# COMPARATIVE STUDY OF JUICES AND WINES OBTAINED FROM FOREST FRUITS AND GRAPES USING $^{1}$H-NMR SPECTROSCOPY

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*Nuclear magnetic resonance (NMR) spectroscopy coupled with Principal Components Analysis (PCA) chemometrical method were used to characterize juices and wines obtained from four types of forest fruits (strawberries, blackberries, raspberries and blueberries) and three varieties of grapes (Muscat Ottonel, Fetească Albă and Burgund). $^{1}$H-NMR spectroscopy provided two sets of data that can be used to classify juices and wines. The first set of data used in PCA was the absolute concentrations of compounds identified in juices and wines. The second set of data was obtained by transforming the $^{1}$H-NMR spectra into strings of numerical values by integrating relevant spectral windows in steps of 0.2 ppm. Those sets of data were further used to discriminate juices and wines according to the row material.*

**Keywords**: juice, strawberries, blackberries, raspberries, blueberries wine, $^{1}$H-NMR, PCA

## 1. Introduction

Juices from forest fruits, such as strawberries, blackberries, raspberries, blueberries, are beneficial to human health and contribute to the prevention of

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degenerative processes caused by oxidative stress [1]. Juices and wines are a complex mixture of components, mainly consisting of water, ethanol, amino-acids, organic acids, carbohydrates and polyphenols.

In addition to these constituents, the complex matrix of juices and wines contains small amounts of ingredients responsible for important characteristics (taste, smell and color). The composition of juices and wines depends on many factors such as fruit variety, yeast strains used in the fermentation process [2, 3], geographical origin (also depending on soil, climate) [4], and wine-making technology [5-7].

Nuclear magnetic resonance spectroscopy (NMR) coupled with multivariate analysis is often used to characterize and authenticate juices and wines from different viewpoints: variety [2, 8], geographical origin [9] and crop year [10]. In this respect, NMR spectroscopy is a suitable analytical tool for the analysis and characterization of juice and wine compounds. In previous studies of Romanian wines, $^{1}$H-NMR spectroscopy was used for the identification and the quantitative measurement of different wine constituents. "Fingerprints" for several Romanian wines have thus been obtained and used in order to establish wine identity [11-13].

In the present study we used $^{1}$H-NMR spectroscopy coupled with multivariate statistical method (*Principal Component Analysis* - PCA) to characterize different varieties of juices and wines from four types of forest fruits (strawberries, blackberries, raspberries and blueberries) and three varieties of grapes (Muscat Ottonel, Fetească Albă and Burgund). We aimed to classify those juices and wines in terms of species (raw material). For this, $^{1}$H-NMR spectroscopy offers two sets of data that can be used to discriminate juices and wines. The first set of data obtained was the absolute concentration of compounds identified in juices and wines. The second set of data was obtained by transforming the $^{1}$H-NMR juice and wine spectra into strings of numerical values by dividing the relevant spectral regions into 0.2 ppm intervals, and the corresponding integrals.

## 2. Materials and method

### *2.1 Chemicals*

Deuterium oxide ($D_2O$) (99.9 atom % D) used for $^{1}$H-NMR analysis was purchased from Sigma-Aldrich, Steinheim, Germany.

### *2.2 Samples*

Forest fruits were purchased from local markets of four geographical area of Romania: strawberries (Satu-Mare), blackberries (Cluj-Napoca), raspberries (Giurgiu) and blueberries (Baia-Mare). All juices and wines from forest fruits were obtained in our laboratory. Authentic juice and wine samples from grapes Muscat Ottonel, Fetească Albă and Burgund from the 2009 crop were provided from Urlați vineyard (the geographic area of Dealu Mare, Romania).

### *2.3 Recording the $^1$H-NMR spectra*

Samples concentration for $^1$H-NMR analysis was in all cases juice or wine/$D_2O$ = 9/1 (v/v).$^1$H-NMR spectra were recorded on a Varian INOVA 400 spectrometer, operating at 9.4 Tesla, corresponding to the resonance frequency of 399.95 MHz for the $^1$H nucleus, equipped with a direct detection four nuclei probe head and field gradients on *z* axis. Samples were analyzed in 5 mm NMR tubes (Norell 507). Typical parameters for $^1$H-NMR spectra were: 45° pulse, 2.05 s acquisition times, 6.4 KHz spectral window, 64 scans, 26 K data points and relaxation delay time 2s. The FID was not processed prior to Fourier transformation. The average acquisition time of the $^1$H-NMR spectra was approximately 4 minutes. Because the concentration of water is very high in comparison with other important components,spectra were also recorded using the "Presat" experiment (water suppression using presaturation at 4.79 ppm). Same parameters as above were used, but the delay time parameter was set at 12 s. The average acquisition time of the Presat spectra was approximately 17 minutes. Each sample was recorded in triplicates.

### *2.4. Statistical analysis*

Statistical analysis (*Principal Component Analysis, PCA*) used to investigate the compositional differences between wines from different species were performed using the XLSTAT 7.5.2 program.

## 3. Results and discussion

### *3.1 $^1$H-NMR spectra of juices and wines*

$^1$H-NMR spectra of raspberry juices and wines are presented in Fig.1 (a) and (b), respectively.

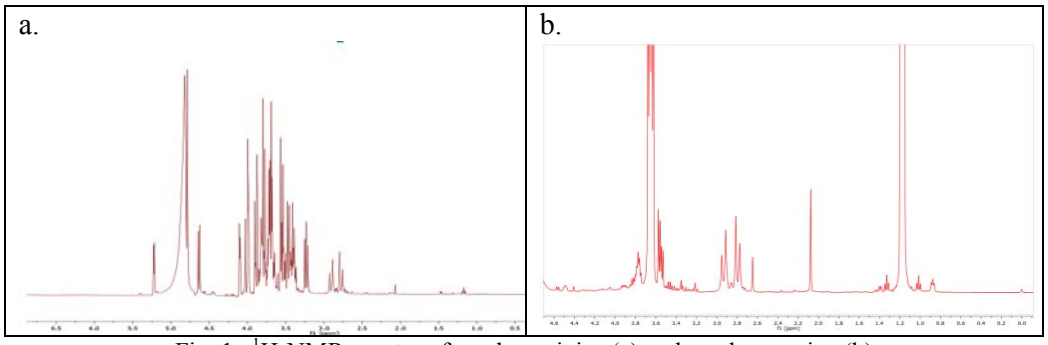


Fig. 1. $^{1}$H-NMR spectra of raspberry juice (a) and raspberry wine (b)

Representative $^{1}$H-NMR spectra of grape juices and wines have been presented in a previous paper of our research group [6].

### *3.2 Quantitative analysis of components identified in juices and wines*

Thirteen components with known structure – valine (Val), alanine (Ala), isoleucine+proline (Ile+Pro), proline+valine (Pro+Val), acetic acid, succinic acid, malic acid, citric acid, glycerol, glucose, fructose, polyphenols, ethanol, were identified in $^{1}$H-NMR spectra of juices and wines according to a method previously described [11]. For a quantitative determination of compounds identified in juices and wines an internal standard (trimethilsilyl sodium propionate,TSP) was used in $^{1}$H-NMR analyses. The concentration of TSP in each sample was 1 mM/L. Based on TSP concentration, the absolute concentration of components identified in juice and wine samples was determined (tables 1 and 2).

*Table 1*

**The concentration of components identified in traditional Romanian forest fruits and grape juices**

| Identified compounds | Concentration (mmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Strawberries | Raspberries | Blackberies | Blueberries | Burgund | Feteasca Alba | Muscat Ottonel |
| Val | 0.7 | 3.3 | 0 | 0 | 1.5 | 1.1 | 6.2 |
| Ala | 1.3 | 0.9 | 2.7 | 0 | 0.3 | 0.4 | 5.1 |
| Ile +Pro | 0 | 2.4 | 0 | 3.3 | 8.5 | 4.8 | 10.5 |
| Val + Pro | 1.1 | 2.3 | 0 | 5.1 | 0.9 | 0.4 | 0.4 |
| Acetic Acid | 1.1 | 4.1 | 5.7 | 0.9 | 0 | 1.2 | 2.1 |
| Succinic Acid | 0.5 | 1.1 | 3.3 | 0.4 | 0.1 | 0.9 | 0.2 |
| Malic Acid | 0.1 | 0.1 | 0.4 | 0.1 | 10.3 | 18.4 | 17.7 |
| Citric Acid | 36.9 | 81.5 | 30.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Glycerol | 28.1 | 23.6 | 17.1 | 23.6 | 68.1 | 47 | 55.6 |
| Glucose | 94.9 | 67.3 | 90.4 | 66.6 | 561.2 | 681.1 | 507.6 |
| Fructose | 38.7 | 43.2 | 60.6 | 36.1 | 256.3 | 319.9 | 338.4 |
| Polyphenols | 7.1 | 11.3 | 9.3 | 9.7 | 0 | 0 | 0 |
| Ethanol | 1.8 | 2.6 | 0.9 | 0.6 | 0.4 | 2.2 | 2.2 |

*Table 2*

**The concentration of components identified in traditional Romanian forest fruits and grape wines**

| Identified compounds | Concentration (mmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Strawberries | Raspberries | Blackberies | Blueberries | Burgund | Feteasca Alba | Muscat Ottonel |
| Val | 0.8 | 3.7 | 2.1 | 0 | 2.2 | 1.1 | 6.1 |
| Ala | 1.4 | 1.1 | 3.1 | 0 | 0.3 | 0.4 | 5.1 |
| Ile +Pro | 0 | 2.4 | 0 | 3.6 | 7.6 | 5.1 | 10.6 |
| Val + Pro | 3.1 | 3.6 | 0 | 0 | 0.9 | 1.1 | 2.9 |
| Acetic Acid | 13.7 | 11.2 | 8.2 | 9.4 | 9.2 | 4.5 | 5.2 |
| Succinic Acid | 12.4 | 3.9 | 3.7 | 1.4 | 11.5 | 10.7 | 5.5 |
| Malic Acid | 0.5 | 0.1 | 0.4 | 0.1 | 10.4 | 17.6 | 16.7 |
| Citric Acid | 185.1 | 42.1 | 156.3 | 30.4 | 0.1 | 0.1 | 0.1 |
| Glycerol | 1.4 | 0.4 | 1.1 | 0.9 | 0.8 | 1.4 | 0 |
| 2,3 Butandiol | 15.2 | 4.3 | 2.1 | 1.3 | 17.7 | 9.7 | 8.3 |
| Lactic Acid | 4.7 | 37.1 | 26.9 | 1.3 | 19.7 | 4.8 | 7.4 |
| Polyphenols | 53.2 | 36.2 | 45.2 | 34.8 | 1.9 | 3.1 | 2.4 |
| Ethanol | 385.9 | 353.9 | 511.2 | 419.7 | 2869.1 | 2243.6 | 2739.9 |
| Methanol | 9.7 | 3.6 | 7.1 | 5.3 | 6.3 | 3.1 | 4.2 |

## Organic acids

The main acids present in forest fruits and grape juices are malic and citric acids. A large amount of malic acid found in wines shows a high content of malic acid originally present in grapes [5]. A high content of lactic acid in wine shows that the malo-lactic fermentation took place; lactic bacteria convert malic and citric acids into lactic acid [6, 10] during fermentation. In this study, we detected a large amount of malic acid and a low amount of citric acid in juices and wines obtained from grapes; on the contrary, a low amount of malic acid and a large amount of citric acid were found in forest fruits juices and wines.

Succinic and acetic acids are non-volatile organic compounds occurring in wines mainly during the alcoholic and malo-lactic fermentation processes. High amounts of acetic and succinic acids were found in strawberries and Burgund wines.

## Amino-acids

Amino-acids from juices and wines have various origins. Some are originating from forest fruits and grapes [7, 14] and can be partially or totally metabolized by yeasts at the end of fermentation or released from dead yeasts, others are produced by enzymatic degradation of grape proteins [15-16]. In the present study, the amounts of amino-acids have a little variation from one species to another; however, the highest levels of amino-acids are present in Muscat

Ottonel juice and the corresponding wine and the lowest in blackberry juice and wine.

**Polyphenols**

Forest fruits skins are known to contain large amounts of phenolic compounds, including anthocyanins, flavonols, chlorogenic acids and procyanidins, with high biological activity and may provide health benefits as dietary antioxidants [17-18]. Polyphenols were globally determined from $^{1}$H-NMR spectra, based on the signals of the 7.0-7.4 ppm region, using a methodology previously described. [11] As reflected from tables 1 and 2, large amounts of polyphenols (ranging from 35 to 53 mmol/L) were detected in forest fruits wines compared to grape wines; on the other hand, the amount of polyphenols found in forest fruit juices is very low compared to wines, and polyphenols are totally absent in grape wines. This apparently paradox situation is due to the fact that grape and forest fruit juices are fermented on fruit skin; the migration of polyphenols from fruit skin to wine improves with the increasing of ethanol concentration.

### *3.3 Conversion of $^{1}$H-NMR spectra into strings of numerical values*

The $^{1}$H-NMR spectrum was converted into a string of 24 numerical values using the MestReNova 6.0.2 program. The area between 4.8-5.1 ppm was eliminated from the study because it contains information which is not relevant for wines discrimination (water). Areas between 0.1-0.8 ppm and 5.5-7.0were also eliminated because those areas lack signals or the signals cannot be integrated. The spectrum range 0.8-4.8 ppm, was divided into 20 regions of 0.2 ppm which were integrated; in this way 20 values were obtained. In the same way another four values between 5.1-5.5 and 7.0-7.4 (every 0.2 ppm) were obtained. The spectral window at -0.1-0.1 ppm containing the TSP signal was used as reference spectral window; the integral value of this window was set at 1.0 for each spectrum. In this manner, each $^{1}$H-NMR spectrum was represented as a 24 values vector.

### *3.4 PCA data analysis*

Using the $^{1}$H-NMR spectroscopy – absolute concentration of compounds with known structure coupled with PCA chemometrical analysis, juices and wines from forest fruits and grapes were discriminated.PCA analysis showed a clear separation between juices Fig. 2 (a) and wines Fig. 2 (b) according to raw material.

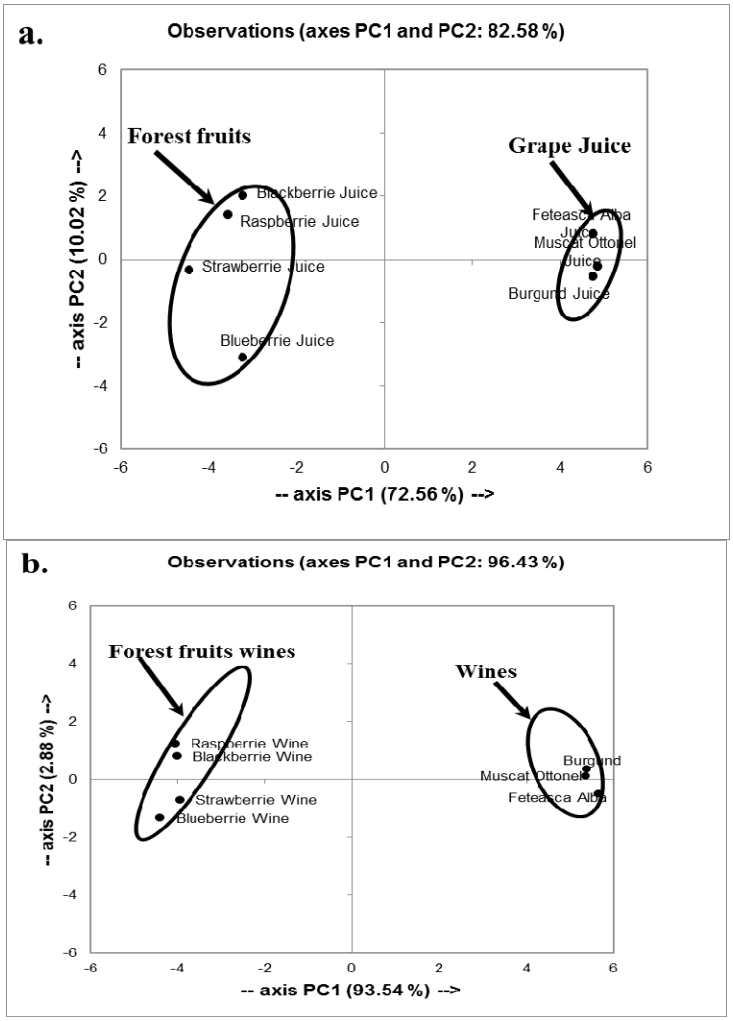


Fig. 2. PCA score plot derived from the absolute concentration of compounds identified in juices (a) and wines (b)

The data obtained from transforming $^{1}$H-NMR spectra into strings of numerical values were also processed using the PCA method. Fig. 3 (a) and (b) shows a good separation of juices and wines, respectively, obtained from different raw materials.

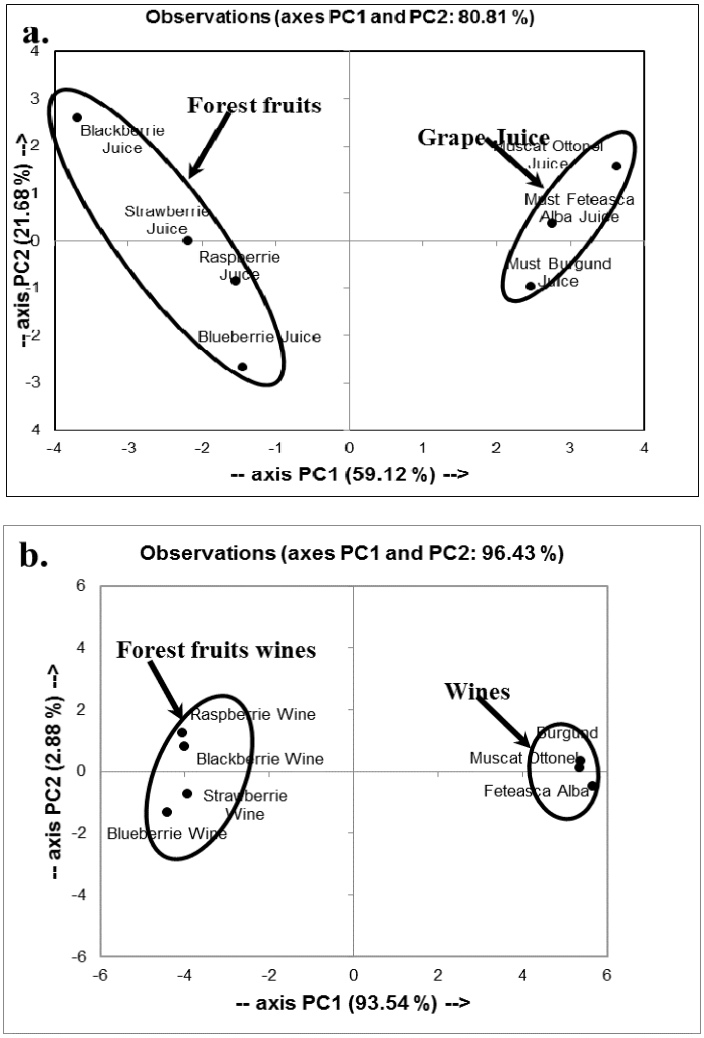


Fig. 3. PCA score plot derived from $^{1}$H-NMR spectra conversion into strings of numerical values: juices (a) and wines (b)

## 1. Conclusions

In the present study, the discrimination possibilities of juices and wines from different row materials (forest fruits and wines) based on $^{1}$H-NMR spectroscopy coupled with Principal Component Analysis technique were investigated. It was interesting to note that, by using different sets of data provided by $^{1}$H-NMR juices and wines spectra, we were able to classify juices and wines according to the raw material.

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