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Journal of Agroalimentary Processes and Technologies 2020, *26*(3), 191-199

Journal of Agroalimentary Processes and Technologies

Current techniques used for Romanian wine characterization- A review

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Abstract

Wine, especially the red one, is grown for more than 10 000 years, worldwide. One important aspect regarding its distribution on the market and consumer's confidence is wine authenticity. Authenticity, in terms of geographical origin, vineyard and "terroir", it is the specific definition of one wine, of each vine. This review aims to summarize the most important analytical techniques used for authentication and characterization of principal parameters of Romanian wines especially in the last ten years. Among other, the review will focus on the most relevant, actual information found on spectroscopic, vibrational techniques, trace elemental analysis, chromatographic methods and isotopic ratios assessments used.

Keywords: Spectral, vibrational, chromatographic methods, isotopic, wine, authenticity, statistical analysis

1. Introduction

Nowadays, there is an increasing trend in offering the "best of" products, in terms of quality, safety and security for consumers. Regarding the wine industry, potential existing frauds raise a lot of questions in people's mind, making them question about about wine's quality, affecting the product image, resulting in sales decreasing [1]. For this reason, as for regular controls made on traceability issues, oenologists are using more and more actual techniques (spectral and chromatographic) for authenticating their products and maintaining the quality standards requested on the market.

There are specific laws in action regarding the specificity of one wine, so that no uncertainties exist on the products, the DOC (Controlled Denomination of Origin) ones. In European Union, including Romania, Regulation No. 251/2014 for presentation, labelling and the protection of geographical indications of aromatised wine products is applicable.

However, disloyal practices as addition of components of the wine (sugar, ethanol, diethylene glycol for sweetness), flavour and colour substances, the non-authorized addition of preservatives and acidity adjustments are still present [1-4]. As an actual example, according to one study published in Journal of Retailing and Consumer Services, because of the social distancing and „staying at home" advice, the process of purchasing wine was transferred to online media. The research focused on the authenticity in terms of visual colours. The results showed that wines with simple colours and low visual impact were viewed as authentic and bought by many of the consumers [2]. However, this new concept of authenticity based on visual perception can, most of the times be ambiguous, thus the need for real authentication is mandatory [1-4].

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This review will focus on the most actual information and experimental results found on spectroscopic (UV-Vis), vibrational techniques (IR - infrared spectroscopy), MIR (mid-IR), NIR (near-IR), trace elemental analysis (ICP-MS (inducted coupled plasma – mass spectrometer), AAS (atomic absorption spectroscopy), chromatographic methods (HPLC - high performance liquid chromatography), HPTLC (high performance thin layer chromatography), GC (gas chromatography) and isotopic ratios assessments ($^{13}C/^{12}C$, $^{18}O/^{16}O$ by IRMS -isotope ratio mass spectrometry) methods, D/H ratio by SNIF–NMR method,^{1}H and ^{13}C by NMR (nuclear magnetic resonance spectroscopy) [5].

The techniques, previously mentioned, were coupled with statistical analysis and multi-elemental data in order to get the best statistical distribution and differentiation over the observed parameters for Romanian wines.

2. State of the art

The detailed information about the method's goal and their application on Romanian wines is based on scientific literature from the last ten years.

a. Spectroscopic techniques

Spectroscopic tools emerged as a need of optimizing costs and preparation of the samples. By employing the mentioned below ones, time is saved, as no preparation of the sample is required, maybe a couple of dilutions needed, no toxic reagents or solvents are used and no changes in sample structure are made.

UV-Vis spectroscopy is a cheap, easy to make determination which in combination with chemometric methods as PCA (Principal component analysis) or PLS-DA (Partial least square – discriminant analysis) is a useful one for wine composition and fingerprinting.

UV-VIS is a measurement technique based on the intensity of the light beam passing through a sample and reaching the detector [6-9].

IR spectroscopy is a versatile, non-destructive, and reliable technique based on vibration energy of atoms from elemental molecules. It has been employed since 1990's, where all it started with NIR for basic determination of wine, but later, the interest came on FT-IR, in MIR region, offering a widely variety of new compounds to be quantified and a larger accuracy for the method.

In 2019, Elisabeta-Irina Geană et al., aimed to classify based on grapes variety and vintage year, 39 different red wines, different production years, starting 2007, until 2019, from Dobrogea Region. Spectroscopic techniques, simple, easy to operate with, UV-Vis and IR were used [6-9].

The UV-Vis spectrum revealed differences between the samples with different harvest periods and different grape varieties. The region of interest was [250-600] nm, specific for volatile compounds, as for polyphenols originating from grape skins and fermentation process.

In IR spectra, the resulted peaks showed similar heights, on the fingerprint region, [1600 – 900] cm^{-1}. For a better image and a simplified understanding of the obtained data, PCA was applied. From the two, the best separation, based on variability of the values, was obtained for FT-IR. Comparison among the two techniques is presented in figure 1.

PLS-DA models, based on data used in PCA determination, revealed a correlation coefficient of [0.813, 0.860] for classification according to grape varieties, respectively [0.626, 0.872] for wine's maturity. Through the calculus of error values in case of calibration and validation of the model, the lowest uncertainty was determined for FT-IR, both for grape variety, as for the harvesting year [10-13].

Another study using IR and multivariate techniques, realized on 15 different, white, rose and red DOC wines, from different regions of Romania, aimed to characterize and prove the authentic features of each wine. Employing FT-MIR method, more specific the ATR (Attenuated Total Reflectance) accessory it was easy enough to identify the phenolic compounds, carbohydrates, amino acids and organic acids, on the selected area of interest. This information, all together, was offering detailed characteristics on selected parameters, for each sample, along with the appropriate sweetness index. After applying the PCA, on the fingerprint region, 600-1800 cm^{-1}, the variability of the 2 components explained 90% from the variability of data sets (figure 2) [14].

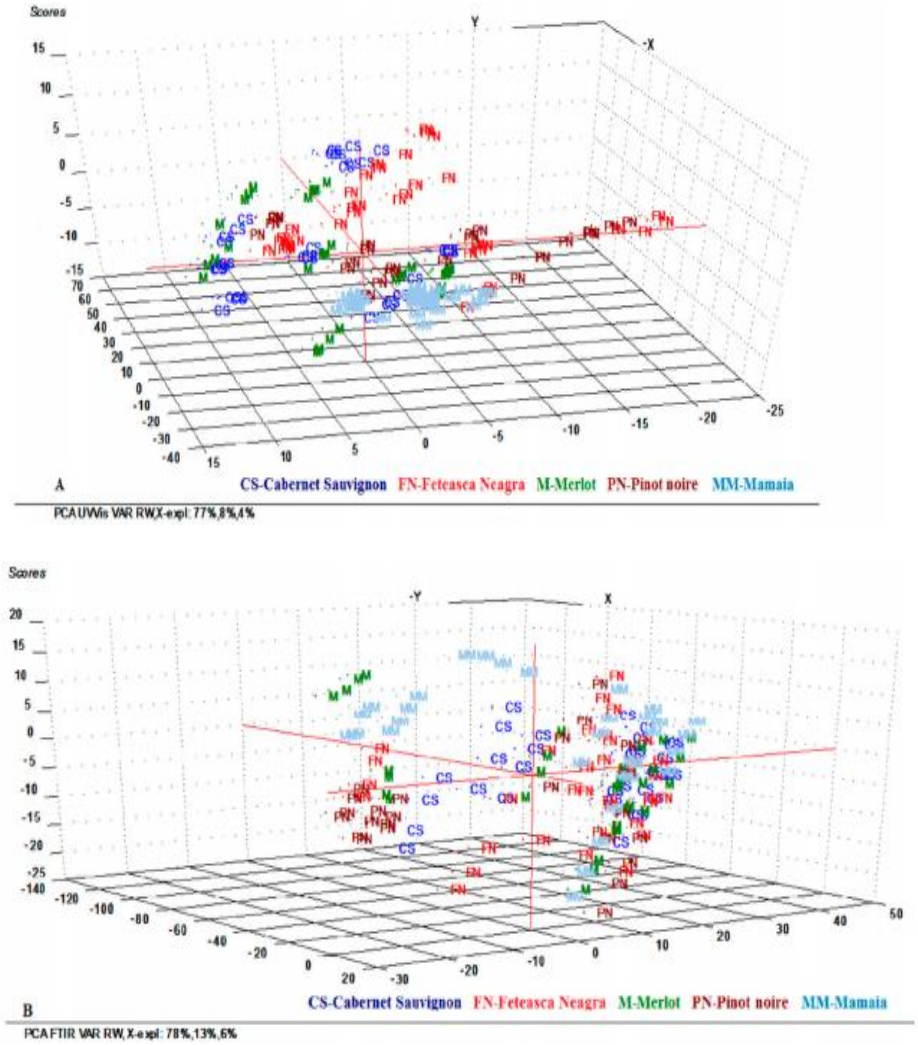


Figure 1. Application of PCA for the data set obtained via UV-Vis (A) and IR (B) (Elisabeta-Irina Geană et al, 2019)

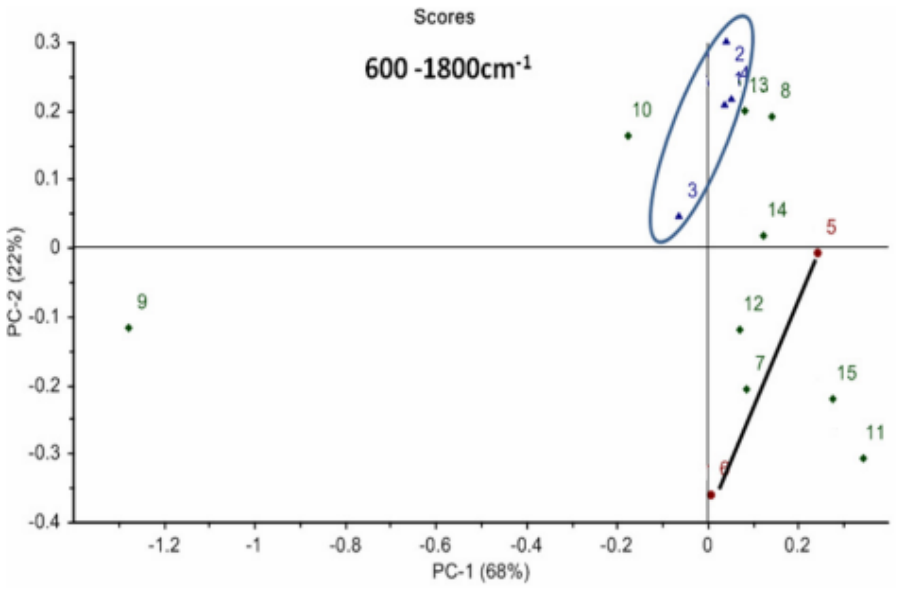


Figure 2. Application of PCA for the data set obtained via IR-ATR (Todasca, M.C., et al., 2010)

b. Chromatographic techniques

Chromatographic techniques are usually used for qualitative and quantitative analysis of principal components present in wine, polyphenols, organic acids, sugars and some minor ones [9]. Polyphenols can serve as indicators for grape varieties, being strongly influenced by climatic factors.

Anamaria Hosu et al., 2015, studied the HPTLC methods ability in determining the phenolic profile, as the antioxidant activity of 27 wines from 3 different varieties. The aim of her research was to classify wines according to grape provenance and vintage year. This method was rather preferred than HPLC because it is faster, more flexible, can quantify many classes of compounds, without purification of the sample. HPTLC system was prepared for running the analysis by selecting the mobile and stationary phase, as setting the parameters, the flow, the temperature, all for a good resolution. After many trials to select the best ratio of mobile phase composition, ethyl acetate : formic acid : acetic acid : water, 20:2:2:4(v/v/v/v)was chosen as being the one offering the best separation among the three wine varieties: Fetească Neagră, Cabernet Sauvignon and Merlot. Concerning the detection method, fluorescence proved better than UV-Vis in showing well defined distinctive bands [15].

GC-MS method combined with e-Nose technique, were used by Laurentiu Mihai Palade et al., 2016, in determining the volatile profile of three Romanian wines (Fetească Neagră, Cabernet Sauvignon and Merlot), as an authentication marker. In order to discriminate the samples, four major compounds determined via GC-MS headspace, 1- butanoic acid methyl ester and 2-ethyl heptanoic acid, respectively terpinen-4-ol and linalool, were summed up after integration. The results were compared, among the three samples, a clear differentiation being obvious.

As example, for Fetească Neagră, % area (the percentage of the peak area as proportion of the total area of the integrated peaks) between first 2 components was 2.39, meanwhile for the Cabernet Sauvignon was 4.28 and for Merlot 1.39. Based on sensor's recognition of aromatic compounds present in wine, e-Nose technique can establish, as well, a clear distinction among samples [16,17].

c. Isotopic techniques

Isotopic methods, based on ratios of D/H, $^{18}O/^{16}O$, $^{13}C/^{12}C$, coming from principal components of the samples, are representative for the „birth" place of wines and the most reliable assays in authentication tries. The most used are: isotope ratio – mass spectroscopy and nuclear magnetic resonance.

In 2015, when the "authenticity" word started to be more and more present/vocal, some researchers struggled to identify five Romanian representative varieties, Cabernet Sauvignon, Merlot, Fetească Neagră, Pinot Noir and Mamaia from Murfatlar vineyard. The method was based on was identification of isotopic ratios. ^{13}C $^{/12}C$, $^{18}O/^{16}O$ from water by IRMS and ^{1}H and ^{13}C by NMR spectroscopy from ethanol [18].

Researches suggested that stable isotopes from water and ethanol can be very useful in determining the geographical origin as they are in direct relation with the environmental conditions. An example is $\delta^{18}O$ which is higher in years with hot temperatures, increasing $^{18}O/^{16}O$ ratio, while in arid ones, it goes in opposite direction, so it depends on climatic changes. It can be a clue in process differentiation of the samples [19].

The NMR analysis was also used in fingerprinting wines based on constituents: sugars, alcohols, and amino acids. After performing ^{1}H or ^{13}C-NMR analysis together with multivariate data analysis, there were four important classes selected for classification of wines according to composition: sugars, glycerol, amino acids and alcohols. The method was also confirmed by the literature in a German study (Godelmann et al., 2013) [20,21].

In 2011, Anamaria Hanganu et al., compared three wine varieties Fetească Neagră (FN), Cabernet Sauvignon (CS) and Merlot (M) with Cadarcă, another wine originating from Miniș area, in order to highlight the similarities and differences among them. The research was conducted using ^{1}H-NMR spectroscopy. To get the most conclusive comparison, the markers signals from the NMR spectra (figure 3), were coupled with data analysis tool. After applying PCA, the variability coefficient of almost 50%, clearly separated wines, figure 4 [22].

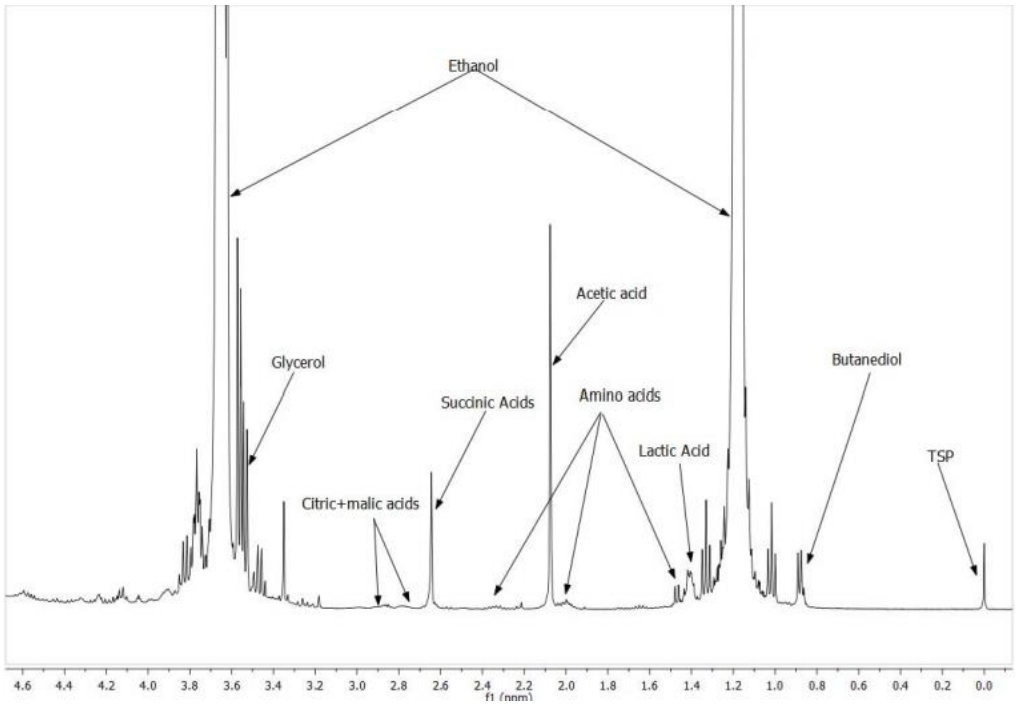


Figure 3.[1] H-NMR profile of Cadarca wine (Anamaria Hanganu et al., 2011)

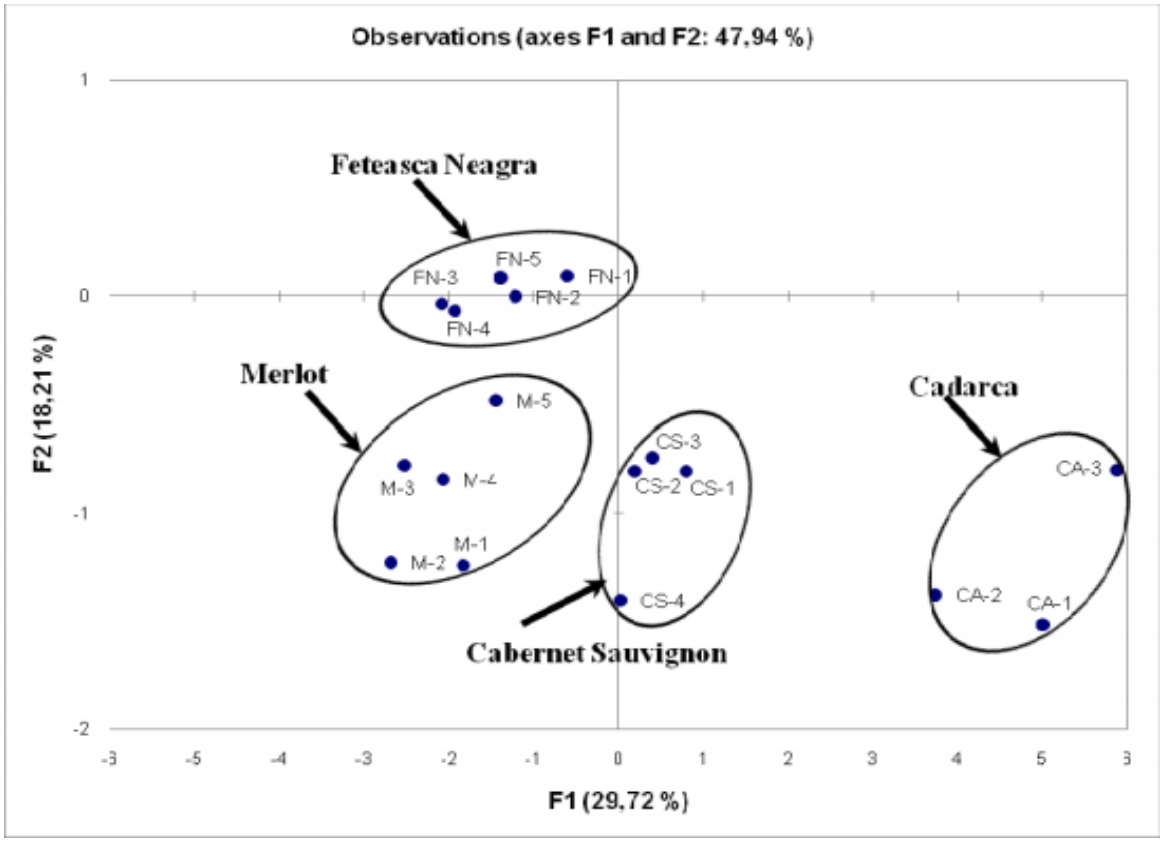


Figure 4. Application of PCA for the data set obtained for the compared samples (Anamaria Hanganu et al., 2011)

d. Trace elemental methods

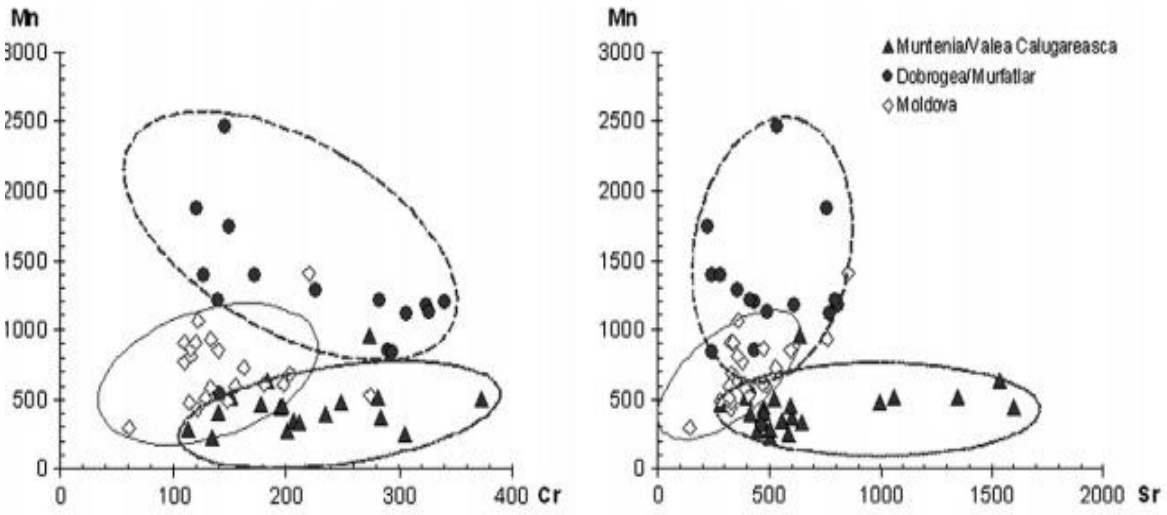
ICP—MS is a method based on identification of traces of certain elements in analysed sample. It is used for liquid samples, being characterized by high sensitivity, sensibility, and very low detection limits.

In wine's case, the soil composition is highly important in defining its elemental charge. The attention was focus on the elements found in small quantities, even traces, as no metabolization or significant modification are happening to them during the vinification process. In Romania, according to (Suhaj and Korenovska, 2005), Mn and Sr are the ones that can offer a specific view on wine's origin or provenance.

According to a study from 2013, Cr, Ni, Rb, Sr, Ag, Zn, Mn, Cu, Co, V, Pb, and Be were chosen as appropriate elements for the determination of wine's origin, by trace element analysis from Muntenia (Valea Călugărească), Dobrogea (Murfatlar) and Moldova (Iași, Cotnari, Bujoru, Panciu, Odobești, Nicorești) vineyards. There were 60 samples (18 grape varieties), 36 harvested in 2010 and 24 in 2009. From the same regions, excepting Moldova, as samples were selected from multiple areas and the collecting process went slowly, samples of soil were analysed [18, 23].

In performing wine analysis, Rb and Sr showed the highest concentrations in red samples, meanwhile in white ones, Co and Cu mostly occurred. As no variations were observed for Mn, Rb, Sr, Ag, Cr and Co, they were compared, statistically, using PCA, with the differentiation of the specific area (figure 5). As the below graphs show, wines from Dobrogea/Murfatlar and Muntenia/ Valea Călugărească regions were well divided, while the wines from Moldova partially overlapped over some wines from the other chosen regions.

Another study from 2014, based on the same determination, aimed to discriminate 22 wines, 16 from Drăgășani (two area Drăgășani S, in the north and Drăgășani I, in the south) and six from Recas. The elements determined were Li, Be, Co, Ni, Cs, U, Pb, V, As, Ba, Cr, Cu, Zn, Al, Mn, Rb, Sr, Fe, Ca, Mg, Na, and K. An important detail is related to major compounds Na, K, Mg, Ca, and Fe which were quantified via AAS, according to official method. After performing the necessary steps, the values showed that the high concentrations of Be, Pb, Ba, Cr, and Ca were found in wines from the Dragașani region, while Li, Ni, Cs, V, Cu, Sr, Fe, Mg, Na, and K were found in wines from the Recaș; Co, U, Zn, Al, Mn, and Rb levels were similar for both vineyards. Following the PCA correlation for the 22 elements, the variability coefficient was around 54%, the distinction between areas based on elemental composition being represented in figure 6 [24, 25].



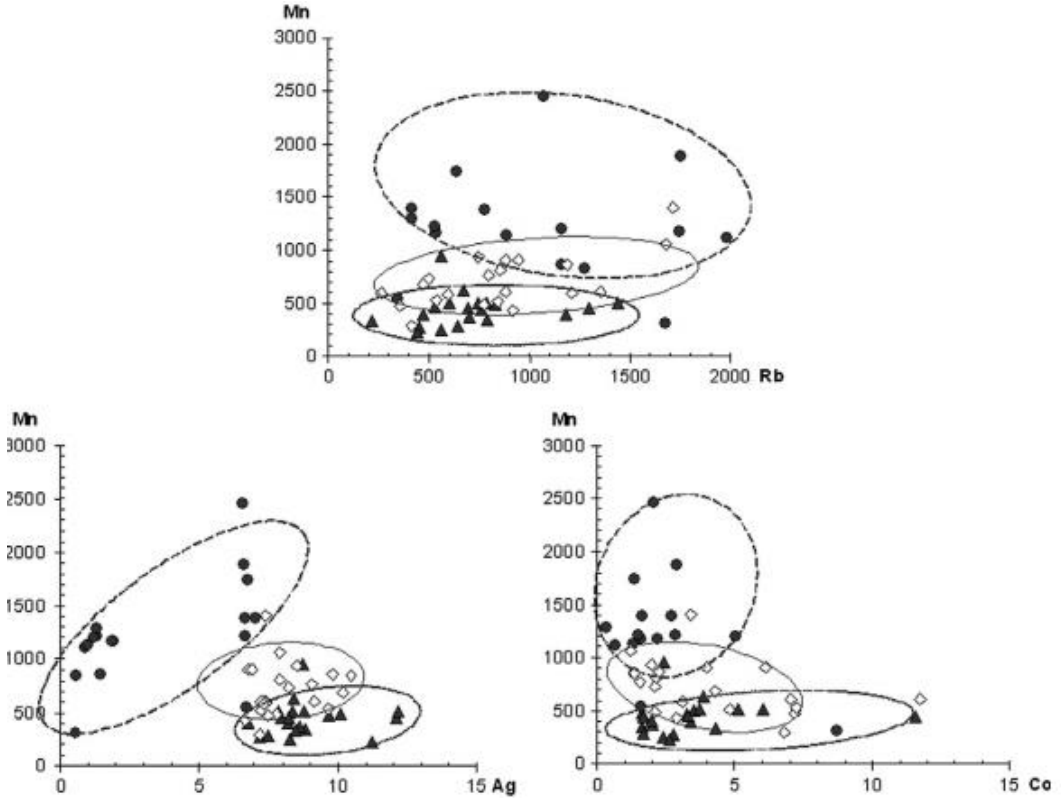


Figure 5. Statistical correlation of Mn content with Cr, Sr, Rb, Ag and Co (Geana. I et al., 2013)

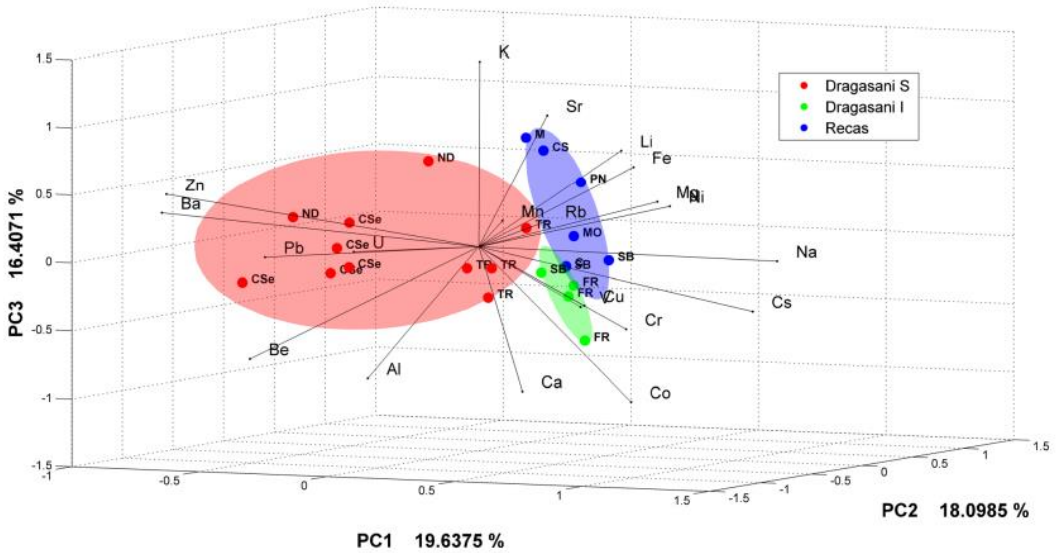


Figure 6. Application of PCA for the data set obtained for the three regions (Palade, M.L et al., 2016)

d. Electrochemical sensors

Elisabeta-Irina Geană et al., 2020 restated the electronic tongues advantages, combined with multivariate data analysis in authenticating wine. Electronic tongues are based on electrochemical sensors (voltametric, amperometric or potentiometric) for multiple aroma detection. It can be used in analysis of many samples and combined with PCA, LDA and some others, it records, process and discriminates mineral waters, milks, teas, wines and beers. In wine's case, phenolic compounds, which are pruned to redox reactions, can generate an electrochemical profile, at electrode's surface.

If the profile is related to specific data records, it can serve in determination of varietal and geographical traceability, wine aging process, vintage year prediction and detection of adulteration.

Besides identification and quantification of phenolic compounds, another important aspect it is related to the evolution of alcoholic and malolactic fermentation. Changes in organoleptic properties and development of new aroma compounds thanks to oak barrels, used for storage and maturation, can be easily recognized as well.

This method has three important advantages: it does not require sample preparation, it is fast and can be used in production of portable device, for easy handling everywhere.

Although it is an unexploited field in Romania, previously mentioned researchers, accepted the challenge of developing voltametric sensors for determining the antioxidant activity of wine, following the maturation process and determining wine's main compounds for authentication. They have used carbon screen printed electrodes modified with polypyrene with addition of potassium hexacyanoferrate (III), sulfuric acid, sodium decansulphonate for authentication purpose. These sensors served for cyclic voltammetry measurements which in combination with statistical analysis, revealed some expected results.

In addition, for evaluating total phenolic content in white and red wines have used a multicomponent enzyme sensor based on carbon scree printed electrodes (SPCE) modified with cobalt phthalocyanine (II) as electron mediator and tyrosinase as biocatalyst. More results of these attempts are still in attendance of being published [26-28].

3. Conclusions

As a summary, it is important to highlight once more the importance of authentication techniques for wines. Although in Romania, in the last years, the high costs of appropriate analysis didn't allow more studies and differentiation of wines; oenologists are becoming more and more conscious about this inquiry. „Easy to use", simple, economic, non-destructive techniques as UV-Vis or FT- IR, can be a potential rapid solution.

In appeals, one or more of isotopic (^{1}H-NMR or IRMS), chromatographic (GC or HPLC) or trace elemental determination (ICP-MS) has to be considered as more costly but more sensitive methods. From now on, although there are some researches on authentication techniques there is need for more complex and deep analysis for proving the Romanian wine's quality and value, to support the recognized taste and tradition.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

Acknowledgement: This study was achieved through Core Program, with the support of the Ministry of Education and Research (MEC), contract 22N/2019 (project PN 19 02 04 02) and project 26 PFE/2018 funded by Ministry of Education and Research trough Program 1- Development of the National R&D System, Subprogram 1.2- Institutional Performance- Projects for Excellence Financing in RDI.

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