ATR-FTIR Spectroscopy Coupled with Chemical and Chemometric Analysis to Distinguish Between Some Sweet Wines

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FTIR Spectroscopy correlated with some chemical characteristics and chemometric analysis have been applied to distinguish between sweet wines obtained from different Romanian varieties and Canadian icewine. Chemical analyses differentiate the two categories of origin in terms of sugar content, acidity and total polyphenol content but are expensive and time-consuming. 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, carbohydrates, polyols content and geographical origin. FTIR spectroscopy coupled with chemometry is a profitable technique for distinguishing between different wines and validates the results obtained by chemical analysis.

Keywords: sweet wine; mid-infrared spectroscopy; principal component analysis, sugar content, icewine

Grape wine has a very complex composition (hydroxy compounds, carboxylic acids, nitrogenous compounds, aldehydes, vitamins, enzymes and many mineral substances) and a relatively low content of ethyl alcohol (less than 15% V/V) so it is the healthiest alcoholic drink for the human body. The very complex chemical composition makes it impossible to classify wines according to rigorous scientific criteria. On the other hand, the chemical composition of a wine is unique not only due to its variety footprint, but also to the geoclimatic conditions and winemaking technology. [1, 2].

Sweetness of a wine is determined by the level of residual sugar, remaining after fermentation stops, in balance with alcohol level, acidity and polyphenol content. According to EU regulation 753/2002 a wine containing more than 45g/L residual sugar is considered sweet [3, 4].

The technology of sweet wine production typically involves the dehydration of grape berries in order to increase the sugar concentration. This can be done in different ways. For example, in Tuscany, Italy, the grapes are dehydrated in specially places under shade and controlled temperatures, while in other Mediterranean countries (Cyprus, Greece, Spain, Turkey) are left to air dry, in open spaces. In France (Bordeaux) or Hungary (Tokay) the grapes are attacked by Botrytis cinereal [5].

In cold regions such as those in Canada, Germany, Austria or the United States, the high level of sugar in the famous icewines is getting by freezing the grapes or the grape juice. For these wines, the highly concentrated must is extracted from the frozen berries under pressure condition. The entire harvesting and pressing process must be carried out below -8°C. Juice will have anywhere from 32-46 Brix [6]. Icewine juice is concentrated in all soluble solids, including glucose and fructose, as well as acids and nitrogenous compounds. The management of fermentation biotechnology is very difficult and the fermentation lasts 2-6 months. However, these wines are included in top international level quality premium wines due to their sensory quality and longevity [7].

In Romania, sweet wines with a registered designation of origin (DOC) are obtained by delayed harvest, being classified as: DOC-CMD (full maturity harvest), DOC-CIB (berries ennoblement harvest) and DOC-CT (delayed harvest). Considering the amount of residual sugar, they can be: semi-sweet, with 12.1 - 50 g/L of residual sugar; sweet, with more than 50 g/L of residual sugar. These sweet wines are obtained in different vineyards, from some varieties such as Busuiocaua, Muscat Ottonel, Tamaioasa Romaneasca, Grasa de Cotnari, sometimes Chardonnay and Pinot Gris, resulting wines for which sugar and acidity harmonize in the happiest way [8].

For the classification and characterization of sweet wines, numerous techniques and analytical methods can be used: the metallic content [9], volatile fraction [10, 11], aromatic profile [12]. Multivariate analysis has been suggested to differentiate or authenticate wines according to their geographical origin or grape variety using based on the compositional profiles [13].

Some fast, low cost and environmentally friendly methods to characterize the different categories of wine was developed by using FTIR-ATR spectroscopy combined with multivariate analysis [14] or mid-infrared (MIR), near-infrared (NIR), visible (VIS) and ultraviolet (UV) spectroscopy and chemometrics [15, 16].

This study aims to investigate some of the chemical characteristics and to distinguish between sweet wines obtained from different native and Canadian varieties by correlating results of chemical analyses (sugars, pH, titratable acidity, total polyphenols and antiradical activity) with FTIR and chemometric analysis.

Experimental part

Wine samples

Eight samples of sweet commercial wine were analysed, five of which were certified in Romania (Cotnari and Mehedinti vineyard) and the other three were icewine produced in Canada.

Table 1 presents some characteristics of wine samples as: name (variety), origin, type of certification and way of obtaining (full maturity harvest-CMD, berries ennoblement harvest-CIB or delayed harvest-CT), year of production, organoleptic properties and ethanol content.

Sugar content

The sugar was determined by the refractometric method using a DigitaleHandrefraktometer DR201-95 which...
calculate the content in Brix degrees (% sucrose, m/m) based on the index of refraction.

Acidity
The acids play a considerable role in wine quality. There are different organic acids in wines, and each of them contributes differently to total wine acidity or indirectly, to wine colour and stability [17].

Titrable acidity (TA) was determined by titration using a 0.1N NaOH solution (known correction factor), using phenolphthalein as a colour indicator. The results were converted and expressed in g / L tartaric acid using the relationship:

$$A_{\text{tartaric acid, g/l}} = 0.075 \times \frac{V_{\text{NaOH}} \times f_{\text{NaOH}} \times C_{\text{NaOH}}}{V_{\text{sample}}} \times 1000$$

where: 0.075 is the amount of tartaric acid corresponding to 1 meq H+/L, $V_{\text{NaOH}}$ is the volume of NaOH solution with the concentration $C_{\text{NaOH}}$ and correction factor $f_{\text{NaOH}}$ used in the titration and $V_{\text{sample}}$ is the volume of sample taken in the analysis.

Actual acidity or real acidity expressed by pH was measured using an Inolab 730 pH-meter.

Total polyphenols content
The total polyphenols content (TPC) was determined by the Folin-Ciocalteu method, that involved the reduction of the F-C reagent by the phenolic compounds in wine with the formation of a blue complex which has a maximum of absorption at 760 nm. The absorbance was interpolated and expressed in gallic acid on a calibration curve obtained with a series of 12.5 - 150 µg/mL gallic acid solution and describe by a linear regression equation. The results were obtained as an average of three consecutive determinations and read at a UV-Vis Jasco 730 spectrometer.

By multiplying the absorption values by 20, in the case of white wines, and by 20 x dilution factor, for the red ones, we have the total polyphenols index (TPI) that allows to classify the wines in: supple taste wines (TPI < 30), well-formed wines (30 < TPI < 50) and astringent, with excess of polyphenols wines (TPI > 50) [8].

Antiradical power
The evaluation of the antiradical power of the wine was made in comparison with 2,2-Diphenyl-picrylhydrazyl (DPPH), one of the most stable free radical organic compounds. It accepts H from the antioxidants present in the sample and the antioxidant effect is proportional to the disappearance of the DPPH radical. DPPH has a strong peak absorption at 517 nm (violet). The colour changes to yellow due to DPPH H formation. The antioxidant effect can be evaluated by decreasing absorbance at 517 nm [18].

The DPPH (394.32 gram /mol) methanolic solution with a concentration of 6x10^{-5} mol/L (2366x10^{-5}g/L) in methanol was obtained and then, the reference solution was prepared by diluting 3900 µL with 100 mL methanol. Radical reaction was initiated by transferring to 25 mL of each wine sample 3975 µL of reference solution (dilution ratio 1: 160). For each sample, the absorbance value was measured after 15 min of incubation in the dark and at the room temperature, at 517nm, on a UV-Vis Jasco 730 spectrometer.

Percent inhibition of DPPH. (I%) was calculated from the decrease in absorbance with the relation:

$$I_{\%} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

where: $A_{\text{blank}}$ is the absorbance for the blank (reference solution of ethanol- DPPH. ethanolic solution) and $A_{\text{sample}}$ is the absorbance for the sample mixed with DPPH reference solution.

The ATR-FTIR spectral analysis
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 analysed 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 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, 19-21]. 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 700 cm⁻¹), performed both on the entire spectral range (4000 to 400 cm⁻¹), and on the MIR “fingerprint” (i.e. 1800 to 700 cm⁻¹), on the peak areas corresponding to regions 940-600 cm⁻¹ (1), 1000-1120 cm⁻¹ (2) and 3000 -2800 cm⁻¹ (3).

Results and discussions
Table 2 comprise the values obtained for the chemical parameters investigated for the eight wine samples.

Table 2

| Sample | Sugar % BRIX | Acidity pH | Titratable acidity (TA) g tartaric acid / l | Total polyphenols μg galic acid /ml | Total polyphenols content (TPA) μg galic acid /ml | TP1 | T% DPPH inhibition |
|--------|--------------|------------|---------------------------------------------|-------------------------------------|-----------------------------------------------|------|-------------------|
|        |              |            |                                             |                                     |                                               |      |                   |
| 1      | 13.6         | 2.99       | 4.84                                        | 46.0455±0.0283                      | 13.33                                         | 68.42|                   |
| 2      | 12.0         | 2.88       | 4.53                                        | 33.1392 ± 0.0945                    | 10.08                                         | 52.39|                   |
| 3      | 10.6         | 2.83       | 5.36                                        | 51.2333 ± 0.0323                    | 14.92                                         | 76.94|                   |
| 4      | 11.1         | 2.86       | 5.31                                        | 40.0688 ± 0.0695                    | 11.91                                         | 56.44|                   |
| 5      | 13.8         | 2.76       | 3.74                                        | 57.2947 ± 0.0308                    | 16.01                                         | 60.47|                   |
| 6      | 29.0         | 3.44       | 7.12                                        | 61.5184 ± 0.0383                    | 17.58                                         | 54.45|                   |
| 7      | 28.1         | 3.62       | 7.35                                        | 125.0695 ± 0.0510                   | 33.88                                         | 82.51|                   |
| 8      | 27.7         | 3.59       | 6.90                                        | 37.2224 ± 0.0156                    | 18.33                                         | 51.83|                   |

The chemometric analysis. Principal Component Analysis (PCA)
The Principal Component Analysis (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 sweet wines related to the two production systems [14, 29, 30].

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 [25, 26].

In all samples, similar spectral features were generally obtained. Figure 1 exhibits the general FT-MIR spectra of all wine residue samples.

In order, to get an overview of the compositional differences that occurred in these eight samples of wine an overlapping image of the of Romanian and Canadian wine samples was taken and it is presented in figure 2. Various spectral bands occur in the region from 1800-700 cm⁻¹, which is part of the fingerprint region, such as those corresponding to the vibration of the C-O, C-C, C-H and C-N bonds. It was possible to consider a few specific regions which can be useful for wines’ characterization [27-29].

Region 1 (690-940) is responsible for polyols (mainly glycerol). Region 2 (1000-1120 cm⁻¹) is responsible to carbohydrates (glucose, fructose and oligosaccharides), and region 3 (2800-3000 cm⁻¹) for polyols (mainly glycerol).

Table 3 includes the FT-MIR absorption wave numbers, specific to the 3 regions of each wine fingerprinting. All spectra were characterized 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.

The chemical composition of the wine samples is an important parameter for their classification. The results of the FTIR analysis of the Romanian and Canadian wines are shown in Table 3.

Table 3

| Region          | Absorption peak cm⁻¹ |
|----------------|-----------------------|
| 1              | 2.83                  |
| 2              | 4.53                  |
| 3              | 5.36                  |
| 4              | 7.31                  |
| 5              | 3.74                  |
| 6              | 7.12                  |
| 7              | 7.35                  |
| 8              | 6.90                  |

The pH values indicating the degree of ionization of acids and bases in wine can involve polyphenols especially in samples with more than 500 g/L sugar concentration [24]. In all the analysed samples, the Cabernet Franc icewine variety stands out with a much higher content of polyphenols and the highest DPPH inhibition power. This variety, a well-formed wine (TP1 > 30), with a brilliant ruby hue, is considered very rare and precious and is specific to the Ontario-Canada area. For the other samples, the values are comparable and place the wines in the category of sweet wine types.
between the groups. The Romanian wine varieties are located on the right of the plot, while Canadian sweet wines are located on the left of the graph.

Figure 4 represents the scores for the region 600-940 cm\(^{-1}\), specific to phenolic compounds (chemical compounds that affect the taste, color and mouthfeel of wine). In this case, the first principal component (PC1) explained 74% of the variability, the second principal component (PC2) explained 23% and the third principal component (PC3) with 2% of the variability, together explaining 99% of the whole variability of wine samples and suggesting a good clustering based on acidity. The type of wine separates: in the right are the Romanian wines (Grasa de Cotnari - Grasa 1, Tamaioasa Romaneasca Cotnari- Tamaioasa - 2) and in the left part of diagram are Canadian wines (Vidal Blanc Aged in Oak, Cabernet Frans and Vidal). Moreover Grasa de Cotnari 1 and 2 are in the lower part of the right of plot and Tamaioasa 1 and 2 and Busuioaca are in the positive quadrates of both PC1 and PC2. Cabernet Franc wine is separated from the other canadian wines. These are in concordance with total polyphenols values obtained by the Folin-Ciocalteu method (table 1).

Figure 5 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...
Fig. 2. Overlapped ATR-MIR spectra for the five sweet Romanian commercial wine samples (left) and three Canadian Icewine (right).

Table 3

| Sample of wine                  | 1 (600-940 cm⁻¹) | 2 (1000-1200 cm⁻¹) | 3 (2800-3000 cm⁻¹) |
|---------------------------------|------------------|--------------------|--------------------|
| Grasa de Cotnari (Grasa 1)      | 677, 719, 775, 817, 864, 918 | 1029, 1096         | 2888, 2936         |
| Tămâioasa Română (Tâmâioasa 1) | 675, 689, 719, 722, 774, 816, 864, 917 | 1025, 1099         | 2888, 3205         |
| Cotnari Inedit-Bunioaca de Bohotin (Bunioaca) | 668, 677, 719, 776, 816, 863, 859, 916 | 1027, 1099         | 2887, 2934         |
| Grasa de Cotnari demiulce (Grasa 2) | 669, 719, 775, 816, 862, 900, 917 | 1028, 1100         | 2886, 2934         |
| Tămâioasa Română (Tâmâioasa 2) | 664, 677, 891, 719, 775, 816, 864, 901, 918 | 1028, 1100         | 2883, 2934         |
| Vidal Blanc Aged in oak         | 642, 659, 719, 776, 816, 862, 891, 916 | 1029, 1031, 1100    | 2888, 2935         |
| Cabernet Franc                  | 612, 624, 839, 719, 776, 816, 864, 917 | 1027, 1046, 1100    | 2889, 2935         |
| Vidal                          | 620, 647, 666, 697, 719, 729, 749, 776, 816, 864, 917 | 1028, 1056, 1101    | 2884, 2937         |

Fig. 3. 2-D scores obtained from PCA of FTIR spectra for the first two PCs based on the FTIR fingerprint region (700-1800 cm⁻¹) a), and PC3 versus PC1 b)
Fig. 4. 2-D scores obtained from PCA of FTIR spectra for the first two PCs based on the FTIR fingerprint region specific to phenolic derivatives (600-940 cm⁻¹).

Fig. 5. 2-D scores obtained from PCA of FTIR spectra for the first two PCs based on the FTIR fingerprint region specific to carbohydrates (1000-1120 cm⁻¹).

Fig. 6. 2-D scores obtained from PCA of FTIR spectra for the first two PCs based on the FTIR fingerprint region specific to polyols (2800-3000 cm⁻¹) a), and PC3 versus PC1 b).
first principle component 2 and 2% respectively of the total variance. The type of wine separates: in the right of the diagram are the Canadian sweet wines. These are in concordance with sugar content values presented in table 1.

Figure 6 represents the scores for the region 2800-3000 cm$^{-1}$, specific to polyols (mainly glycerol). The first PC explains 53% while the second and the third principle component 14% and 11% respectively of the total variance. Vidal Blanc Aged in oak is separated from the rest two Canadian wines. This result may be due to processes that occur during wine aging, such as: the release of some compounds from the oak wood into the wine; chemical reactions involving both wine and wood compounds as well as oxygen permeation through the wood; evaporation of volatile compounds with the concentration of non-volatile ones [31, 32].

Polyols especially glycerol afford fullness to wine and decrease the perception of acidity, particularly in dry white wines. Also glycerol significantly contributes to the complexity of wine and the length of the finish. Its contribution to sweetness was considered significant, but only when present at high concentrations [33].

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 sweet Romanian and Canadian wine, that can be observed by MIR spectroscopy and used to distinguish wines, following PCA.

The chemometric instruments are rapid and allow an accurate separation of the wine by the sugar content, phenols, polyols, with very good results from the spectrum of a drop of wine in the absence of the chemical analysis of the sample necessary for these determinations.

The chemical analyzes show that the Romanian sweet wines and Canadian icewines are distinguished by the sugar content, acidity and the total content of polyphenols. The differences are particularly remarkable in terms of sugar content where the values are double in icewines versus the Romanian ones. All wines demonstrate antiradical activity without major differences between the two groups of origin. However, chemical analyzes are laborious, time consuming and expensive. In contrast, ATR-MIR spectroscopy combined with multivariate analysis proved to be a quick and effective technique to distinguish between different samples of sweet wine.

The separation of wines by chemometry according to the content of carbohydrates, phenols, polyols is in good concordance with that obtained by chemical methods.

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