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ROMANIAN WINE STUDY USING IR SPECTROSCOPY IN COMPARISON WITH ^{1}H-NMR

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Determinarea compozţiei vinului într-un timp cât mai scurt şi cu rezultate cât mai exacte şi reproductibile este o problemă ce revine mereu în actualitate pe măsura dezvoltării tehnicilor de analiză. În această lucrare s-a urmărit compararea metodei de analiză a compoziției vinurilor româneşti utilizând ^{1}H-RMN cu spectroscopia IR, cunoscut fiind faptul că spectroscopia IR este mult mai accesibilă şi mai rapidă. Au fost analizate şi metodele specifice de dozare a componentelor din vin prin integrarea benzilor de absorbție din spectrul IR, specifice diferitelor grupe de substanțe organice. Au fost supuse acestui studiu 22 de probe de vin roşu românesc din soiurile: Cabernet Sauvignon (CS), Merlot (M), Fetească Neagră (FN), Pinot Noir (PN) şi Burgund (Bu).

A faster wine composition study and with more accurate results is a current challenge since analytical methods are developed. In this paper we compared the method for wine analysis, ^{1}H-RMN, with the IR spectroscopy. It is well known the fact that the IR spectroscopy is more accessible and faster then the ^{1}H-RMN method. There were also analyzed the specific methods for quantitative measurement by the integration of absorption bands from the IR spectrum, specific for different organic compound groups. There were studied 22 red wine samples of Cabernet Sauvignon (CS), Merlot (M), Fetească Neagră (FN), Pinot Noir (PN) and Burgund (Bu) varieties.

Keywords: wine composition, IR, ^{1}H-NMR

1. Introduction

The wine composition analysis is a constant concern since the authentication of wine is required by the European Union and by more demanding and cultivated consumers. This requirement appeared since the diversification of the wine producers and of the more or less correct winery techniques.

Romania has an old tradition in producing wine; some of the earlier signs appeared in Dacian's times. Today Romania is the sixth European wine producer, so faster and accurate methods of analysis for the Romanian wine are mandatory.

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In some earlier studies of Romanian wines [1] we established a method used for the identification and the quantitative measurement of some compounds usually found in wines, based on the ^{1}H-NMR spectroscopy. Through the identification and the quantitative measurement of 14 minor components, we obtained “fingerprints” for several Romanian wines. These fingerprints were used to establish wine identity.

Researchers working in the field of food chemistry and especially in wine analysis, from different countries, focus their efforts in the authentication of their national wines. Studies are conducted using the ^{1}H-NMR not only for wine (the final product) but as well for must (raw material) during his fermentation process. [2-9]

The results obtained using ^{1}H-NMR are very good, but in the same time the accessibility of an NMR instrument can be a little bit difficult. Following that idea we evaluated the possibility of transferring the method for wine analysis which implies the ^{1}H-NMR method to another one that uses the IR spectroscopy which is more easily reached.

The IR spectroscopy had some applications in wine or other beverages analysis. IR was used for detection of ethanol in wine [10] and some other beverages [11] and as well FT-IR spectra were employed 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 producing countries. [12]

Another group of researchers used the region of wavenumbers 1200–800 cm^{-1} of the FT-IR spectra for wine polysaccharides quantification. [13]

2. Experimental part

Twenty two red wine samples from Cabernet Sauvignon (CS), Merlot (M), Fetească Neagră (FN), Pinot Noir (PN) and Burgund (Bu) varieties, produced in Romania were subjected to this study.

An important problem while recording IR spectra of wines is due to the existence of a large amount of water in wine samples, generating an absorption band which overlaps other existing bands in the spectrum, making them difficult to identify. For this reason, wine samples can’t be directly subjected to analyze, but only after a preliminary treatment leading to water removal from the sample. Therefore, IR spectra of wine samples were recorded only on the wine evaporate, obtained from 5 mL of wine subjected to evaporation on an electric heater for 4 hours at 60ºC.

Following the procedure used for identification and quantitative measurement of the compounds present in Romanian wines by the ^{1}H-NMR spectroscopy, first step in the analysis of the IR spectra of wine evaporates was to establish characteristic bands for some of the major compounds of wine. Thus, spectra of several compounds existing in wine were analyzed by superposition with the IR spectrum of a wine evaporate. Due to the fact that in the IR spectrum the compounds can't be differentiated one from another like in the ^{1}H-NMR spectrum, because their bands are not well separated, they were analyzed by groups.

Organic acids such as acetic, lactic, citric, malic and succinic acid were analyzed first. The band at 1719 cm^{-1} was chosen to highlight the organic acids usually existing in wine (Fig. 1). The intensity of this band will be used in the following computation.

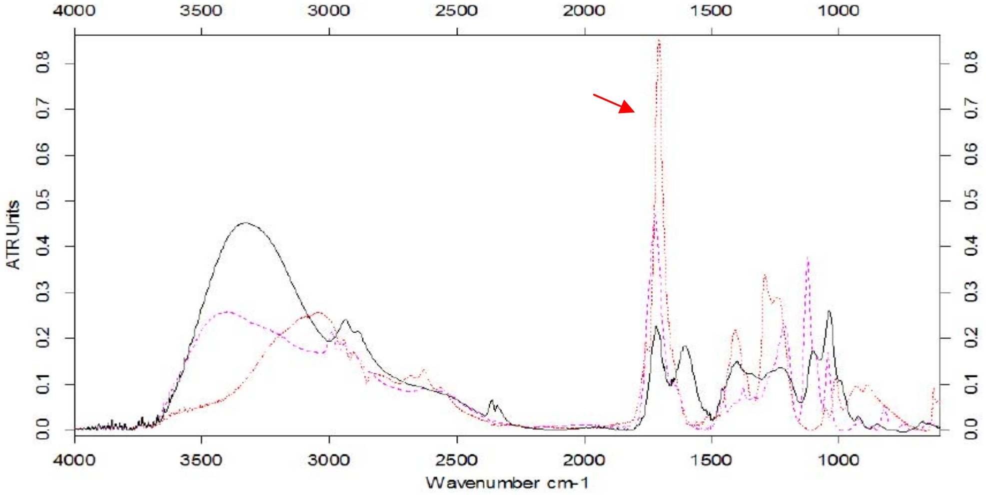


Fig. 1. IR spectrum of a red wine evaporate (continuous black line), overlaped with acetic acid and lactic acid spectra (dot lines).

Next there were analyzed the amino acids normally occurring in wine, which were identified and measured by the ^{1}H-NMR method.

It was noticed that all aminoacids display in their IR spectra the band at 1604 cm^{-1}, and so this band was chosen to distinguish amino acids in wine evaporates and used in computation (Fig. 2).

Fig. 2 shows the IR spectrum of a wine evaporate overlaped with the IR spectra of aminoacids (alanine, valine, arginine).

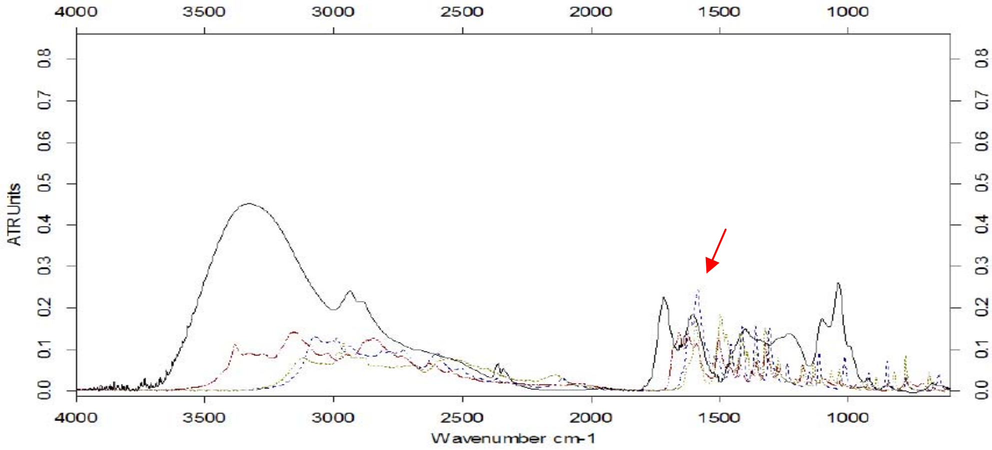


Fig. 2. IR spectrum of a red wine evaporate (continuous black line), overlaped with aminoacids IR spectra (dot lines).

Next there were studied the absorption bands of glycerin. Two characteristic zones for glycerin were noticed. The band at 2938 cm^{-1} usually appears in IR spectra of polyols, but the major polyol in wine is glycerin, that's why this band can be assign to this triol (Fig. 3).

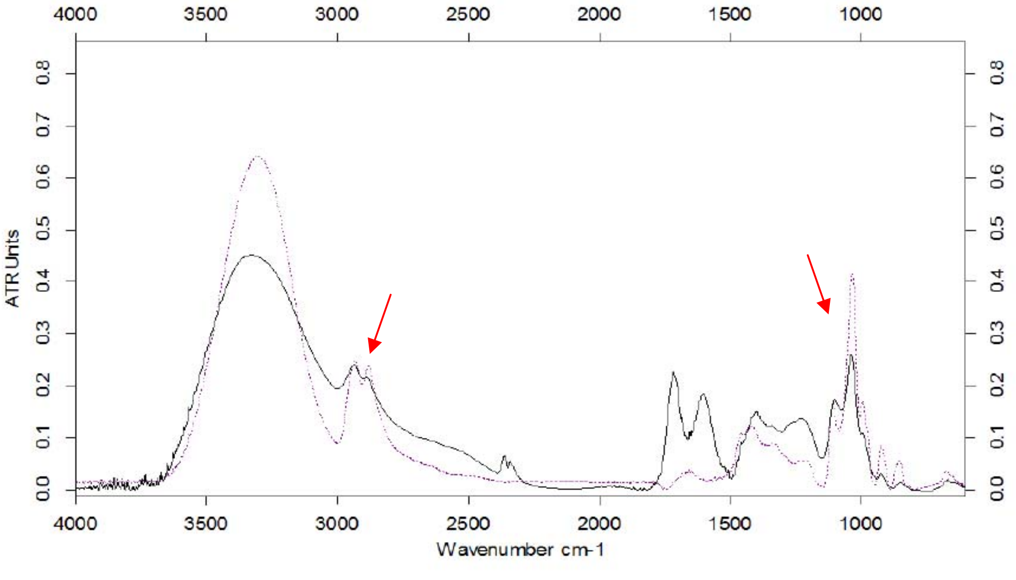


Fig. 3. IR spectrum of a red wine evaporate (continuous black line), overlaped with glycerin IR spectrum (dot line).

There is another band associated to glycerin, at 1039 cm^{-1}, but it also appears in the IR spectrum of the common monosaccharides.

In this way there were identified several IR bands generated by different compounds occurring in wine evaporates. Fig. 4 shows the main absorption bands of the usual components of wine.

In the IR method there are less absorption bands assigned to specific compounds than signals established as markers in the ^{1}H-NMR method [1]. Therefore the IR method can be used only for the rapid determination of concentrations for different classes of compounds using the absorption bands previously mentioned about, but it doesn't provide necessary data in order to differentiate wine samples.

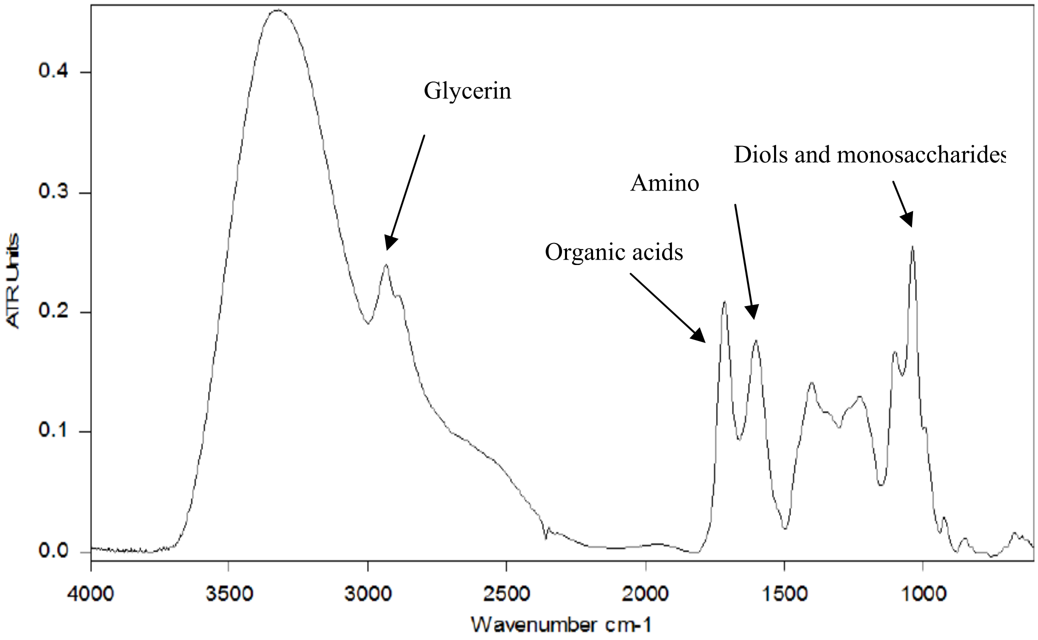


Fig. 3. IR spectrum of a red CS variety wine evaporate.

The computation of some wine components concentration is based on the characteristic bands previously identified correlated with the concentrations of different components determined by the ^{1}H-NMR method applied on wine evaporates. OPUS software package used to record IR spectra can also be used in order to obtain some calibration equations. These equations are further used to rapidly determine the concentration of different classes of compounds, based only on IR data.

By using the characteristic absorption bands described in the experimental part, a 10 sample batch of Romanian wine evaporates, CS variety, were used and different calibration equations were obtained.

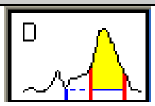
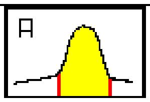
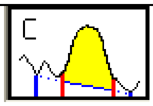
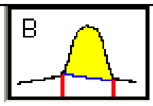
1) Thus, using the characteristic band of glycerin (at 2938 cm^{-1}) it can be obtained the linear dependence between the integral value of the glycerin IR absorption band and the concentration of this compound obtained from the ^1H-NMR spectra recorded on the same wine evaporate.

Several calibration equations depending on the integration procedure are obtained. All calibration equations are linear with variable correlation coefficients, presented in Table 1 in their increasing order.

$$Y = a + bX$$

Table 1.

Calibration equations obtained depending on the measurement type of the IR integral

Current No.	Integration procedure	a value	b value	Correlation coefficient
1.		+69.8	-1.3	0.1
2.		+300	-5.78	0.4
3.		-46.2	16.4	0.7
4.		-49.3	17.7	0.8

It can be noticed that in the fourth case – procedure B in IR spectrum – the best correlation index between spectra used for the calibration curve is obtained.

By using the previously defined calibration curves in order to determine the glycerin concentration in a red wine FN sample, the values presented in Table 2 were obtained.

Table 2.

Glycerin concentrations computed by the fourth calibration equations.

Integration procedure	Computed glycerin concentration (theoretical) mmol/L	Glycerin concentration (practical) mmol/L
C	63.35	65.75
D	66.64	
A	68.85	
B	61.49	

Because of the fact that the best correlation was obtained by using the B procedure of integration, the other identified absorption bands were analyzed by using this kind of band integration.

The band at 1039 cm^{-1}, corresponding to polysaccharides and polyols (glycerin and 2,3-butanediol), was investigated too. Since there are no measurable traces of monosaccharide in the analyzed CS wines, we can assume that this band is only due to glycerin and 2,3-butanediol.

In this case, a calibration equation of this type was obtained:

$Y = -26.291 + 4.629X$, with a correlation factor of 0.89.

2) The absorption band characteristic to organic acids (at 1719 cm^{-1}) was also investigated. Since the bands of different acids are overlapped in the IR spectrum, organic acids in wine can't be differentiated one another, so they will be globally reported as "total acidity".

In order to obtain the calibration curve, the concentrations of the organic acids identified and measured according to the ^{1}H-NMR method were totalized, for each wine evaporate sample.

The following calibration equation was obtained:

$Y = +18.17 + 1.02X$, with a correlation factor of 0.53. The correlation factor is much lower in comparison with the other compounds correlation factors possibly because of the slightly different pH values of samples.

3) The characteristic band for amino acids (1604 cm^{-1}) was also investigated. In this case were totalized, as well, the concentration values obtained by the ^{1}H-NMR method for different isolated and measured amino acids.

The calibration equation in this case is:

$Y = +38.34 - 1.1X$, the correlation coefficient in this case was enhanced (0.88) for this group of spectral data using the B integration method.

In the light of things presented above we can conclude that the IR spectroscopy offers only 3 values, which are not enough for discrimination between varieties of wine. But due to the fact that IR is a very accessible and fast method, it can provide valuable information that combined with other physical methods can be a useful tool for the discrimination of wines.

3. Conclusions

In conclusion, the IR spectroscopy, in spite of being rapid and relatively cheap, it doesn't provide enough and relevant information to specifically assign wines. It can only be obtained semi quantitative information regarding the content of related compounds in wine samples. But correlating the ^{1}H-NMR spectroscopy results with the IR spectroscopy a quick method for wine analysis can be established offering good results for routine analysis.

4. Acknowledgments

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