

Rapid Method for the Discrimination of Romanian Wines Based on Mid-Infrared Spectroscopy and Chemometrics

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ATR-FTIR Spectroscopy combined with multivariate data analysis have been applied for the discrimination of 10 different Romanian wines (white and red wines), produced in 2 wineries from Romania: Reca' and Stefanesti-Arge's from different cultivars. Principal Component Analysis were performed using different regions of FT-MIR spectra for all wines. Principal Component Analysis of their chemical parameters indicated that the wines can be discriminated based on their different phenolic, glucides, acidity content and geographical origin.

Keywords: white and red wine; mid-infrared spectroscopy; principal component analysis, Recas, Stefanesti-Arges

Wine is a complex mixture of several hundred compounds present at different concentrations. The main compounds are: water, ethanol, glycerol, sugars, organic acids, salts; aliphatic and aromatic alcohols, amino acids and phenolic compounds are present at much lower concentrations. Chemical analysis of a complex mixture such as wine is becoming of great importance for quality control to both the winemaking industries and the consumers.

Various analytical techniques are used in wine analysis. Conventional analytical methods such as: chromatographic analytical methods, mass spectrometry and capillary electrophoresis are relatively expensive in instrumentation, time-consuming in sample preparation and measurement and are not suitable for fast measurement. Recent advances in infrared spectroscopic instrumentation, together with the introduction of sampling techniques such as attenuated total reflection (ATR), have made spectral methods increasingly attractive for the rapid analysis of complex matrices across many scientific fields.

A MIR spectrum contains information about the chemical composition and physical state of the material under analysis, yielding structural information that constitutes the fingerprint of a sample. Each biological material presents unique spectrum signals [1].

ATR-FTIR requires no sample preparation, so samples can be analyzed quickly, non-destructively and at low cost [2]. Compared to spectra in the near infrared region, the MIR region (4000 to 400 cm^{-1}) typically comprises more spectral peaks, which are better defined and therefore, more easily interpreted [3]. As such, MIR spectroscopy has become a popular technique for qualitative analysis [4].

Related to wine, mid-infrared spectroscopy combined with multivariate data analysis has also been explored for:

- classification of sparkling wine by style and quality [4];
- differentiation and classification of wines and brandies during their ageing process [5];
- discrimination between different red, rosé and white Romanian wine varieties [6], or between the organic and non-organic ones [2];
- to discriminate extracts due to the polysaccharide composition. Based on the bands from 1600 cm^{-1} , the polyphenolic content was also evaluated [7];
- to develop a rapid and simple method for wine authenticity [8];

- authentication with a feasibility study on variety, type of barrel wood and ageing time classification of wine [9];
- authentication by comparing the phenolic extract spectra of unknown wines with those of authentic wines. Tarantilis et al. created libraries of spectra using wines samples from three *grape* varieties to discriminate them from each other [10];

- evaluate the ripening of grapes collected at different stages between the *veraison* and harvest periods [11].

This study aims to investigate the use of mid-infrared (MIR) and multivariate data analysis techniques to classify some Romanian wines produced from autochthonous cultivars.

Experimental part

Wine samples

Ten commercial Romanian wines, including white (4 samples) and red (6 samples), of different sweetness indexes (4 samples dry, 5 samples half-dry and 1 sample half-sweet) were investigated. These wines of controlled origin were produced in 2 wineries from Romania: Reca' and Stefanesti-Arges.

Table 1 includes data from each wine sample, the specific cultivar, the year of production, the denomination (as dry, half-dry, half-sweet) and their region of origin. All wine samples were centrifuged at 3000 rot/min for 15 min and then used directly for recording the ATR-FTIR spectra.

Analysis of wines by Attenuated Total Reflectance Mid-Infrared (FT-MIR) Spectroscopy

The ATR-FTIR spectra were recorded in a range between 4000-400 cm^{-1} using a FTIR Jasco 6300 spectrometer, detector TGS, apodization Cosine. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. Each sample (10 μL) was spread uniformly through on the surface of the diamond ATR crystal using a micropipette.

At the same time, the viscous residues were also obtained after 1 mL of each sample was dropped on a pan and volatilized for 8 h. For each viscous and liquid wine samples the IR spectra were recorded and analyzed for classification.

The spectral data were processed with JASCO Spectra Manager II software. Liquid samples of wine, without any preparation, were scanned at 4 cm^{-1} resolution, accumulation: 100 scans. For each subsample, three

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	Cultivar (Variety)	Vineyard	Year	Wine type	Alcohol Content
White wine					
1	Riesling	Recas	2015	Dry	12%
2	Sauvignon Blanc	Recas	2016	Dry	12%
3	Fetească Regală	Recas	2016	Half-dry	11,5%
4	Fetească Regală	Ștefănești	2016	Half-dry	10,5%
Red wine					
5	Fetească Neagră	Recas	2016	Dry	12,5%
6	Cabernet Sauvignon	Recas	2016	Dry	13,5%
7	Merlot	Recas	2016	Half-dry	13,5%
8	Pinot Noir	Recas	2016	Half-sweet	12,5%
9	Cabernet Sauvignon	Ștefănești	2016	Half-dry	11%
10	Fetească Neagră	Ștefănești	2016	Half-dry	11%

Table 1
ROMANIAN WINE SAMPLES ANALYSED

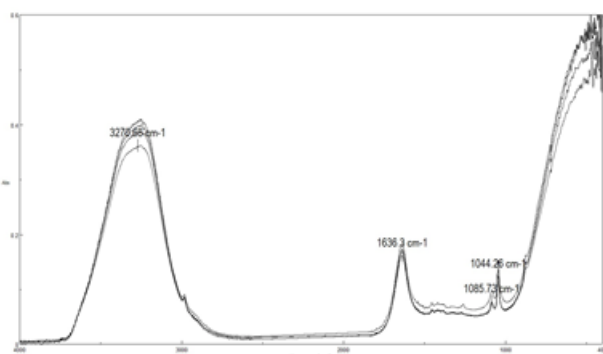


Fig. 1. The ATR-MIR spectra (4000-400 cm^{-1}) of a white wine liquid samples

replicate spectra were recorded to ensure the spectral reproducibility and assess analytical precision and the average spectrum was done. Background reference spectra were recording using air after every sample to minimize the interference due to carbon dioxide and water vapor in the atmosphere. Between measurements, the ATR crystal was carefully cleaned using acetone then dried with a soft tissue.

Data Analysis

Infrared Spectra were exported from Spectra Manager, in ASCII (dx) format, into the Unscrambler Software (Edition X 10.3, Camo. Oslo Norway) for chemometric analysis. Spectra were pre-processed using the second-derivative transformation, the Savitzky-Golay derivation. The use of spectra derivatives with Savitzky-Golay algorithm as a chemometric pre-processing technique is widely reported in most classification based on FTIR spectroscopy [12-14]. The principal component analysis (PCA) model was developed using cross validation. PCA was performed both on the entire spectral range (4000 to 400 cm^{-1}), and on the MIR *fingerprint* (i.e., 1800 to 900 cm^{-1}), on the peak areas corresponding to regions 1000-1120 cm^{-1} (1) and 1600-1720 cm^{-1} (2).

Results and discussions

FT-MIR spectral fingerprinting of wine samples

The ATR-MIR spectra of commercial wines showed moderate to strong absorbance peaks at 1044, 1085, 1636-1638 and around at 3270 cm^{-1} (fig. 1) with peaks at 3270 and 1636-1638 cm^{-1} corresponding to the O-H stretching and bending respectively, associated with water [15, 16]. The MIR region between 1120 and 1000 cm^{-1} has previously been attributed to C-O vibrations of sugars, such as glucose and fructose, and alcohols (ethanol, glycerol), phenols, esters and lactones, compounds which are constituents of wine [17].

In all samples, similar spectral features were generally obtained. Figure 2 exhibits the general FT-MIR spectra of all wine residue samples.

Identification of specific functional groups and molecules in wines, depending on their color and sweetness

Sugars are very important for winemaking. ATR technique combined with IR spectroscopy was also considered as a powerful method for measuring sugars in wine.

Volatile Compounds. The measurement of volatile compounds can provide significant information of wine [18]. These volatile compounds are results of biochemical and chemical reaction pathways during berry crushing and metabolism, fermentation, wine aging and storage, which are also responsible for wine aroma.

Acid is another important component of wine products, which greatly affects mouthfeel of wine. The most common organic acids are the carboxylic acids, whose acidity is associated with their carboxyl group -COOH. Regmi et al., demonstrated direct determination of organic acids (tartaric acid, lactic acid, acetic acid, malic acid, citric acid and succinic acid) in wines using FT-IR spectroscopy and chemometric analysis [19].

The phenolic content in wine refers to the phenolic compounds, including a large group of several hundred chemical compounds that affect the taste, color and mouthfeel of wine [20]. They are important to the organoleptic properties of wines, and their analysis and quantification are of great significance. The most important phenolic compounds in the white wine are the hydroxycinnamic acids and tannins and anthocyanins, respectively in red wines, which are located in grape skins and seeds. Anthocyanins has become an important object of enology, because these polyphenolic compounds have a decisive influence on an organoleptic feature that determines the quality of a wine: the color. Moreover, anthocyanins have beneficial effects upon human health such as antioxidant and anti-inflammatory properties, important to prevent many diseases. Tannins are responsible for the stabilization of the color and the sensory characteristics of the wines due to their astringent and bitter properties.

Glycerol is quantitatively a major component of wine, and its determination at various stages of the winemaking process provides important information regarding issues that are directly or indirectly related to quality control [21].

In order, to get an overview of the compositional differences that occurred in the ten samples of wine an overlapping image of the of white and red wine samples was taken and it is presented in figure 3. It was possible to consider a few specific regions which can be useful for wines' characterization [6, 22]. Region 1 (1000-1120 cm^{-1}) is responsible to carbohydrates (glucose, fructose and oligosaccharides), region 2 (1600-1720 cm^{-1}) to free amino acids and organic acids and region 3 (2800-3000 cm^{-1}) for polyols (mainly glycerol). In addition, the phenol signal due to ring vibrations, stretching and bending C-C

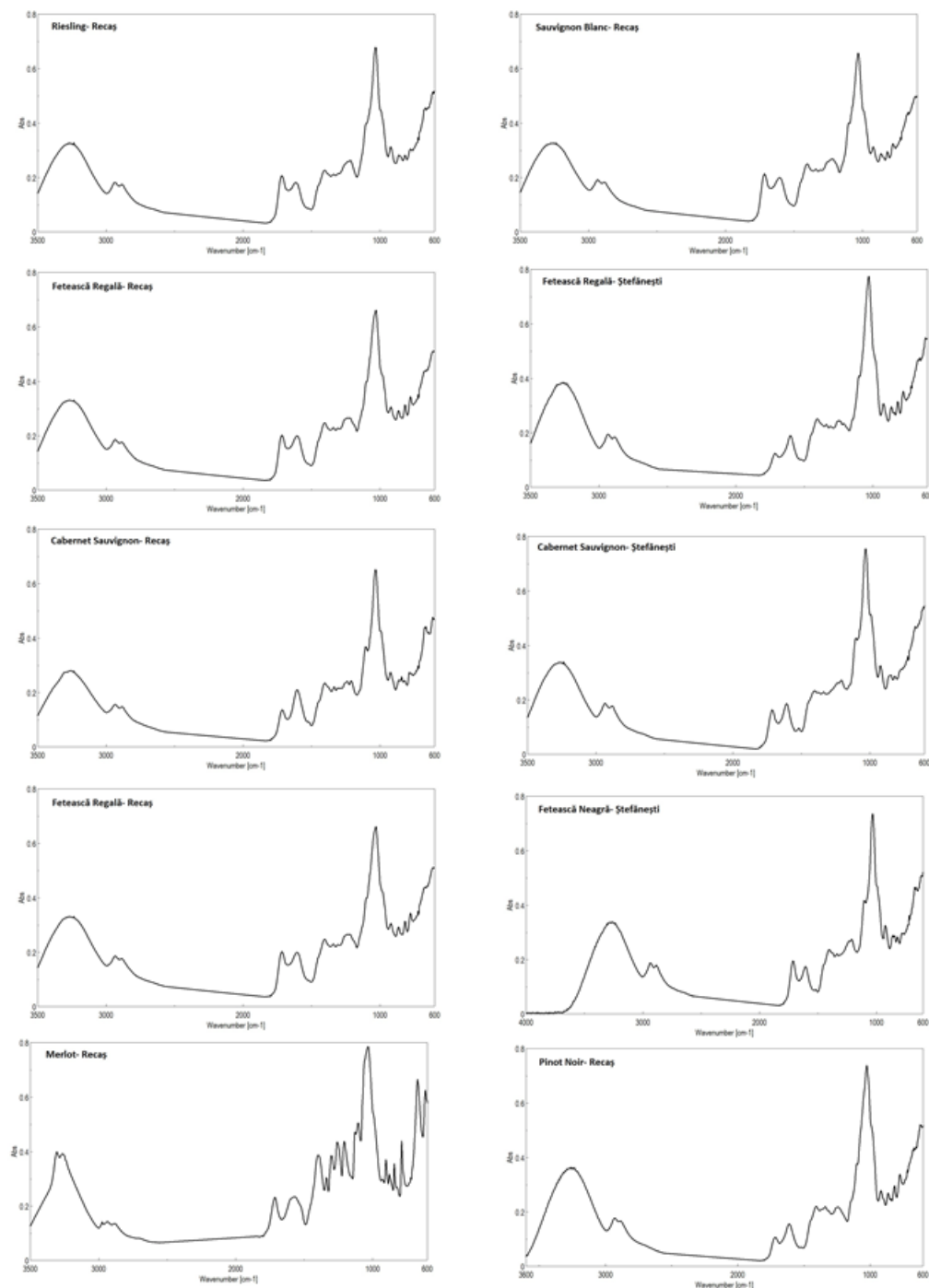


Fig. 2. ATR-MIR spectra registered for all 10 wine samples, in the region 600-3500 cm^{-1}

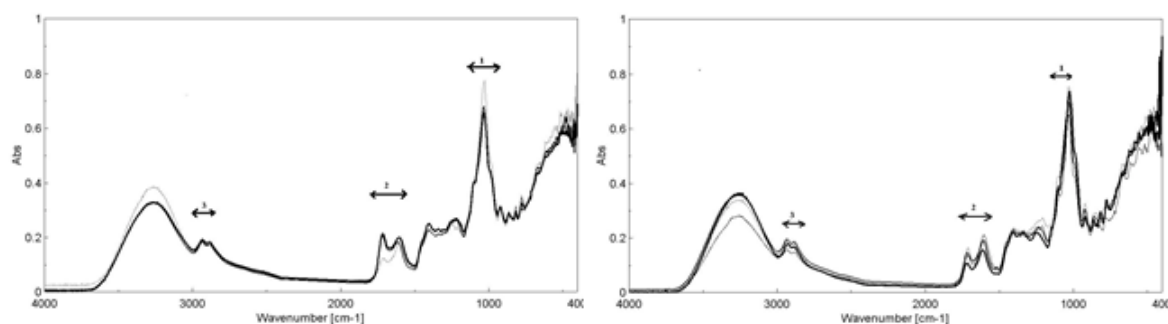


Fig. 3. Overlapped ATR-MIR spectra for the four white wine samples (left) and six red wine samples (right)

vibration bands typical for aromatic molecules, CH bending and CH_2 wagging vibrations, deformation of $-\text{CH}_2-$ groups, stretching vibration of C-O, bending of O-H, can be found in the 900-1680 cm^{-1} region [23].

Table 2 includes the FT-MIR absorption wave numbers, specific to the 3 regions of each wine fingerprinting.

All spectra were characterised by a similar profile. No visual differences were observed between the MIR spectra of wine samples analysed. It was observed that water and

ethanol absorption peaks dominate the spectrum. Chemometric analysis allow the differentiation of wines.

Region 1800-900 cm^{-1} was selected for working range since the RSD (relative standard deviation) between absorption values for the samples were high in this region as shown in figure 4, where an overlay of all investigated samples is presented.

Sample of wine	1 (1000-1200 cm^{-1})	2 (1600-1720 cm^{-1})	3 (2800-3000 cm^{-1})
Fetească Regală-Recaș	1029, 1099	1604, 1715	2884, 2934
Riesling - Recaș	1030, 1100	1605, 1715	2883, 2934
Sauvignon Blanc- Recaș	1030, 1100	1605, 1715	2883, 2934
Cabernet Sauvignon - Recaș	1032, 1103	1604, 1714	2884, 2933
Fetească Neagră - Recaș	1028, 1101	1605, 1715	2884, 2933
Merlot- Recaș	1032, 1105	1637, 1713	2883, 2935
Pinot Noir - Recaș	1024, 1100	1612, 1715	2883, 2929
Cabernet Sauvignon - Ștefănești	1029, 1101	1607, 1714	2882, 2933
Fetească Neagră- Ștefănești	1031, 1104	1607, 1714	2884, 2936
Fetească Regală- Ștefănești	1028, 1100	1601, 1715	2887, 2936

Table 2
ATR-MIR WAVE NUMBERS SPECIFIC TO
THE REGIONS OF EACH WINE
FINGERPRINTING

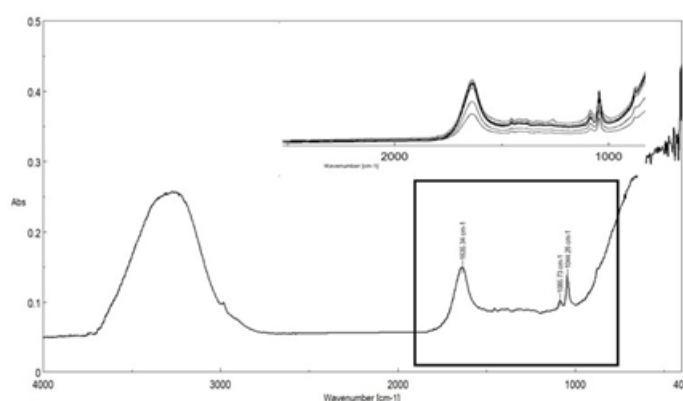


Fig. 4. Typical ATR-FTIR spectrum for a sample of wine (Cabernet Sauvignon-Ștefănești): zoom of the selected wavenumber range with an overlay of all sample of wine

facilitates the understanding of similarities and differences among samples. PCA is a well-established analysis technique which works by finding the correlation between a set of variables and then creating a new set of uncorrelated variables named principal components (PCs). PCA was performed on the MIR spectra to examine qualitative differences within the set of red and white wines related to the two production systems. PCA is a frequently used method and has been successfully applied to the analytical results both for individual compounds and component combinations to establish differences among the grape varieties [2, 24].

Figures 5-7 present the results of PCA analysis and scores. PCA was carried out between the domain of the selected spectral region 1800-900 cm^{-1} for wine (discriminate wine types: red and white fig. 5). The first three principal components (PCs) represent 94% of the total variance (PC1 = 83%, PC2 = 8% and PC3 = 3%). This indicates that these three components were sufficient to provide a clear separation between the groups. The white wine varieties are located at the top right, while the red

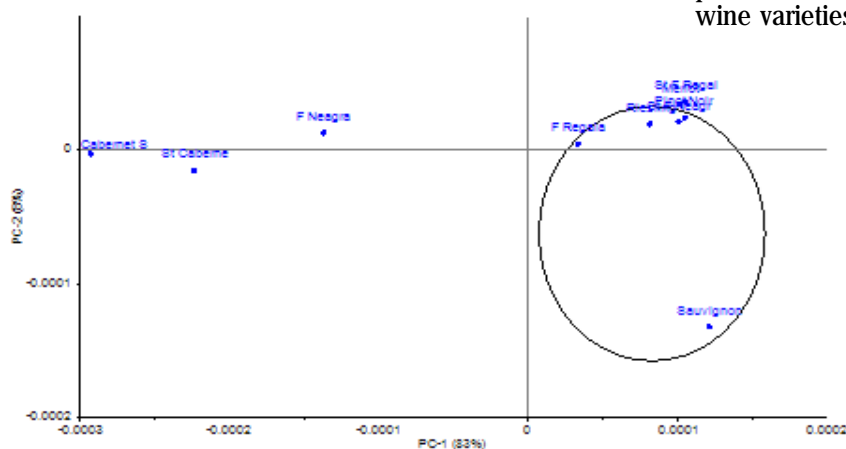
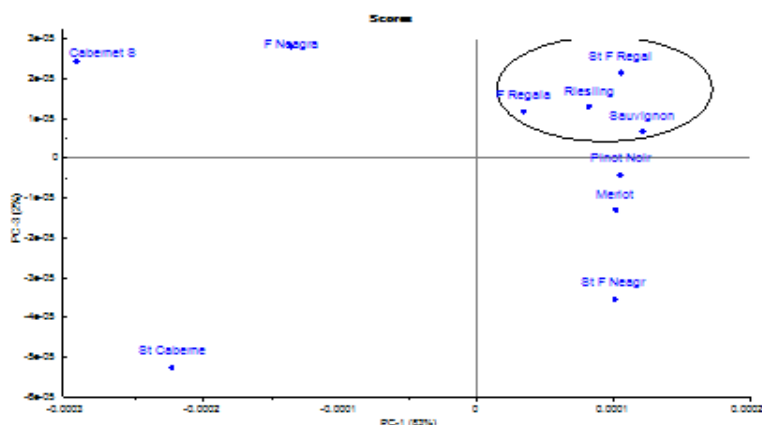


Fig. 5. 2-D scores obtained from PCA of FTIR spectra for the first two PCs based on the FTIR fingerprint region (900-1800 cm^{-1}).



The chemometric analysis. Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) represents one of the most frequently used chemometric tools that

wines are located on the left of the graph. Color is the most important parameter for the categorization of wines based on the first main component. Phenolic content from wine

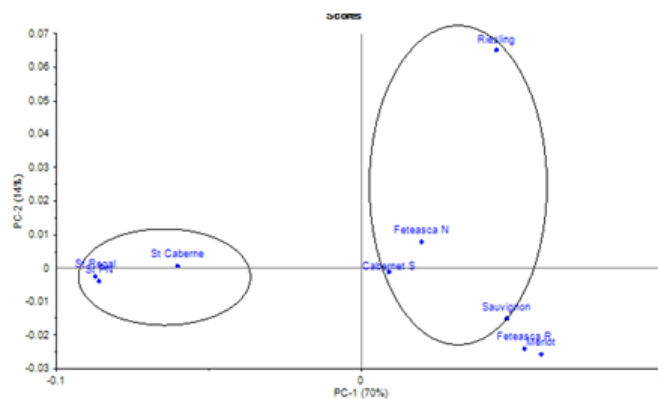


Fig.6. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region specific to acids and their derivatives (1600-1720 cm^{-1})

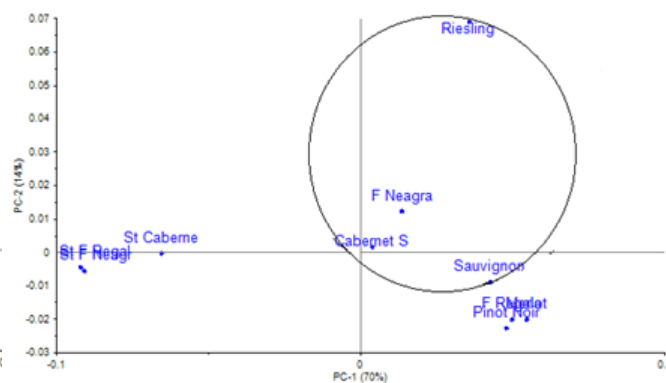


Fig.7. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region specific to carbohydrate derivatives (1000-1120 cm^{-1})

seem to be the determining parameter that could separate the samples according to the wine color.

Figure 7 which represents the scores for the region 1000-1120 cm^{-1} , specific to carbohydrates derivatives, revealed a good clustering of wines based on sweetness index. The first PC explains 70% while the second and the third principle component 14% and 5% respectively of the total variance. The type of wine separates: in the right corner of the diagram are the dry wines (Riesling, Fetească Neagră, Cabernet Sauvignon, Sauvignon Blanc).

Conclusions

This study demonstrated the capacity for ATR-MIR spectroscopy (combined with multivariate analysis) to broadly classify wines. The results demonstrated qualitative compositional differences between red and white, dry and half-dry wines that can be observed by MIR spectroscopy and used to distinguish wines, following PCA.

ATR-MIR could be used as a rapid method of screening, authentication and discrimination between of wine varieties, in the classification of different samples or identification of unknown samples of wine.

The advantages of this method (e.g. fast, non-destructive and with no-sample preparation) allows implementation as an additional control for wine producers in monitoring the wine quality and for the consumers in assessing the authentication with a simple and rapid procedure. The proposed method is promising because it is simple, fast and economical. Therefore, in order to build a more complete model, several sets of samples, including wine for many years and harvests from different wine factories in Romania, would be needed.

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