

Romanian Wines Quality and Authenticity Using FT-MIR Spectroscopy Coupled with Multivariate Data Analysis

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Abstract

Fourier Transform Mid-Infrared Spectroscopy (FT-MIR) combined with multivariate data analysis have been applied for the discrimination of 15 different Romanian wines (white, rosé and red wines), obtained from different origin-denominated cultivars. Principal component analysis and hierarchical cluster analysis was performed using different regions of FT-MIR spectra for all wines. The general fingerprint of wines was splitted in four characteristic regions, corresponding to phenolic derivatives, carbohydrates, amino acids and organic acids, which confer the wines quality and authenticity. By qualitative and quantitative evaluation of each component category, it was possible to discriminate each wine category, from red, to rosé and white colours, to dry, half-dry and half-sweet flavours. The multivariate data analysis based on absorption peaks from FT-MIR spectra demonstrated a very good, significant clustering of samples, based on the four main components: phenolics, carbohydrates, amino acids and organic acids. Therefore, the ATR-FT-MIR analysis proved to be a very fast, cheap and efficient tool to evaluate the quality and authenticity of wines, and to discriminate each wine category, based on their colour and sweetness, as consequence of their biological (cultivar) specificity.

Keywords: carbohydrates and acidity, Fourier Transform Mid-Infrared Spectroscopy, phenolics, Principal Component Analysis, red, rosé and white wines

Introduction

Grapes, grape juices and wines, consumed in moderate quantities, proved to contain a large number of phytochemicals with health benefits promoting effects (German and Walzem, 2000), from essential amino acids, minerals, organic acids, aromas, stilbenes (resveratrol), vitamins, and especially a large variety of pigments (flavonoids and anthocyanins) as well catechins, procyanidins and phenolic acid derivatives (Jackson, 2008).

Therefore, the quality and authenticity of the varietal origin of grapes and wines is of great interest to both the wine industry and the consumer (Cozzolino *et al.*, 2003; Cozzolino *et al.*, 2009). The main secondary metabolites found in grapes, with important roles in plant resistance to pathogens, allelopathy, oxidative stress and plant growth regulation, are the phenolic derivatives (Ogbemudia and Thompson, 2014). These derivatives include monophenolic compounds (phenolic acids, as free or derivatized as esters or glucosides, e.g. benzoic and cinnamic acid derivatives), and

especially polyphenolics (anthocyanins and flavonoids, catechins, procyanidins, and their derivatives) key-biomarkers of grapes and wines' quality, having antioxidant, antimutagenic, antiproliferative and antimicrobial properties (Guerrero *et al.*, 2009). Phenolic acids are derived from benzoic and cinnamic acids and are found as free acids and esters, glycosides or bound complexes, which have also beneficial effects on human health (German and Walzem, 2000; Mateus *et al.*, 2001; Socaciu, 2008).

The modern analytical methods to evaluate phenolic derivatives are usually based on reversed-phase high-performance liquid chromatography (HPLC), or another separation techniques such as gas chromatography or capillary electrophoresis, followed by ultraviolet (UV), electrochemical (EC), fluorescence (F) or mass spectrometric (MS) detection (Lorrain *et al.*, 2013). Commonly, for wines direct analysis or solid-phase extraction followed by reversed phase HPLC-UV-VIS or HPLC-MS methods were used to analyze the phenolic

fingerprint providing detailed information regarding individual phenolics and their quantity, enabling high resolution and accurate measurement of monomeric anthocyanins and catechins (Ginjom *et al.*, 2011), but also the discrimination of cultivars (Muccillo *et al.*, 2014), the influence of fermentation, vinification, maturation during storage, formation of oligomeric and polymeric pigments (García-Falcón *et al.*, 2007; Ivanova *et al.*, 2012). In spite of so many factors of influence, the differences in the overall quality of wines, their phenolic fingerprint still remains characteristic for each cultivar (Avar, 2007; Da Costa *et al.*, 2000; Gruz *et al.*, 2008).

In contrast to chromatography, spectroscopic techniques (UV-Vis, IR), when applied to mixtures, are less selective but may contain information about the complete phenolic composition of the wines under investigation (Gorinstein *et al.*, 1992). UV-Vis spectrometry coupled with infrared spectroscopy, proved to be a cheap and rapid analytical method to be used for wine composition, quality and authenticity evaluation (Linskens and Jackson, 1988; Somers and Verette, 1988).

The use of infrared (IR) spectroscopy for routine analysis of wine began with near infrared spectroscopy (NIRS) as preferred method in the early 1980's (Hashimoto and Kameoka, 2008; Ough and Amerine, 1988). Since that time, the focus for quantitative analysis of grapes and wine has moved towards Fourier transform infrared (FT-IR) technology in the mid-infrared region, since it offers better accuracy in determination and more constituents and properties can be quantified compared to NIRS (Dubernet and Dubernet, 2000; Eichinger *et al.*, 2004; Patz *et al.*, 2004; Soriano *et al.*, 2007). Modern infrared spectroscopic instrumentation is fitted with chemometric software packages that facilitate the establishment of calibration models which can be used to quantify many components simultaneously, thereby reducing the analysis time and cost (Eichinger *et al.*, 2004). FT-IR spectroscopy technology and chemometric techniques for analysis of grapes and wine were implemented in South Africa in the early 2000's (Bauer *et al.*, 2008) and several qualitative and quantitative applications were developed in the last few years.

Near-infrared (NIR), as well as mid-infrared (MIR) spectroscopic techniques combined with multivariate data analysis, proved to be very promising for their good reproducibility in routine analysis (Irudayaraj and Tewari, 2003; Schulz and Baranska, 2007). While NIR absorptions reflect only overtones and combination bands of fundamental transitions, less distinct, the mid-infrared (MIR) absorption bands are related to defined vibrational transitions and are better resolved. The significant improvements in the IR instruments design and auxiliary optics have made modern MIR spectrometers robust for routine applications for liquids, MIR spectra being conveniently recorded using the attenuated total reflection (ATR) technique (Wilson and Tapp, 1999).

The Fourier Transform Infrared Spectroscopy (FT-IR) technique, in combination with chemometrics, is a fast and reproducible technique for identifying the authenticity and adulteration of different food and beverage products (Da Costa *et al.*, 2004). The FT-IR is increasingly used for the authentication of alcoholic beverages (Galignani *et al.*,

2005; Kupina and Shrikhande, 2003) or to verify the authenticity of different fruit juices (Leopold *et al.*, 2011).

During the last two decades, extended studies on grapes and wines were reported, using NIR and FT-IR coupled with biostatistical tools, either to evidenciate saccharides, alcohols or other quality parameters, or their authenticity and traceability, related to origin (cultivar or region) (Chedea *et al.*, 2010; Coimbra *et al.*, 2002; Da Costa *et al.*, 2004; Edelmann *et al.*, 2001; Edenharder *et al.*, 2001; Orbán *et al.*, 2006; Peña-Neira *et al.*, 2000). Using the FT-IR spectra of the wine polysaccharide (1200 and 800 cm^{-1}) of the dry extracts coupled with PCA and CCA chemometric methods, it was possible to discriminate extracts based on the polysaccharide composition. Based on the bands from 1600 cm^{-1} , the polyphenolic content was also evaluated (Gorinstein *et al.*, 1992).

Mid-infrared spectroscopy and UV-Vis spectroscopy combined with multivariate data analysis have been applied also for the discrimination of different red wine cultivars ('Cabernet Sauvignon', 'Merlot', 'Pinot Noir', 'Blaufrankisch', 'St. Laurent', and 'Zweigelt'). Both authentic wines and their phenolic extracts were investigated by ATR-MIR spectroscopy and UV-Vis spectroscopy (Edelmann *et al.*, 2001). The main components (sugar and organic acids) failed to offer satisfactory classification of wines, but by using phenolic extracts, a complete discrimination of all cultivars investigated was achieved.

The FT-IR spectra were also used for the differentiation and classification of wines and brandies during their ageing process, as well as for the characterization and differentiation of distilled drinks from several countries (Palma and Barroso, 2002), good linear regression coefficients (0.995) between the ageing scale and the FT-IR data being obtained (Eichinger *et al.*, 2004).

The oenological wine parameters (alcoholic degree, volumic mass, total acidity, glycerol, total polyphenol index, lactic acid and total sulphur dioxide) were investigated by combined NIRS and FT-MIRS. In this case, NIRS yielded better results, but the use of both methods improved the determination of glycerol and total sulphur dioxide. The validation sets used for developing general equations were built with samples from different "appellation d'origine" (Urbano Cuadrado *et al.*, 2005).

A fast and accurate study was reported on 22 Romanian red wines of 'Cabernet Sauvignon', 'Merlot', 'Fetească Neagră', 'Pinot Noir' and 'Burgund' varieties, comparing $^1\text{H-NMR}$ with the IR spectroscopy (Todasca *et al.*, 2007). By IR spectroscopy, characteristic bands for amino acids (1604 cm^{-1}) and organic acids (1719 cm^{-1}) were investigated, but the method could not provide good differentiations. Meanwhile, the NMR method is considered to be more accurate, but much more expensive.

This study aims to investigate the use of mid-infrared (MIR) and multivariate data analysis techniques to classify Romanian wines produced from autochthonous cultivars. In this respect, the Fourier Transform mid-infrared (FT-MIR) spectroscopy was applied to characterize 15 different Romanian wines (white, rosé and red wines), obtained from different authentic, origin-denominated cultivars, found in different Romanian regions. The wines were investigated by

attenuated total reflectance (ATR) and the most important components (phenolics, carbohydrates, amino acids and organic acids) were localized in specific fingerprint regions of the spectra. Based on the differences between the FT-MIR spectra, by multivariate data analysis (Principal component analysis), were identified the specific discrimination factors useful to authenticate the biological (cultivar) and regional origin, as well their sweetness index.

Materials and methods

Wine samples

Fifteen Romanian wines, including white (n=4), rosé (n=2) and red (n=9), of different sweetness indexes (5 samples dry, 7 samples half-dry and 3 samples half-sweet) were investigated. These wines originated from different cultivars and were produced in ten different wineries from Romania, namely Jidvei, Cotești, Panciu, Huși, Recea, Ceptura, Tohani, Sarica-Niculitel, Murfatlar, and Sadova Corabia. The wines, packed in glass bottles and produced in the vintage years 2008-2012, were purchased from local supermarkets and stored at room temperature until analyzed. Tab. 1 includes data from each wine sample, the specific cultivar, the year of production and 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, then used directly for recording the FT-MIR spectra.

Tab. 1. Numbering of wine samples: white (1-4), rosé (5-6), red (7-15) with references to the sweetness index (dry, half-dry, half-sweet) and the production year

No.	Cultivar (Variety)	Vineyard	Year	Wine type
White wine				
1	Feteasca Alba	Cotesti	2011	Half-dry
2	Feteasca Alba	Jidvei	2011	Dry
3	Feteasca Regala	Jidvei	2011	Half-dry
4	Feteasca Regala	Recas	2012	Half-dry
Rosé wine				
5	Babeasca Rose	Panciu	2012	Half-dry
6	Busuioaca de Bohotin	Huși	2011	Half-sweet
Red wine				
7	Babeasca Neagra	Panciu	2012	Half-dry
8	Babeasca Neagra	Huși	2009	Dry
9	Babeasca Neagra	Sarica-Niculitel	2011	Dry
10	Babeasca Neagra	Sadova Corabia	2010	Dry
11	Feteasca Neagra	Cotesti	2008	Half-sweet
12	Feteasca Neagra	Panciu	2011	Half-dry
13	Feteasca Neagra	Ceptura	2012	Dry
14	Feteasca Neagra	Tohani	2010	Half-dry
15	Feteasca Neagra	Murfatlar	2011	Half-sweet

Fourier Transform Mid-Infrared (FT-MIR) Spectroscopy

The FT-IR spectra were obtained with a Shimadzu IR Prestige-21 spectrometer including a HATR device and an internal reflection accessory (10 reflections) made of Zinc Selenide (ZnSe). The samples were recorded by co-adding 64 scans. Each spectrum was registered from 3500-600 cm^{-1} . As reference, the background spectrum of air was collected. The total phenolic extracts' contents were measured without any preparation, directly on the ZnSe ATR (Attenuated Total Reflection) crystal. Between measurements, the ATR crystal was carefully cleaned using acetone.

Multivariate data analysis

Advanced chemometrics was applied to discriminate between different wine samples, based on their colour, content of phenolic derivatives, amino acids and organic acids, as well as carbohydrates. Therefore, the discrimination was performed using the whole FT-MIR spectra (600-3500 cm^{-1}) as well as the regions identified (1-4) at frequencies of 600-940 cm^{-1} (1), 970-1100 cm^{-1} (2), 1600-1716 cm^{-1} (3) and 2800-3000 cm^{-1} (4). To discriminate among these samples and regions, the principal component analysis (PCA) was applied on the peak areas corresponding to each region, using Unscrambler X 10.1 Software, version 10.1.

The correlation factors were calculated using Origin software (OriginLab, version 8.0).

Results and discussion

FT-MIR spectral fingerprinting of individual wine samples

Fig. 1 exhibits the general FT-MIR spectra of all wine samples, from white (samples no. 1-4), to rosé (samples no. 5, 6) and red wines (samples no. 7-15), according to their list in Tab. 1 in the whole IR region range, from 600 to 3500 cm^{-1} . In all samples, similar spectral features were generally obtained, but with specific quantitative modifications in the fingerprint region (1800-600 cm^{-1}). Comparing the shapes of spectra, there were identified four different regions (1-4) which can show specific differences, related to the colour of wines (white, rosé and red wines), and to the sweetness (dry vs half-dry and half-sweet).

Tab. 2 includes the specific wave numbers and fingerprint regions used to identify the stretching and bending vibrations, depending on the functional groups, as reported in literature (Coimbra *et al.*, 2002; Gorinstein *et al.*, 1992; Todasca *et al.*, 2007). Such data were useful in the interpretation of the FT-MIR absorption peaks obtained in

Tab. 2. Characteristic IR wave numbers (cm^{-1}) for stretching and bending vibrations in wines, according to literature reports (Coimbra *et al.*, 2002; Gorinstein *et al.*, 1992; Todasca *et al.*, 2007)

Fingerprint regions (cm^{-1})	Characteristic frequencies (cm^{-1})	Assignment
< 1000	690, 745, 961, 964	Phosphates, Phenolics, Mono-substituted phenyl derivatives, Unsaturated lipids, incl. carotenoids
970-1100	976, 1030, 1060, 1065, 1079	Glucose, Oligo- and polysaccharides, incl. methoxylated derivatives Alcohols (ethanol) Organic acid stretching vibrations at 1700 cm^{-1}
1500-1716	1566, 1574, 1650, 1662, 1655, 1700	Amide I, II Aromatic ring stretching at 1600 cm^{-1} Stretching vibrations of Amino acids and their derivatives at 1530-1600 cm^{-1} Esters and flavours 1715 cm^{-1}
2800-2935	2854, 2920, 2934	Fatty acids, Polyols (glycerol), Free phenolic acids and catechins, Cholesterol, Carotenoid pigments
3300-3500		OH stretching from water and other hydroxylated molecules (alcohols and phenols)

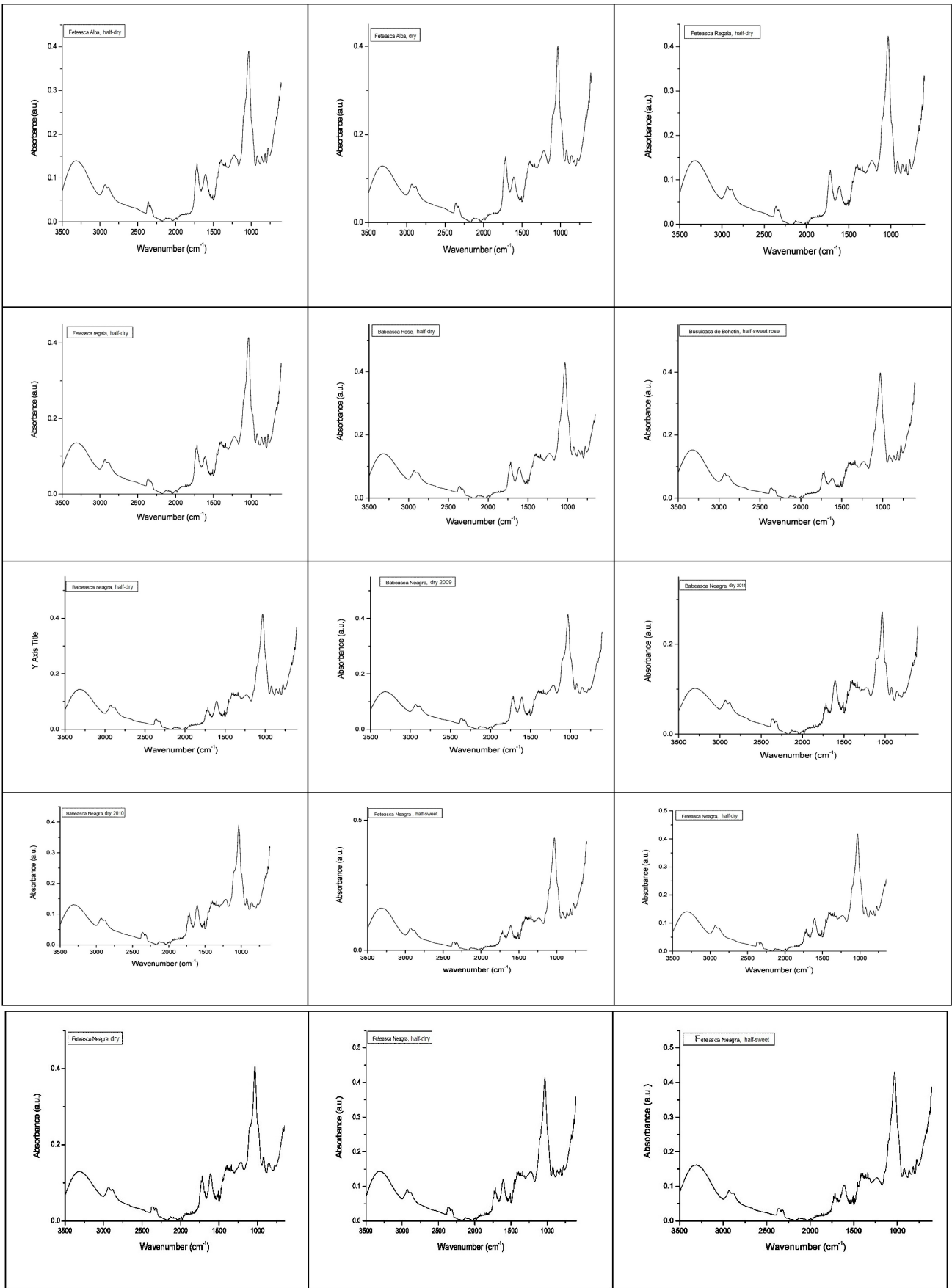


Fig. 1. Individual FT-MIR spectra registered for all 15 wine samples, in the region 600-3500 cm⁻¹

all wine spectra. The region 750-900 cm^{-1} corresponds to absorptions of phenyl/aromatic derivatives, including phenolics. Aromatic derivatives have also OH- stretching bands between 1200-1250 cm^{-1} . The region 1030-1100 cm^{-1} is specific to absorptions of -C-OH groups found in carbohydrates, e.g. 1030 cm^{-1} for glucose and 1060 cm^{-1} for fructose (Leopold *et al.*, 2009). Amino acids like proline, alanine, arginine, glutamic acid, γ -aminobutyric acid absorb at 1608 cm^{-1} and simple phenolics like gallic acid and catechins absorb around 1600 cm^{-1} . The absorption band at 1716 cm^{-1} is characteristic to organic acids found in wines (tartaric, acetic, lactic, citric, malic acids). The absorption band at 2938 cm^{-1} is usually related to polyols, like glycerol and other compounds like flavours. Generally, wines contain water, alcohols, glycerol, sorbitol, mannitol, amino acids, esters, minerals, sulfites, phenols, sugars, organic acids such as tartaric, malic and citric acids, aldehydes, as well as volatile acids as common ingredients (Cerdán *et al.*, 2004). Similarity of the most important components gave rise to similar peak positions in the FT-IR spectra of the studied Romanian wines.

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

According to Fig. 2, representing a generic FT-MIR spectra of wines, it was possible to consider 4 specific regions which can be useful for wines' characterization: region 600-940 cm^{-1} (1) corresponds to phenolics derivatives (including esters), region 2 (970-1100 cm^{-1}) to carbohydrates (glucose, fructose and oligosaccharides, mainly saccharose), region 3 (1600-1716 cm^{-1}) to free amino acids, peptides and organic acids and region 4 (2800-3000 cm^{-1}) for polyols (mainly glycerol). Tab. 3 includes the FT-MIR absorption wave numbers, specific to the 4 regions of each wine fingerprinting: 600-940 cm^{-1} (1), 970-1100 cm^{-1} (2), 1600-1700 cm^{-1} (3). In the 4th region (2800-3000 cm^{-1}), all wines absorbed at the same wave numbers, 2887 and 2931 cm^{-1} . Generally, the range 600-1800 cm^{-1} was considered as "fingerprint region", useful to differentiate wines according to their sweetness index (dry *vs* half-dry and half-sweet). One can notice that in region 1, the absorptions are determined mainly by the polyphenols, region 2 was specific

to carbohydrates, mainly glucose (1033 cm^{-1}), but also fructose and sucrose (1100 cm^{-1}); region 3 was specific to amino acids (1602-1608 cm^{-1}) and organic acids (1700 cm^{-1}).

FT-IR spectra of wines showed absorption bands at different frequencies and these bands can be attributed to the various functional groups. IR spectral peaks are related to the bonds in the compounds, thus they could be assigned to the composition of the specific food sample such as its phenol, alcohol, aldehyde, higher alcohol, polyol, acid, sugar, volatile acid and amino acid content (Lee *et al.*, 2009).

Tab. 4 shows detailed information regarding the values of peak intensities at 1033, 1100, 1600, 1700 cm^{-1} , which can be used as quantitative markers of differences between the individual wines and wine types (based on colour and sweetness index). The ratios 1033/1100 cm^{-1} and 1600/1700 cm^{-1} were also used to characterize quantitatively the sweetness claimed on the label of these wines (dry, half-dry and half-sweet). Fig. 3 represents the differences between mean values registered from peak intensities corresponding to glucose (G), fructose+ sucrose (F+S), amino acids (AA) and organic acids (OA). Characteristic absorbance peaks of sugar components (970-1100 cm^{-1}) as well as amino acids and organic acids (1600-

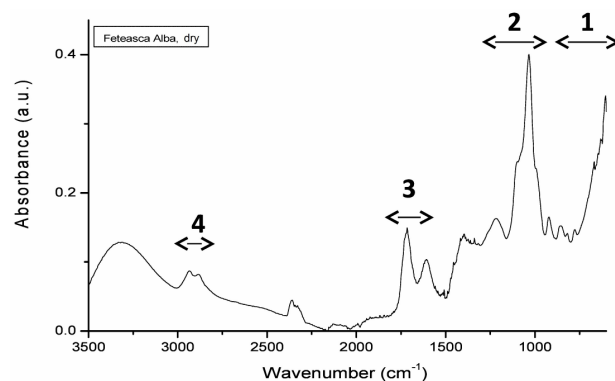


Fig. 2. The FT-MIR spectra of a wine sample: 1 - spectral region 600-940 cm^{-1} ; 2 - spectral region 970-1100 cm^{-1} ; 3 - spectral region 1600-1716 cm^{-1} ; 4 - spectral region 2800-3000 cm^{-1}

Tab. 3. FT-MIR absorption wave numbers specific to the 4 regions of each wine fingerprinting: 600-940 cm^{-1} (1), 970-1100 cm^{-1} (2), 1600-1700 cm^{-1} (3). In the 4th region (2800-3000 cm^{-1}), all wines absorbed at the same wave numbers, 2887 and 2931 cm^{-1}

Wine no.	1 (600-940 cm^{-1})	2 (970-1100 cm^{-1})	3 (1600-1700 cm^{-1})
1	630, 777, 817, 864, 920	1033	1602, 1716
2	775, 817, 854, 921	1033	1608, 1716
3	605, 667, 777, 817, 862, 920	1031	1602, 1716
4	630, 779, 817, 864, 921	1033	1608, 1716
5	603, 775, 817, 860, 920	1031	1602, 1716
6	630, 775, 815, 864, 898, 898	1028	1608, 1716
7	603, 630, 775, 817, 862, 920	1033	1602, 1716
8	605, 630, 775, 817, 923	1033	1608, 1716
9	771, 854, 923, 995	1033	1602, 1716
10	771, 854, 923	1033	1608, 1716
11	775, 815, 862, 920	1033	1602, 1716
12	603, 775, 815, 862, 921	1033	1608, 1716
13	607, 630, 775, 854, 921	1033	1602, 1716
14	605, 775, 817, 862, 921	1033	1608, 1716
15	603, 775, 815, 862, 920	1033	1602, 1716

Tab. 4. Peak intensities which can be used as quantitative markers of differences between the individual wines and wine types (based on colour and sweetness index)

Wine no.	1033 cm ⁻¹ (glucose)	1100 cm ⁻¹ (fructose and sucrose)	1033/1100	1600 cm ⁻¹ (amino acids)	1700 cm ⁻¹ (organic acids)	1600/1700	Wine type
1	0.3901	0.246	1.5858	0.1081	0.1332	0.812	Half-dry
2	0.4006	0.242	1.6554	0.1038	0.1494	0.695	Dry
3	0.4233	0.2387	1.7734	0.0853	0.1223	0.697	Half-dry
4	0.4142	0.2534	1.6346	0.0986	0.1299	0.759	Half-dry
5	0.43052	0.2378	1.8104	0.0974	0.1151	0.846	Half-dry
6	0.3978	0.2125	1.8720	0.0633	0.0854	0.741	Half-sweet
7	0.4154	0.2265	1.8340	0.1018	0.0777	1.310	Half-dry
8	0.414	0.2495	1.6593	0.1163	0.1206	0.964	Dry
9	0.2714	0.169	1.6059	0.1192	0.0685	1.740	Dry
10	0.3903	0.2388	1.6344	0.1290	0.1045	1.235	Dry
11	0.4318	0.2347	1.8398	0.0952	0.0765	1.244	Half-sweet
12	0.4183	0.2345	1.7838	0.1163	0.0785	1.481	Half-dry
13	0.4039	0.2518	1.6041	0.1237	0.1183	1.045	Dry
14	0.4135	0.2385	1.7338	0.1196	0.0955	1.252	Half-dry
15	0.4282	0.2291	1.8691	0.1044	0.0790	1.322	Half-sweet
Mean values (MV)	0.4028	0.2335	1.7263	0.1054	0.1036	1.0762	

1716 cm⁻¹) appeared and the intensity of these peaks increased with the increase of sugar and acid content. Thus, according to the characteristic peaks in the infrared spectra in the range of 970-1100 cm⁻¹ and 1600-1716 cm⁻¹, dry, half-dry and half-sweet wines can be divided according to the amount of sugar, amino acids and organic acids, that can be evaluated in accordance with such spectra. Tarantilis *et al.* (2008) also reported differentiation between wine samples based on small differences between spectra of their phenolic extracts, the spectral region between 1800-900 cm⁻¹ being chosen for wine fingerprinting. Also, Chen *et al.* (2009) have achieved discrimination between dry and sweet red wine samples using 2D correlation spectroscopy and MIR.

Multivariate Data Analysis by Principal Component Analysis

PCA was performed on the MIR spectra, to examine qualitative differences within the set of red, rosé and white wines. Figs. 4-7 present the results of PCA analysis and

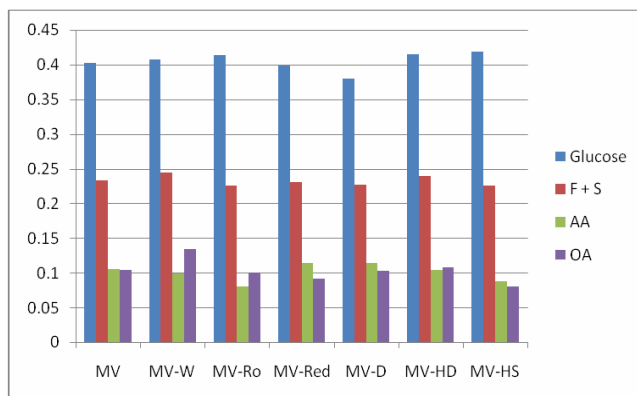


Fig. 3. Mean values of peak intensities registered for: glucose, fructose and sucrose (F+S), amino acids (AA) and organic acids (OA). Abbreviations: MV-mean value; D-dry, HD-half-dry; HS-half-sweet; W-white; Ro-rosé

scoring, to discriminate between wine types, based on the general FT-IR fingerprint (Fig. 4, from 600 to 1800 cm⁻¹) considering as well the three regions of IR absorption specific to phenolic derivatives (Fig. 5, 750-940 cm⁻¹), carbohydrate derivatives (Fig. 6, 970-1100 cm⁻¹) and amino acids and organic acids (Fig. 7, 1600-1716 cm⁻¹).

As presented in Fig. 4, for the whole fingerprint region, the first principal component (PC1) explained 68% of the variability and the second principal component (PC2) explained 22% of the variability; while together PC1 and PC2 explained 90% of the whole variability of wine samples and showed a good similarity for white wines (grouped in the positive quadrante), also for rosé wines (close in the inferior quadrante). Red wines were more heterogeneous in the composition and were spread, not clustered in a specific group. Nevertheless, the red, half-sweet wines (11 and 15) were close to the rosé wines, which proved to be superior in sweetness.

As presented in Fig. 5, for the region 750-940 cm⁻¹, specific to phenolic derivatives, the first two components

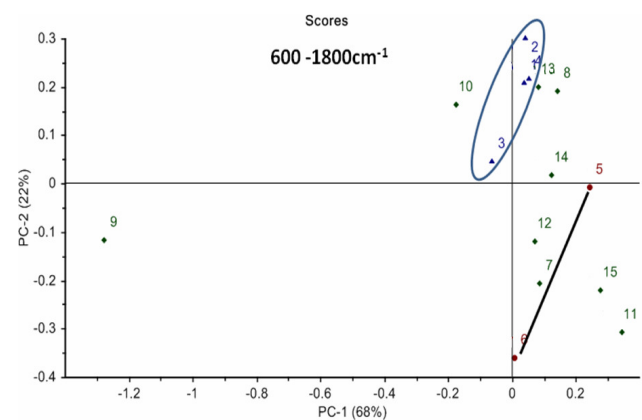


Fig. 4. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region (600-1800 cm⁻¹). Sample numbering as in Tab. 1

(PC1 and PC2) together, explained 98% of the whole variability of wine samples. Although the variance explained was high (98%), a weaker clustering of wines based on their different colours was obtained. In this case, the wines were discriminated along the axis PC2, depending on the type of wine to which they belong (white, rosé or red), the three groups being partially overlapped with each other. Such overlaps are due to the closer relationship between some of wines which come from the same vineyard (eg. sample no. 1 overlapped red wines, close to the sample no. 11) or the same manufacturer (eg. sample no. 5 overlapped red wines, close to the samples no. 7 and 12). A weaker clustering of wines can also be explained by the fact that PCA is sensitive to the number of samples in the data set and it requires relative equality in group sizes to adequately discriminate between groups with very similar characteristics (Bevin *et al.*, 2008).

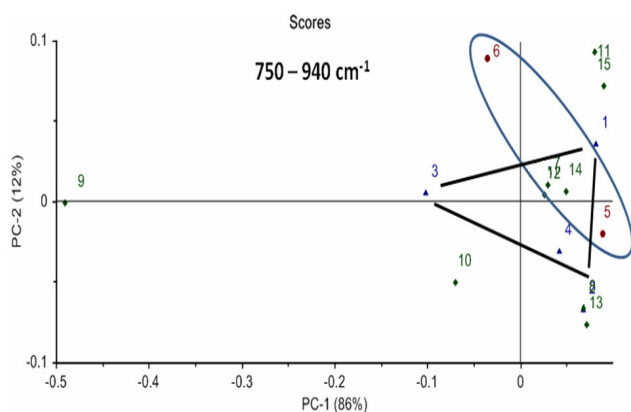


Fig. 5. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region specific to phenolic derivatives (750-940 cm^{-1}). Sample numbering as in Tab. 1

Fig. 6, representing the scores for the region 970-1100 cm^{-1} , specific to carbohydrates derivatives, revealed an excellent clustering of wines, not only based on colour, but especially considering their sweetness index, excepting wine no. 9. The first principal component (PC1) explained 90% of the variability and the second principal component (PC2) explained 7% of the variability; together, PC1 and PC2 explained 97% of the whole variability of wine samples.

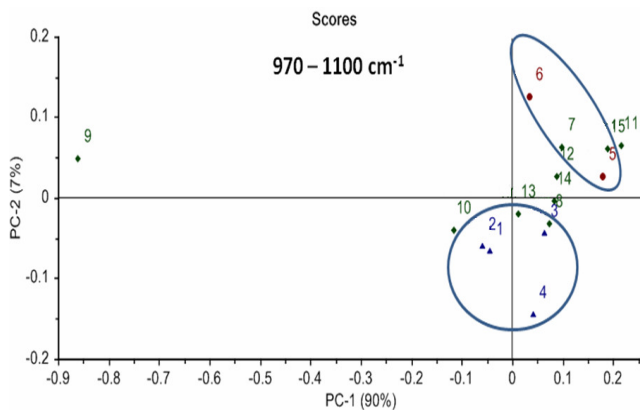


Fig. 6. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region specific to carbohydrate derivatives (970-1100 cm^{-1}). Sample numbering as in Tab. 1

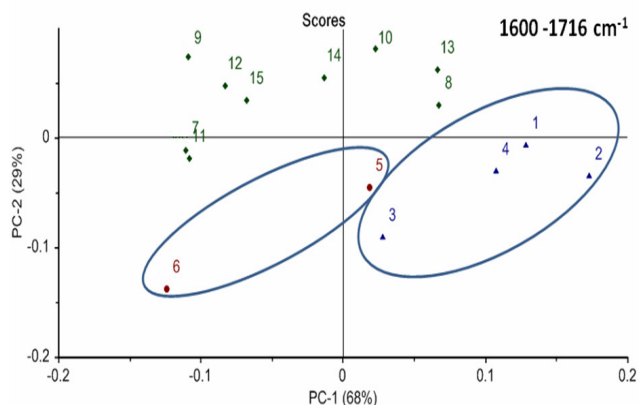


Fig. 7. The PCA scoring to discriminate between wine types, based on the FTIR fingerprint region specific to amino acids and organic acids derivatives (1600-1716 cm^{-1}). Sample numbering as in Tab. 1

Fig. 7 represents the scores for the region 1600-1716 cm^{-1} , specific to amino acids and organic acids derivatives. In this case, the first principal component (PC1) explained 68% of the variability and the second principal component (PC2) explained 29% of the variability, together PC1 and PC2 explaining 97% of the whole variability of wine samples and suggesting a good clustering based on acidity. Thus, we can notice higher PC1 values for white wines (samples no. 1-4, clustered in the positive quadrants of PC1 and negative quadrants of PC2), followed by rosé wines (samples no. 5-6, clustered in the negative quadrants of both PC1 and PC2), being more acidic, compared with red wines, less clustered and located all along the first axis (PC1), in the upper part of the plot, in the positive quadrants of PC1 and PC2. Interestingly, samples no. 8, 10, 13, excepting 9, were more acidic, belonging to the dry wines category.

The combination of MIR and chemometric techniques presented in this study allowed almost complete discrimination between samples. Although some samples did overlap, generally, a separation was observed between samples, according to their colour and sweetness index, which demonstrates that the high information content of MIR spectra has been used successfully for the fast classification of different types of wine. The ability of the MIR model to discriminate or identify wine samples was based on the vibrational responses of chemical bonds to MIR radiation (Cozzolino *et al.*, 2009). As has been reported before by Zhang *et al.* (2010), using PCA as pre-processing method, dry and sweet wine samples can be classified correctly. The same authors suggested that the identification of glycerol, carboxylic acids or esters by MIR spectra analysis could serve to discriminate the different manufacturers and evaluate the quality of wine samples. Also, Cozzolino *et al.* (2009) have managed to achieve discrimination, within a set of red and white Australian wines, according to the two production systems (organic *vs* non-organic), although some samples were overlapped. In contrast, Bevin *et al.* (2008) have not succeeded to adequately discriminate 73 white wines belonging to different varieties, by means of the PCA score plot.

Finally, we can point out that multivariate data analysis showed that all four components of wines (phenolics,

carbohydrates, amino acids, organic acids) which contribute to the colour, sweetness and acidity, are responsible for the good, significant clustering of these wines. Although some of the wines were of the same variety, they came from different manufacturers; therefore, the PC plots of FT-IR data showed that the components of these wines are different and wines are clustered in their own individual groups, based on the colour and sweetness, and are apparently differentiated by their geographical origin.

Conclusion

This study has demonstrated that mid-infrared spectroscopy, coupled with principal component analysis represents a very powerful tool for distinguishing groups that have very similar properties, but have consistent overall differences, and might be used as a technique for the discrimination between different red, rosé and white wine varieties.

The results suggested that mid-infrared spectroscopy is an overall and effective method that offers the possibility to evaluate the authenticity of wines, without the need for costly and laborious chemical analysis.

The use of this fast technique can offer benefits for the wine industry by being a robust rapid screening tool for the discrimination of different types of wine, based on their colour and sweetness, and also being capable of measuring wine quality and assuring consumers of the quality of the final product to be enjoyed.

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