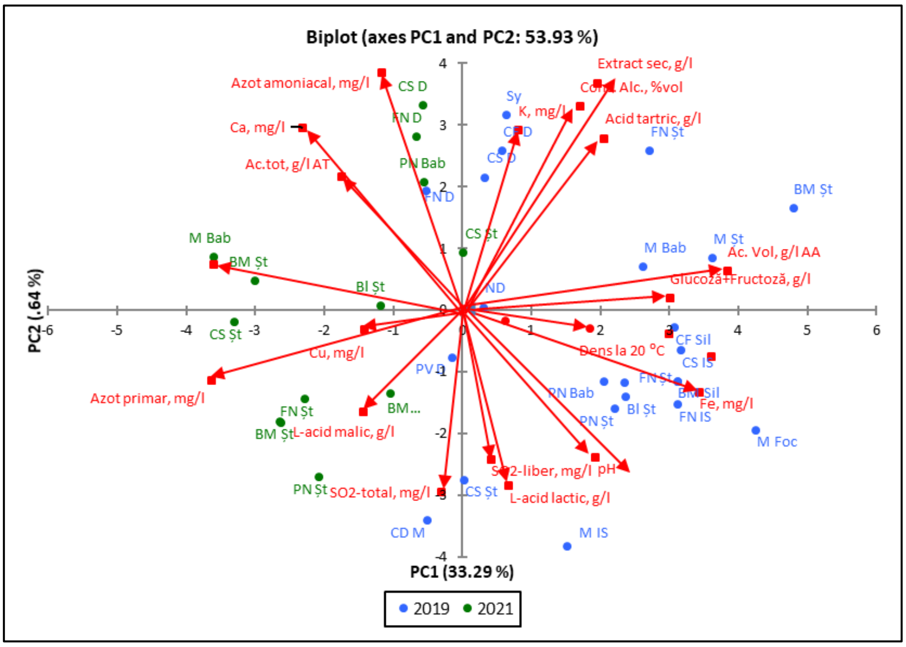


Fig.30 Biplot in discriminatory analysis using analytical parameters over the study years (A) of red wine varieties (B)

The most crowded varieties are from 2019 and the Cotnari, Silagiu, Iasi and a few varieties from Stefanesti. In Figure 30, the varieties are very well defined for the production years, in the I and IV quadrants, which is supposed that 2019 was a better year for wine than 2021, having occupied the dials II and III. In 2021 the red wine varieties are scattered on a fairly wide discriminatory area, those in 2019 are more grouped. On a discriminatory area there are varieties from Silagiu, Iasi, Focsani, some from Stefanesti and Babadag. Other varieties from Stefanesti are found in the area of discrimination with those from Dragasani but more scattered (figure 30).

Figure 31 shows the hierarchical dendrogram for the grouping of the 35 wine samples. It can be concluded that the two specific models of descriptors of interest determine the quality of the wine samples. Two similarity models could be easily distinguished— lower cluster 1 includes samples 24-35 and upper cluster 2-samples 1–23.

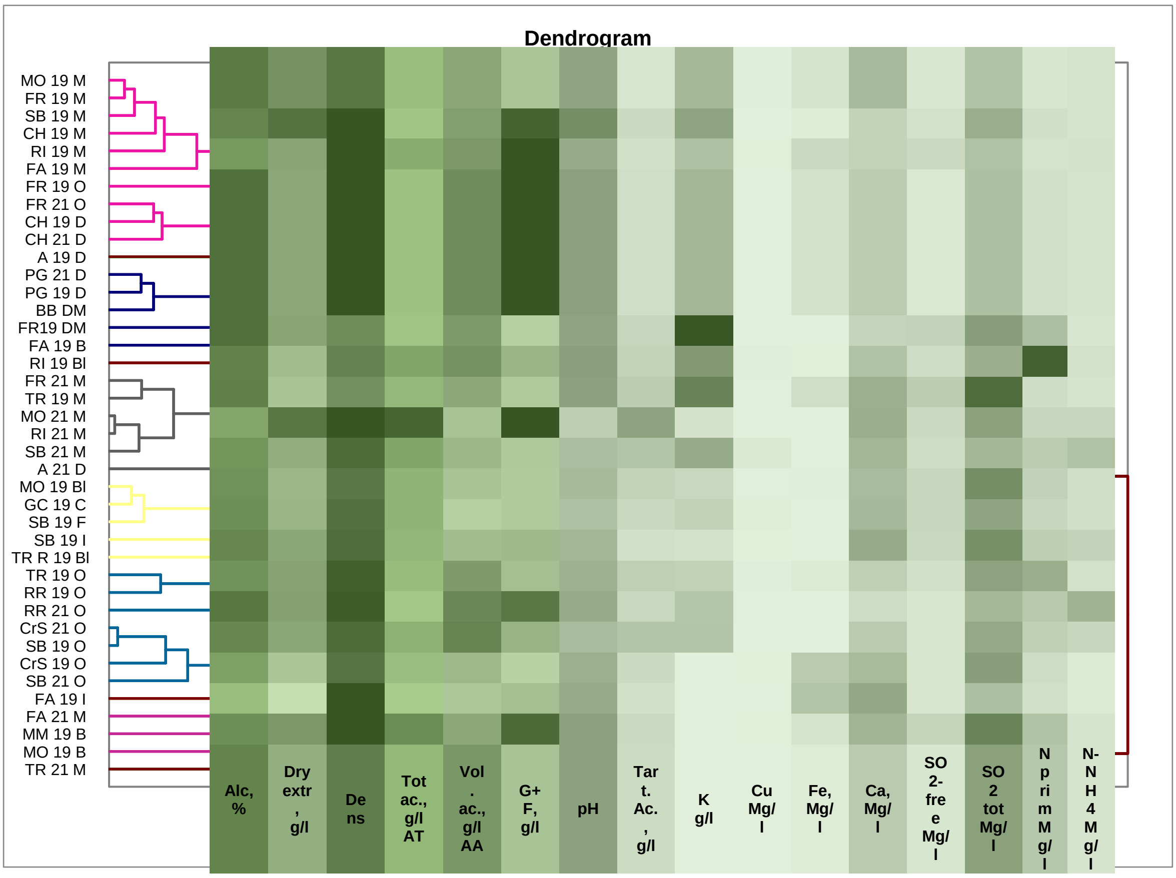


Fig.31 Heat map and dendrogram represent the cluster analysis corresponding to the 35 monovarietal white wines. The relative content of each compound is illustrated by a chromatic scale (from light green, minimum, to dark green, maximum). Also included was the dendrogram for HCA results (hierarchical cluster analysis).

Both groups are well separated and represent two different types of wine quality. This separation is reasonable if the input data is carefully checked. Wine samples from 1 to 35 are white wines, dominant from the Riesling, Feteasca Regală, Sauvignon Blanc and Muscat Ottonel grape types are in large numbers in terms of variety, since the other 23 (1-23) have a fairly high alcoholic concentration of over 13,5% vol.

Figure 32 shows the hierarchical dendrogram for the grouping of the 35 samples of red wine. It can be concluded that three specific models of descriptors of interest determine the quality of wine samples. There are three different clusters, two similar to 2 wine varieties and one with the most homogeneous wine varieties in the studied parameters. Three models could easily be distinguished— the lower cluster includes two wine samples, the upper cluster 2-samples, cluster 2 contains most wine varieties 3-32, and the final cluster 3 – two wine samples. Both groups are well separated and represent two different types of wine quality. Wine samples from 1 to 35 are red wines, dominant from the types of grapes Burgund Mare, Cabernet Sauvignon, Fetească Neagră, Merlot, Pinot Noir. In the 2nd cluster wine varieties are in large numbers in terms of quantity depending on the alcoholic strength.

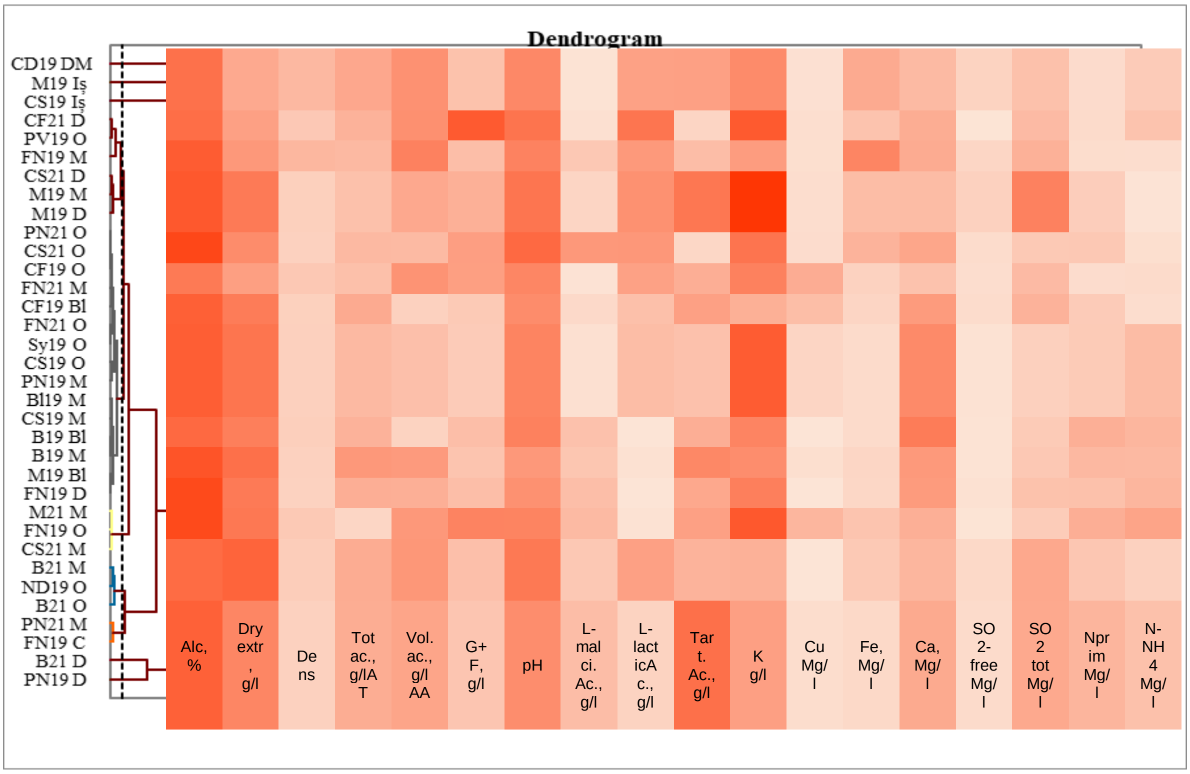


Fig. 32 Heat map and dendrogram represent the cluster analysis corresponding to the 35 monovarietal red wines. The relative content of each compound is illustrated by a chromatic scale (from light pink, minimal, to dark orange-red, maximum). Dendrogram was also included for HCA results (hierarchical cluster analysis).

3.2. Own results regarding the investigation of the mineral profile of wines
3.2.1. Investigating the mineral profile in soils

Within the thesis were investigated the soils from 4 important geographical areas: the wine center Ștefănești (Muntenia), the wine center Iași (Moldova), the wine center Blaj (Transilvania) and the Babadag vineyard (Dobrogea). The assessment of the degree of contamination or pollution of the soil system, the obtained results were compared with the reference values provided by the Order of the Minister of Water no. 756/1997, Forests and Environmental Protection, in the case of soil, and the values obtained in the case of wine analysis were reported to the Law on Vine and Wine but also to the regulations stipulated in O.I.V.

Table 16. Concentration of macrominerals in the soils of the studied wine centres/podgoras and their standard deviation

Denumire proba	Sol Stefanesti	Sol Babadag	Sol Blaj	Sol Iasi	Mean	Std.Dv.
Cr mg/kg	48.343	0.148	14.447	34.002	24.235	21.234
Co mg/kg	1.165	0.031	1.356	1.201	0.938	0.610
Ni mg/kg	3.561	0.046	3.565	3.286	2.615	1.717
Cu mg/kg	5.428	0.120	3.578	135.455	36.145	66.243
As mg/kg	0.888	0.028	0.608	0.453	0.494	0.359
Cd mg/kg	0.138	0.009	0.430	0.167	0.186	0.176
Pb mg/kg	4.655	4.367	19.760	5.073	8.464	7.536
Hg mg/kg	0.045	0.043	0.052	0.046	0.046	0.004
Li mg/kg	14.375	19.190	12.175	14.872	15.153	2.935

Be mg/kg	0.154	0.002	0.110	0.173	0.109	0.077
V mg/kg	53.491	0.178	18.816	37.509	27.499	23.077
Se mg/kg	42.248	0.566	0.661	0.826	11.075	20.782
Mo mg/kg	1.313	0.013	0.252	0.375	0.488	0.570
Ag mg/kg	0.030	0.027	0.055	0.070	0.046	0.020
Sr mg/kg	34.636	0.089	15.276	30.627	20.157	15.767
Mn mg/kg	546.814	489.525	407.533	450.671	473.636	59.173
Zn mg/kg	397.480	333.637	299.852	305.754	334.181	44.698
Fe mg/kg	3.968	13.918	14.552	13.605	11.511	5.044
Al mg/kg	13487.159	15307.121	7738.072	11602.419	12033.692	3238.634
Mg mg/kg	327.042	280.565	184.171	225.283	254.266	62.560
Na mg/kg	177.949	184.487	115.739	127.003	151.295	34.959
Ca mg/kg	978.886	650.460	472.114	541.789	660.812	224.389
K mg/kg	502.500	493.046	513.354	554.129	515.757	26.893

Figures 63 and 64 show the content of macrominerals in all 4 vineyards.

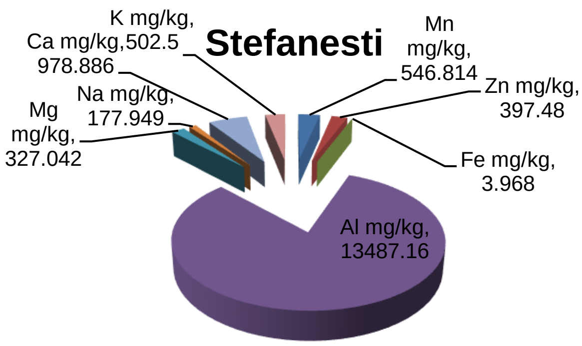


Fig. 63 Macromineral content in soil in Babadag vineyard

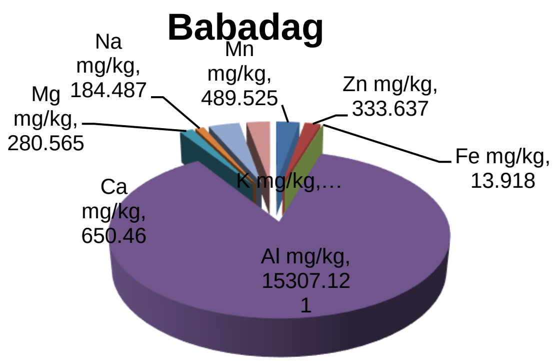


Fig. 64 Macromineral content in soil in Ştefăneşti wine-centre

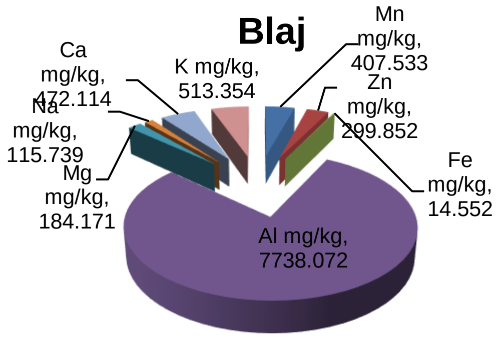


Fig. 65 Macromineral content in soil in Blaj wine-centre

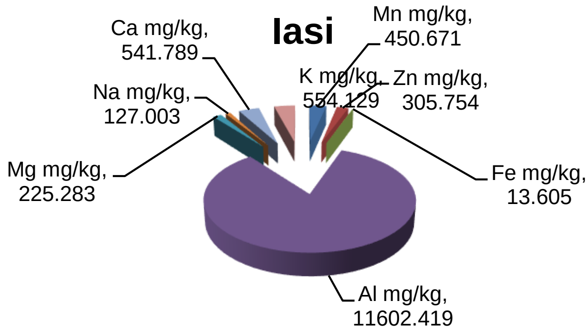
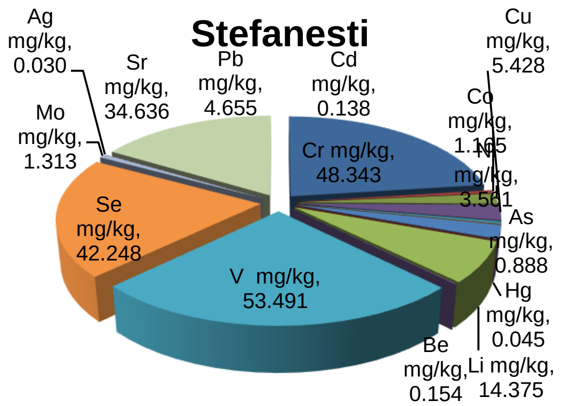


Fig.66 Macromineral content in soil in Iași wine-centre

In the region of Muntenia - Stefanesti wine center is observed the highest content of Sr (34,634 mg/kg fig 67) of the 4 regions studied and the lowest in the region of Dobrogea – Babadag vineyard (0,089mg/kg). The following content of Sr is found in the region of Blaj (15,276mg/kg) and followed by the wine-growing centre of Iasi (region of Moldova, figure 70) with a content of Sr of 30,627mg/kg. The highest concentration of Li among all the studied regions is found in the Babadag vineyard (19,190mg/kg), figure 68, and the lowest in Blaj (12,175mg/kg- figure 69). The highest concentration is observed in the Ștefănești region (42,248 mg/kg, fig. 67) and the lowest in the Babadag vineyard (0,566 mg/l, fig. 68), higher than the average value determined by Lacatusu et al. in 2013 (143µg/kg).



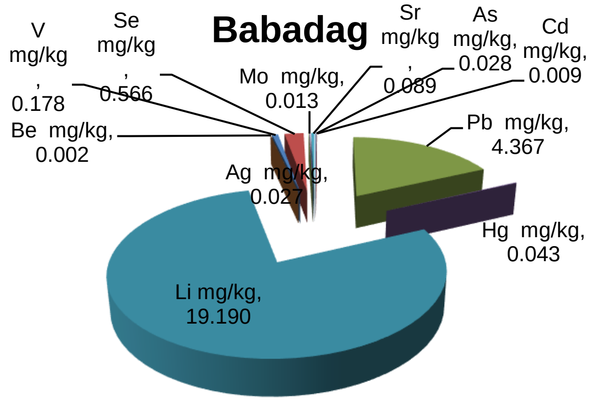
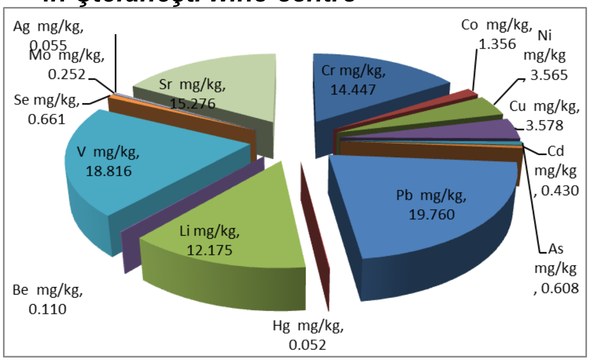


Fig. 67 The content of microminerals in the soil in Ştefăneşti wine-centre

Fig.68 The content of microminerals in soil in Babadag vineyard



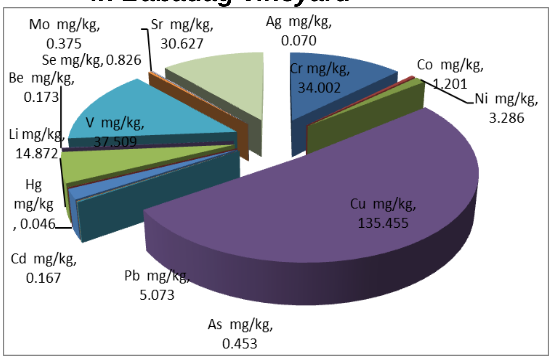


Fig.74 The content of microminerals in soil in Blaj wine centre

Fig.75 The content of microminerals in soil in Iaşi wine centre

In Figures 73 B and 74, a very high amount of Pb (19,760mg/kg) is observed in the wine-growing centre of Blaj, due to the proximity to the A7 motorway and the industrial zone, compared to the other areas where the Pb content falls between 4,367mg/kg (podgoria Babadag) and 5,073mg/kg wine-growing centre Iași.

3.2.2. Investigation of the mineral profile in wines

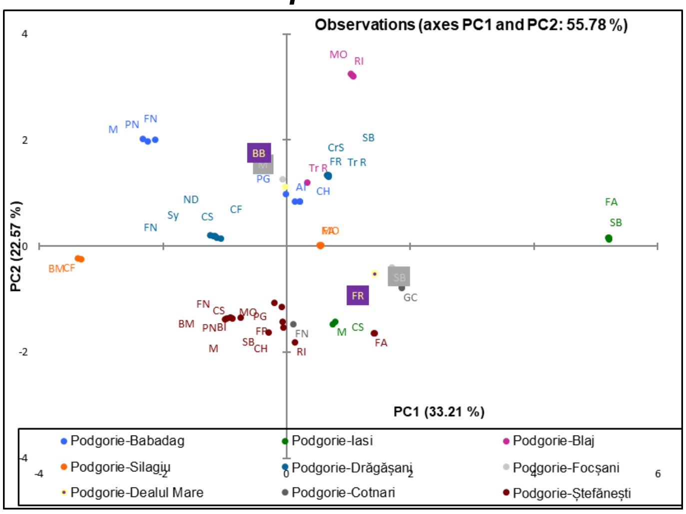


Fig. 78 Discriminatory analysis using macrominerals of wine varieties (A) on geographical areas (B)

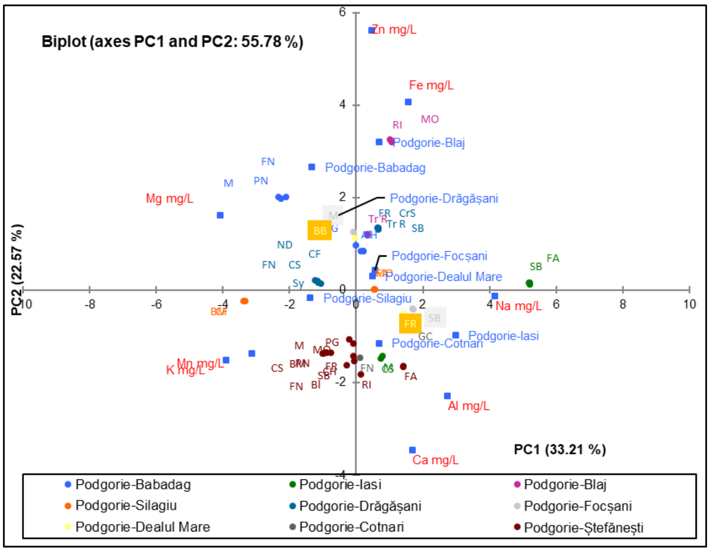


Fig.79 Biplot in discriminatory analysis using macrominerals of red wine varieties (A) on geographical areas (B)

In fig.78 and 79 both white and red wine varieties are exposed, after the macrominerals and metals determined from wine varieties and on geographical areas, the correlation of the areas has a fairly high score of 55.78%, to be noted are all white wine varieties from the Blaj wine center (Riesling Italian and Muscat Ottonel) it is also worth noting the significant difference of white varieties in the wine center of Iasi (Sauvignon Blanc and Feteasca Albă).

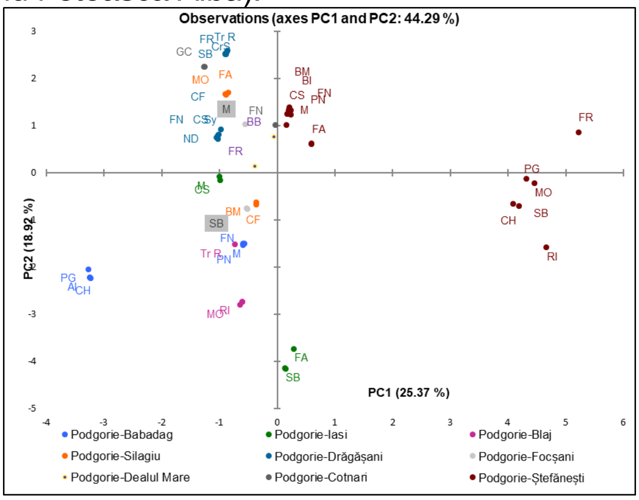


Fig. 80 Discriminatory analysis by microminerals and metals of red wine varieties (A) on geographical areas (B)

In Figure 80 both white and red wine varieties are exposed, after the microminerals determined from wine varieties and on the geographical areas, the correlation of the areas has an acceptable score of 44.29%, it is worth noting that most of the varieties in the geographical areas are grouped, white in a certain area and red varieties in the opposite domain. The red wine varieties are best delimited in Drăgășani vineyard (Negru de Drăgășani, Syrah, Cabernet Sauvignon, Cabernet Franc), are also grouped the red varieties from the Ștefănești wine center (Burgund Mare, Feteasca Neagră, Cabernet Sauvignon, Pinot Noir) from the remarkable and the significant difference of the white varieties from the Iasi wine center (Sauvignon Blanc and Feteasca Albă).

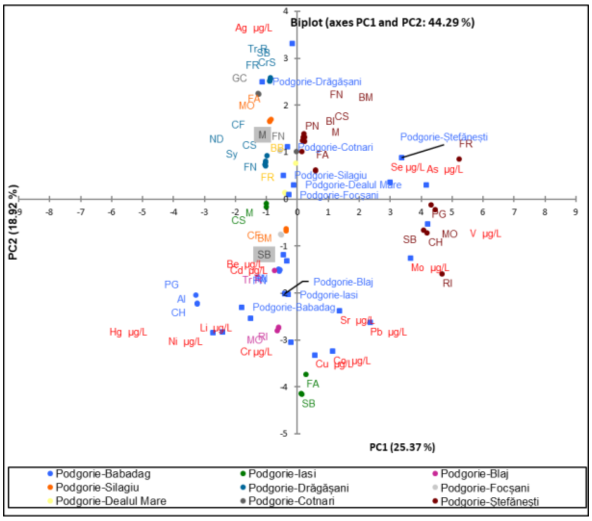


Fig.81 Biplot in discriminatory analysis using micro-minerals of wine varieties (A) on geographical areas (B)

Figure 81 shows the linear correlations between wine varieties from the studied geographical areas and the analysed (micro-mineral) parameters.

3.3. Own results regarding the investigation of the polyphenolic profile of wines

Table 26 shows total polyphenolic compounds in 35 samples of white wine in parallel with the concentration of catechins, tannins and antioxidant activity. According to the results obtained, all white wine varieties have shown strong antioxidant activities, the lowest is found at Sauvignon Blanc from Stefanesti in the year of production 2019 (24,678±0,235mg GAE/l) and the highest at Muscat Ottonel from Stefanesti in the year of production 2021 (73,88± 0,78mg GAE/l). The average total polyphenols is 602,984 mg GAE/l, catechins of 0.5856 mg/l, tannins of 1.08346 mg/l and the antioxidant activity in white wines has an average of 64.3672 mg GAE/l.

Tab 26 Polyphenolic compounds in white wines

Soi vin	PFT mgGAE/l	Catechine, mg/l	Taninuri, mg/l	A. A., mg GAE/l
Al_B 21	330,22±1,414	0,0552±0,0014	7,324±0.063	54,837±0.283
Al_B 19	240,22±6,364	0,2153±0,004	6,4365±0,0064	65,705±0,877
CH_B 19	834,29±0,827	0,2882±0,002	8,6065±0,003	71,986±0,162
CH_B 21	397,89±2,351	0,1696±0,0011	15,1819±0,349	70,47±0,389
PG_B 19	734,07±0,735	0,3524±0,0055	8,8862±0,066	53,93±0,354
PG_B 21	1349,44±19,17	0,8937±0,024	9,0982±0,081	56,34±0,827
FA_Sil 19	871±0,707	1,2541±0,003	0,5271±0,001	27,341±0,158
MO_Sil 19	1058±9,192	1,5728±0,009	0,4798±0,004	38,74±0,361
RR_D 19	644,4±1,534	0,1061±0,005	0	73,073±0,123
SB_D 19	293,07±0,127	0,4787±0,001	0	50,645±0,275
CH_D 21	542±1,414	0,3897±0,001	0,0051±0,000	58,27±0,318
CrS_D 19	703,05±0,120	0,4957±0,001	0	58,35±0,375
CrS_D 21	512±7,778	0,4107±0,023	0,0045±0,001	51,56 ±1,103
FR_D 21	328±1,577	0,6262±0,009	0,0023±0,000	50,99 ± 0,190
TR_D 19	730,85±1,697	0,5534±0,002	0	52,89 ±0,163
TR_D 21	560,33±0,636	0,1248±0,003	1,76421±0,008	50,16 ±0,608
FA_IS 19	528±0,707	1,3214±0,006	0	34,562±0,146
SB_IS 19	693±0,707	1,4102±0,014	0	38,12± 0,226
SB_F 19	314±0,708	0,7152±0,000	0,2741±0,000	31,135± 0,071
FA_Șt 19	245,848±0,75	0,1705±0,001	0	20,183 ±0,633
FA_Șt 21	730,17±1,52	0,1328±0,014	0	49,54±1,089
FR_Șt 21	402,56±6,208	0,1534±0,004	1,6979±0,019	53,238±0,177

MO_Șt 19	455,96±0,565	0,1317±0,015	0	28,086± 0,169
MO_Șt 21	429±0,313	0,163±0,003	1,4028±0,018	73,88± 0,778
RI_Șt 19	394±1,414	0,501±0,001	0	54,32 ± 0,283
RI_Șt 21	392±0,364	0,0863±0,004	0,91908±0,006	73,83 ± 0,878
SB_Șt 19	273,292±1,199	0,6379±0,004	0	24,678±0,235
SB_Șt 21	983±0,536	0,1248±0,011	1,76421±0,010	78,97±0,693
TR_Șt 21	560,33±0,325	0,1248±0,007	1,7621±0,006	59,822±0,123
RI_Bj 19	1156±0,949	1,5077±0,008	0	34,78 ±0,481
MO_Bj 19	1183±0,536	2,1548±0,002	0	38,14 ± 0,064
TrR_Bj 19	1086±0,657	2,0119±0,007	0	37,59 ±0,198
FR_T 19	586±0,535	0,2654±0,003	0	51,745±0,008
GC_C 19	682±0,707	0,5247±0,007	0	36,63±0,247
MM_T 19	364±0,414	0,1031±0,005	0,062±0,006	50,2 ± 0,862

Table 27 shows total polyphenolic compounds (PFTs, anthocyanins, catechins and tannins) in parallel with antioxidant activity, colour intensity and tent in 35 red wine samples from two years of production (2019 and 2021).

Tab 27 Polyphenolic compounds in red wines

Soi	PFT mgGAE/l	Catechine, mg/l	Taninuri, mg/l	A. A., mg GAE/l	Antociani (mg/l)	IC	T
M_B 19	3432,017±0,01	2,0602±0,04	3,437±0,00	95,24±0,49	502±1,41	8,2474±0,35	0,7225±0,02
M_B 21	9754,44±1,10	3,951±0,03	14,473±0,16	77,47±0,29	670±2,12	4,484±0,03	0,8721±0,01
PN_B 19	2372,57±0,40	1,0183±0,00	6,578±0,01	88,63±0,18	247±2,83	4,6434±0,00	0,5964±0,04
PN_B 21	1194,44±1,01	4,2415±0,00	6,5108±0,00	78,64±0,29	329±2,83	6,0033±0,23	0,9853±0,00
BM_Sil 19	4166±1,41	12,1859±0,01	2,2969±0,00	94,4753±0,03	488±2,82	8,3093±0,06	1,1139±0,01
CF_Sil 19	4353±2,12	9,3643±0,02	4,8874±0,04	96,713±0,28	615±2,12	7,9329±0,04	1,02435±0,01
FN_D 1	3087±1,41	1,9738±0,00	2,2922±0,00	90,1275±0,00	225±2,12	6,1476±0,45	0,8757±0,01
Sy_D 19	2816,461±1,74	1,0791±0,00	2,0897±0,00	74,952±0,03	260±2,82	7,848±0,28	0,9715±0,03
CS_D 19	4234,79±2,12	1,5161±0,06	1,4553±0,00	89,78±0,64	299±0,71	5,9414±0,03	1,0504±0,00
CS_D 21	3320±1,41	2,973±0,01	2,7842±0,02	88,32±0,4	312±2,82	7,2103±0,02	1,2622±0,02
M_D 19	3265,55±0,39	5,7645±0,02	2,9246±0,01	89,02±0,07	405±1,41	8,4624±0,03	1,0226±0,01
ND_D 21	4043,33±0,14	9,4319±0,00	9,4319±0,02	94,23±2,16	1023±2,82	3,6919±0,16	0,9602±0,17
ND_D 19	3638,13±0,18	1,615±0,01	4,6127±0,4	96,67±0,06	605±2,82	7,7018±0,01	1,0385±0,03
CS_IS 19	1966±0,41	0,5454±0,02	0,4584±0,04	65,76±0,39	293±2,13	1,143±0,01	0,835±0,02
M_IS 19	2198±0,54	0,7129±0,00	0,5463±0,00	72,54±0,3	288±0,25	0,8474±0,00	0,7982±0,01
M_Foc 19	2707±0,12	5,0957±0,00	2,0707±0,00	54,32±0,01	179±1,21	6,565±0,01	1,0978±0,00
BM_Șt 19	3401±0,71	0,8954±0,00	1,944±0,00	67,335±0,01	279±1,41	3,6855±0,00	0,8581±0,00
BM_Șt 21	4216,67±0,37	3,675±0,01	5,4127±0,01	85,676±0,16	291,9±0,72	6,3092±0,00	0,9794±0,00
CS_Șt 19	2425±0,41	0,4012±0,00	2,1215±0,00	56,33±0,1	280±1,82	1,9397±0,00	1,1005±0,01
CS_Șt 21	3352,77±0,32	5,5494±0,00	9,8742±0,00	85,21±0,08	608,0±40,05	6,5856±0,01	1,3081±0,00
FN_Șt 19	2908±0,41	0,656±0,00	1,369±0,00	59,669±0,01	264±0,71	4,1793±0,00	0,8402±0,00
FN_Șt 21	3027±0,12	5,1286±0,00	4,4721±0,00	62,46±0,01	288±0,71	4,8963±0,00	1,2375±0,01
M_Șt 19	3505±0,13	0,862±0,00	4,8121±0,00	72,53±0,1	438±0,21	4,3617±0,00	0,9541±0,01
M_Șt 21	5212,78±0,03	4,4725±0,00	4,2327±0,00	90,78±0,33	670±0,83	4,6839±0,00	0,8586±0,01
PN_Șt 19	2217,02±0,53	0,3934±0,01	0,8727±0,00	52,877±0,02	152±0,12	2,3136±0,01	1,4003±0,02
PN_Șt 21	6482,78±0,03	7,8342±0,01	4,3348±0,00	88,07±0,57	1391±0,82	5,852±0,02	1,1846±0,01
CD_M 19	2786±0,41	9,4751±0,02	4,5865±0,02	70,236±0,09	936±2,81	6,4177±0,05	0,9802±0,04
FN_C 19	3339±0,12	4,2813±0,01	1,9515±0,00	87,945±0,03	240±0,82	5,9924±0,01	0,8365±0,02
PN_T 19	2132,22±0,55	5,012±0.56	1,898±0,01	85,44±0,32	453±0,53	3,935±0,13	1,1846±0,04
BB_DM19	880±0,41	0,8765±0,04	0,2765±0,01	46,88±0,47	101±0,41	0,2409±0,01	0
PV_D 19	2397,78±0,65	4,0028±0,01	1,6648±0,00	72,87±0,016	1032±2,12	7,3098±0,04	0,9545±0,02
PV_D 21	1778,33±0,26	2,249±0,14	1,1614±0,01	86,32±0,54	871±0,41	4,5309±0,01	0,9492±0,01
CF_D 19	3271,461±0,5	1,1571±0,00	2,2278±0,01	82,28±0,31	294±2,83	7,2798±0,01	1,3173±0,01
CF_D 21	2103,89±0,12	3,2554±0,01	0,731±0,00	85,77±0,18	1348±0,12	7,4103±0,19	0,7617±0,01
PN_D 21	2406,67±0,37	2,0936±0,02	1,1897±0,01	79,56 ± 0,23	475±0,41	3,6919±0,00	0,9602±0,01

The total polyphenols of red wines have an average of 2910,932 mg GAE/l and are found between 9754,44mgGAE/l (Merlot 2021 from Babadag) and 1194,44mg GAE/l (Pinot Noir from Babadag from 2021 production). The catechins are found between 12.1859 (Burgund Mare from Silagiu production year 2019) mg/l and 0.4012 mg/l (Cabernet Sauvignon from Ștefănești, production year 2019). The tannins range from 14,473 mg/l (Merlot from Babadag, 2021) and 0.2765 (Busuioacă de Bohotin from Dealul mare, 2019). The anthocyanins are comprised in the range of 1023 mg/l at the Negru de Drăgășani of 2021 and 179 mg/l at the Merlot of 2019 from Focșani (Table 27).

3.3.2. Statistical analysis and discussions on the polyphenolic composition of wines from the studied geographical areas

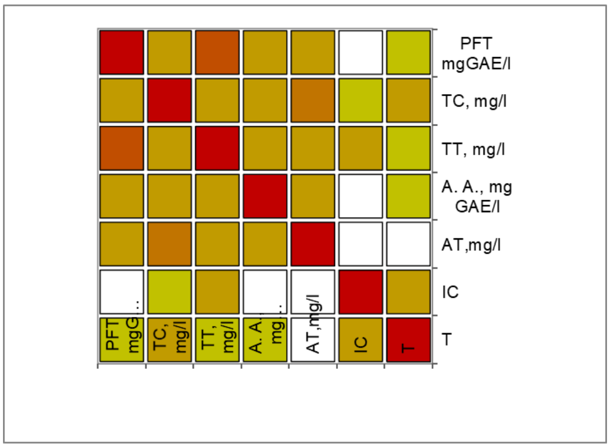


Fig.87 Map of correlation matrix, by varieties, for polyphenolic and chromatic compounds of red wine (PFT – total polyphenolic compounds, CT – total catechins, TT – total tannins, AA – antioxidant activity, AT – anthocyanins, IC –colour intensity, T-tent) (Tița, 2025)

There are only positive but weak correlations (figure 87) in red wines, between PFT and anthocyanins (r^2 = 0.254), color intensity (r^2 = 0.011), but also with tinge (r^2 = 0.190). Such correlations exist between anthocyanin tannins (r^2 = 0.202), AA (r^2 = 0.295) and tint (r^2 = 0.122); between dye intensity and tint (r^2 = 0.241).

Figure 88 correlates and shows in the correlation matrix, the polyphenolic compounds (PFT, CT, TT, AA, AT, IC and T) in red vines over the production years (2019 and 2021).

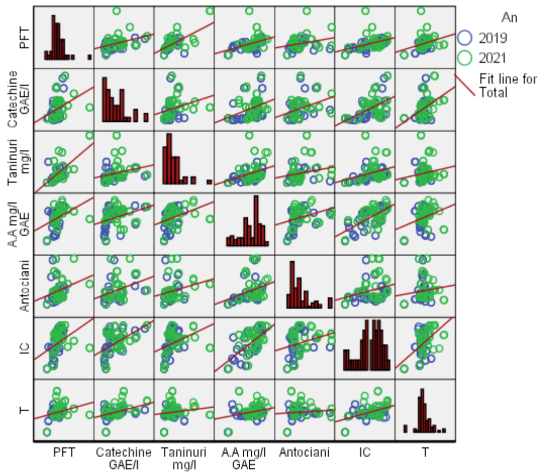


Fig.88 Map of Pearson correlation matrix by years, of polyphenolic compounds in red wine

Figure 92 shows linear correlations between polyphenolic compounds of the polyphenolic profile. It can be seen that the analysis of the main components of the 12 main polyphenolic compounds: gallic acid, 3,4-hydroxybenzioic acid, 4-hydroxybenzoic acid, catechin, epi-catechin, syringic acid, p-coumaric acid, ferulic acid, resveratrol, ellagic acid, abscisic acid, cinamic acid, have shown that the discrimination of wines is depending on the geographical area, but also after the variety.

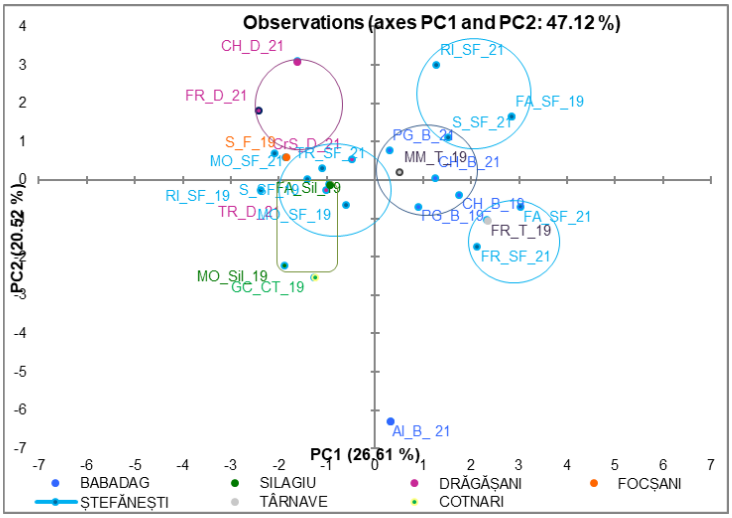


Fig.92 Discriminatory analysis by polyphenolic profile of white wines (A) on geographical areas (B)

Figure 92 shows linear correlations between polyphenolic compounds of the 25 white wine varieties (A) on the studied geographical areas (B).

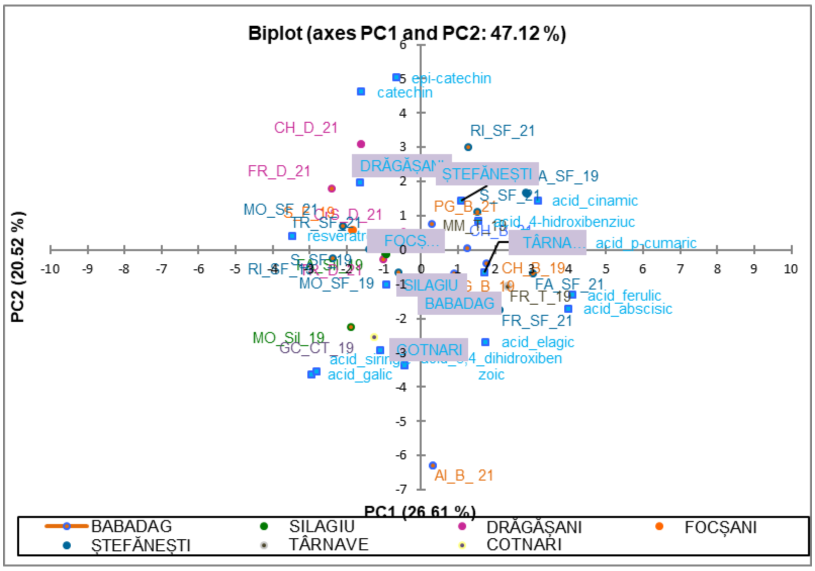


Fig.93 Biplot in discriminatory analysis by the polyphenolic profile of white wines (A) on geographical areas (B)

Figure 93 shows linear correlations between wine varieties in the geographical areas studied and the parameters analysed, with an acceptable score of 47.12% (26.16% PC1 and 20.52% PC2). Polyphenolic parameters of white wine varieties are represented by geographical areas of study.

Figure 98 shows linear correlations between compounds polyphenolic profile of 26 red wines (A) on the studied geographical areas (B).

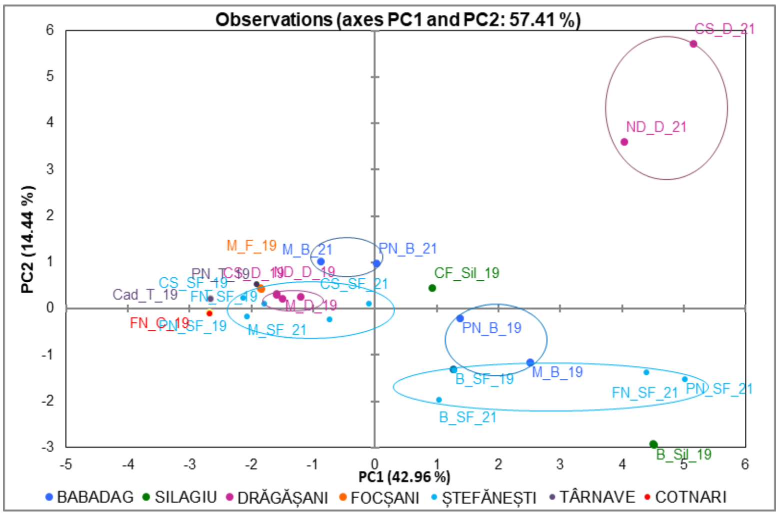


Fig.98 Discriminatory analysis by polyphenolic profile of red wines (A) on geographical areas (B)

In the correlation map of figure 99, the 16 polyphenolic compounds belonging to the polyphenolic profile are represented in the bible, including resveratrol stilben. From figure 98 and 99, it is noted that the wines from Drăgășani have the highest content of polyphenolic compounds, followed by the wines from Babadag, Silagiu, Ștefănesti. The lowest values of the content of polyphenolic compounds are in wines from Focșani, Târnave and Cotnari.

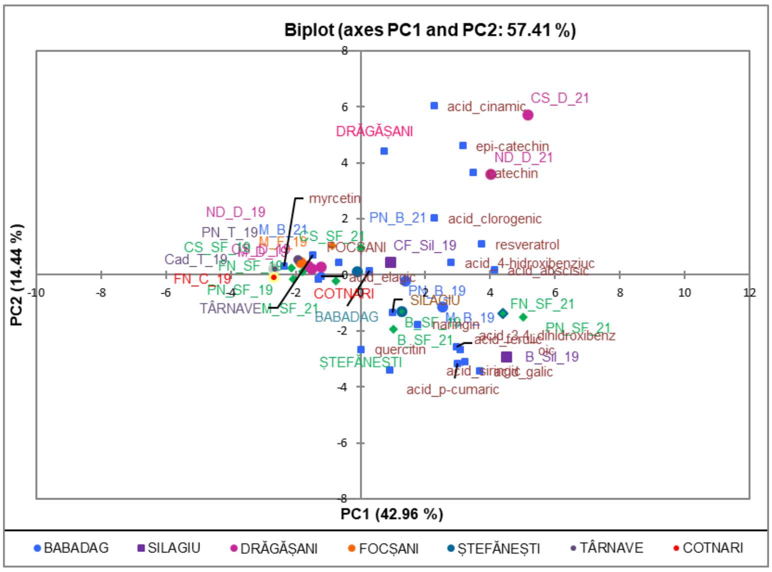


Fig.99 Biplot in discriminatory analysis by the polyphenolic profile of red wines (A) on geographical areas (B)

At the polyphenolic profile, it is noted that the most abundant phenolic substances detected in white wines are at: Aligote from Babadag 2021, syringic acid (103.71±2.12mg/l) and gallic acid (10.93±0.02mg/l). To red wines syringic acid (3129.48±70.71mg/l) to Burgund wine 2019 from Silagiu, gallic acid (168.47±1.41mg/L) to Pinot Noir wine 2021 from Ștefănești, (+)-catechine (94.71±1.18 mg/L) to Negru de Drăgășani wine 2021. In the phenolic profile of white wines, of the same variety, there were found differences for wines from ștefanești and Babadag areas, per year.

In red wines, depending on the polyphenolic profile on the geographical areas, the analysis of the main components shows that the first main component (PC1) explained 42.96% of the variability and the second main component (PC2) explained

14.44% of the variability. Together PC1 and PC2 explaining 57.41% of the entire variability of red wine samples and suggesting a good grouping based on polyphenolic profile compounds. Higher values of PC1 are observed for red wines from samples belonging to the Dragasani geographical area, only wines from the 2021 production years and are grouped in the positive quadrants of PC1 and PC2, high values are also observed here, except Pinot Noir from Ștefănești, 2021 production year and Cabernet Franc from Silagiu, 2019 production year. For red wines from samples belonging to the Stefaneti geographical area are grouped in the positive quadrant of PC1 and the negative quadrant of PC2, only wines from the 2021 production years, and Babadag wines from the 2019 production year.

3.4.1 Own results on wine DNA investigation

We used the same classification system as Agrimonti and Marmiroli (2018) to present the correspondence between products amplified from a particular variety (DNA) and products amplified from must or wine obtained from that variety. For monovarietal wine samples, the 4th method (Işçi et al, 2014 modified) with two stages of CTAB treatment was the most effective.

Of the varieties, it was more difficult to obtain DNA extracts with good purity in samples of riesling, Chardonnay, Burgund and Pinot Noir wine. The quantity and quality of DNA obtained from each extraction process (must or wine) were determined with BioPhotometer plus.

3.4.2. Statistical analysis of data and discussions

Although DNA obtained with extraction protocols was not detectable by agarose gel electrophoresis, after amplification with SSR primers amplicons were obtained in all samples analyzed (Onache, 2021). Comparative results of SSR locations amplified from must samples and wine of the same variety are shown in Table 39. All the extractions were done in three repetitions. The difference between the four methods was analysed with the Duncan test in order to highlight the significant „" differences at a significance level of ≤ 0.05.

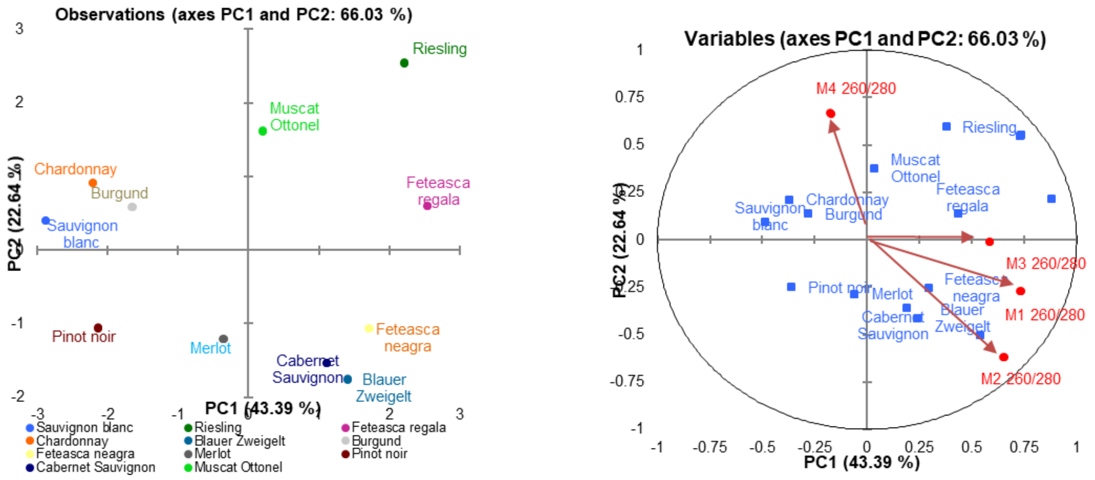


Fig. 104 Discriminatory analysis by methods (A) on the DNA of must varieties (B)

From figure 104 A, it is noted that the DNA in the musts of Chardonnay, Sauvignon Blanc and Burgund are in the same dial, having similar peculiarities, and from figure B are better highlighted, M1(Kit Qiagen), M 2 (Savazzini and Martinelli, 2006 modified), method 3 (autohtonne).

Figure 105 shows the linear correlations between the methods of determining DNA with its purity and the concentration of the obtained DNA. The score is very good at 68.10% (45.69% PC1 and 22.41% PC2). The DNA of wine varieties and its purity are represented, depending on the method of determination. From figure 105 A, the wine retains some characters with the wort, the difference is to the Royal Feteasca falling within quadrant 1 and the most suitable method for determination is M3. Red varieties retain their characters very well, the most suitable methods are M1, M2 and M4.

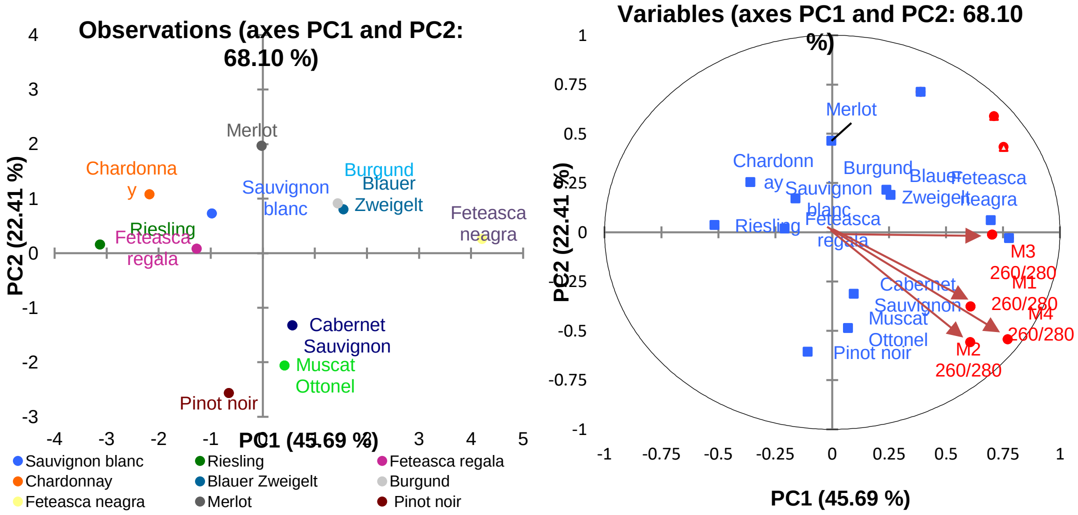


Fig. 105 Discriminatory analysis by methods (A) on the DNA of wine varieties (B)

CHAPTER IV – CONCLUSIONS

Wine is a well-known food, but also easy to fake, because of this the study of mineral, organic elements, polyphenolic composition and elemental composition are the often used instruments in authentication and classification of wines.

Molecular analysis and DNA determination of wine is a useful tool for authentication and determination of their origin and year of production. The study of the phenolic composition of the wine may allow the establishment of one or more biomarkers specific for a particular type of variety.

The chemical composition and sensory characteristics of the wine are strongly influenced by the geographical origin and climatic conditions, the soil, the geography of the plantations of production, the sun exposure, the degree of ripening of the grapes, the impact of agrotechnical measures, the technology of winemaking, the grape variety and the genetic contribution of the vine variety its ability to accumulate compounds with a strong aromatic effect in the grape grain and transfer it to wine.

The content of the elements, which can be used to characterize wines by their geographical origin, the minerals being the most appropriate elements for authenticity and typicity, depending on the geographical origin, as they bring a direct relationship with the composition of the soil on which the vines are grown. The analysis of certain wine elements is of particular interest due to their toxicity in case of excessive consumption and also to the effect they have on the organoleptic properties of the wine. The presence of micro, macro and heavy metals can influence the winemaking process, change the taste, but also the quality of the final product

Investigations on wine varieties analyzed from the 2019 harvest year correlated with the specific climatic conditions this year have shown that there is a clear differentiation for white wines from Silagiu vineyard (Muscat Ottonel - 16,33±0,01% vol and Feteasca Alba - 15,11±0,01% vol) and Ștefănești wine center (Feteasca Albă - 15,35±0,05% vol and Riesling Italian - 14,55±0,03%vol). For the other wine varieties from the other vineyards studied are specific values of white wines.

For red wines, high alcoholic concentrations for the 2019 harvest year were found in the wine centre Ștefănești, Pinot Noir (15,7±0,01%vol) and Merlot (15,6±0,03%vol), Feteasca Neagră (15,5±0,02%vol) from Babadag, Cabernet Franc 2019 (15,5±0,01% vol) from Drăgășani and from Silag vineyard, Burgund variety 16,53 ±0,01% vol.

From an extractive point of view, the 2019 harvest year determined higher values for red wines, this parameter being correlated with the winemaking technology used.

For white wines, the total acidity is between 4.00- 6.5, except for Riesling de Rihn with an acidity of 9.25 g/l tartaric acid. The alcoholic strength and high acidity values also ensure better stability and preservation of the wine obtained. Pedological and climatic conditions also influence the composition of wines, in this case on total acidity.

It was found that the content of free SO_2, total SO_2, primary NO_2 nitrogen and ammonia nitrogen in wines depends primarily on the technology of winemaking and less on the pedo-climatic conditions.

The content in the K, Fe, Cu and Ca elements identified in wine are dependent on the characteristics of the geographical area, but the content of Cu and Fe also depends on the soil and the treatments applied to the vines and soil.

Based on the discriminatory analysis, from the geographical areas studied on the analyzed wine varieties, a similarity between Silagiu, Focșani and Babadag vineyards, the same observations for Ștefănești and Drăgășani vineyards also intertwine with the Babadag area. At the same time, more specific values were observed for the Târnave vineyard and Iași wine centre.

Based on the discriminatory analysis, from the geographical areas studied on the analyzed wine varieties, a similarity between Silagiu, Focșani and Babadag vineyards, the same observations for Ștefănești and Drăgășani vineyards also intertwine with the Babadag area. At the same time, more specific values were observed for the Târnave vineyard and Iași wine centre.

The hierarchical dendrogram for the grouping of the 35 samples of white wine shows that the two specific models of descriptors of interest determine the quality of the white wine samples.

The hierarchical dendrogram for the grouping of the 35 samples of red wine, renders three specific models of descriptors of interest that determine the quality of the samples of red wine.

By analyzing the soils coming from the 4 wine-growing areas (the wine-growing center of Iași, Blaj, Ștefănești and the Babadag vineyard) and correlating with the chemical profile of the wines corresponding to these areas, it was found that some parameters can be correlated with the soil type, but others not.

Based on the determinations made using the specific equipment, a series of results were obtained, and the low content of heavy metals in wine was found to cause them to precipitate and remove a certain percentage of wine. Another observation related to the content of Pb has its explanation in proximity to A10 highway and the industrial area.

Analysis of other minerals Cr, Ni, Sr, Hg, Mn indicated values below the maximum level of contamination, according to the OIV, valid for all geographical areas studied.

The presence of these minerals has a beneficial effect on the body's nutritional needs as expected the content of polyphenolic compounds in red wines is higher than in white wines. Their presence increases the antioxidant properties of wine and also makes it clear that there is a series of hormone that can amplify the content of polyphenolic compounds, specific to fruits. In terms of correlations between total polyphenolic compounds in white wines. In this research, there is a higher content of polyphenolic compounds in red wines than white wines.

For wines from the 2021 harvest, high values were found in polyphenols and therefore their antioxidant activity was high.

At the polyphenolic profile, it is noted that the most abundant phenolic substances detected in white wines are at: Aligote from Babadag 2021, syringic acid (103.71±2.12mg/l) and gallic acid - 10.93±0.02mg/l). To red wines syringic acid (3129.48±70.71mg/l) to Burgund wine 2019 from Silagiu, gallic acid (168.47±1.41mg/L) to Pinot Noir wine 2021 from Stefanesti, (+)-catechine (94.71±1.18 mg/L) to Negru de Drăgășani wine 2021. In the phenolic profile of white wines, of the same variety, there were found differences for wines from Ștefănești and Babadag areas, each year.

Differentiation of wines using the phenolic profile was achieved by the statistical method specific to PCA. Thus the analysis of the main components reported that the first main component (PC1) explained 26.61% of the variability and the second main component (PC2) explained 20.52% of the variability; together PC1 and PC2 explaining 47.12% of the entire variability of white wine samples and suggesting a good grouping, based on the polyphenolic profile of white wines.

For the selection of the most effective methods of DNA extraction from must samples and monovarietal wine, the concentration of the final extract in the DNA and its integrity are important.

Of the tested methods modified in our laboratory, in the case of monovarietal must samples, the best results were obtained with the Savazzini and Martinelli method (2006), and in the case of monoclonal wine samples with the method recommended by Işçi et al 2014.

The reliability of DNA determination methods depends very much on: its purity and its concentration. The score is very good at discriminatory analysis of methods for determining DNA and is 68.10%.

After fermentation, the wine retains some must-like characters. There is, however, a difference in the Royal Feteasca variety, which falls within quadrant 1 (figure 105 B) and the most suitable method for determination is M3 (autochtonous). Red varieties retain their characters very well compared to that of the musts from which they are obtained, and the most suitable methods for determining the DNA in red wines are M1, M2 and M4.

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CHAPTER V - ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS, FURTHER DIRECTIONS TO FOLLOW IN RESEARCH

- A very well documented complex study has been carried out on the current state of research on the methods of qualitative certification of wines, related to the methodical and legislation in force;
- An pedoclimatic and oenological assessment of the 8 geographical areas in Romania under study was carried out;
- A detailed presentation of the chemical composition of 70 varieties of wine wines was made highlighting the positive contribution of moderate wine consumption, health and protective action on the body, based on recent studies and research; Determination of micro-micro-minerals and heavy metals in wine, and establishment of correlations starting from a series of peculiarities regarding the microclimate, variety, etc.
- The most appropriate techniques of analysis and control regarding the determination of the elemental profile have been chosen and used, for the evaluation of the wine terroire and for the determination of micro, macrominerals and heavy metals in wine;
- Determination of the typicality and authenticity of wines of 70 varieties of wine from the 8 geographical areas of Romania, based on qualitative markers with the help of the polyphenolic profile;
- Use of molecular techniques of "Fingerprinting of vines" to determine their typicality and authenticity using DNA extraction methods based on identified molecular markers;
- Own working procedures have been carried out on the technique of DNA extraction from monovarietal wine;
- By achieving the assumed objectives, a completion of the "Database Fingerprinting of wines" in general and for the wine-growing areas under study in particular was ensured.

The results of the research undertaken within this doctoral thesis were disseminated through the elaboration and publication as first author of a number of 15 scientific articles, of which, 12 published in ISI indexed journals (1-Q1, 2-Q2, 1-Q3 and 4-Q4); co-author of 2 scientific articles published in ISI indexed journals; co-author of 2 scientific articles published in ISI indexed journals co-author of the publication of 5 scientific papers in journals indexed in national and international databases; first author of 2 book chapters; I participated in 15 international scientific events.

Continuing research can be done by:

- Development of molecular and DNA analysis in other monovarietal wine varieties;
- Developing and performing molecular and DNA analysis, at known coupages from different wine varieties, in order to be able to differentiate them and to ensure the authentication of the blended varieties;
- Study and identify other wine markers that can help to quickly and accurately assess the authenticity of wine varieties;
- Establishing other correlations regarding the influence of the peculiarities of the wine production area on the quality and authenticity of these wines.

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