

Rapid Quantitative Analysis of Ethanol and Prediction of Methanol Content in Traditional Fruit Brandies from Romania, using FTIR Spectroscopy and Chemometrics

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Abstract

Fourier Transform Infrared (FTIR) spectroscopy in combination with chemometrics were applied for the quality control of 26 fruit brandies made by traditional technology in Romania. Firstly, for the identification and quantitative evaluation of methanol and ethanol in such samples, 4 mixtures of methanol and ethanol standard solutions and a spiked sample were fingerprinted in the 1200 - 950 cm^{-1} FTIR spectral range, identifying specific wavelength of 1020 and 1112 for methanol, 1047 and 1087 for ethanol. Then, the FTIR spectra of all brandy samples in the range 3500 - 750 cm^{-1} was registered and the methanol and ethanol concentrations were determined according to the previous calibration. By PCA (Principal component analysis) of FTIR areas (1170 -1000 cm^{-1}), the variability and discrimination between samples was possible, discriminating between the biological and geographical origin of brandy samples. Based on peak areas and intensities, it was predicted the concentration of methanol in all samples, using Partial least squares regression (PLS). The correlation between FTIR and the reference method (GC-FID) was well correlated and significant ($p < 0.05$). It was demonstrated that FTIR technique offer a good prediction and statistical correlation with GC-FID technique for methanol quantification.

Keywords: ethanol, fruit brandies, FTIR, methanol, PCA, PLS, Romania

Introduction

The Central and East European countries have an old tradition in producing different types of fruit brandies. In Romania, there is a great interest to produce home-made traditional fruit brandies obtained by a double distillation of fermented fruits. *Țuica* and *pălinca* are the two most popular, traditional distilled fruit beverages made from plums or apples, pears, other fruits respectively. Besides their volatile composition, the principal differences of these two types of fruit brandies are the ethanol content which reaches values between 24 up to 86% v/v, for *țuica*, and 40 up to 70% v/v, for *pălinca*. The determination of methanol in these types of fruit brandies it is an important approach, related to its safety for consumers. Depending of the type of fruit brandy, the maximum accepted methanol content was established to be 1350 mg/100 ml anhydrous alcohol and in the case of *țuica* and *pălinca*, the methanol content should not exceed 1200 mg/100 ml anh. alc. (REG. 110/2008).

In the context of quality control of alcoholic beverages a range of different analytical methods are used, such as distillation and picnometry (Tešević *et al.*, 2009), electronic densimetry (Coldea *et al.*, 2011), colorimetry (Joshi

and Sandhu, 2000), photometry (Borges Sivanildo *et al.*, 2006), by fluorescent chemical sensor (Bozkurt *et al.*, 2010). The most advanced techniques are the chromatographic ones, either GC-FID, GC-MS or HPLC (Diban *et al.*, 2009; García-Llobodanin *et al.*, 2008; Hernández-Gómez *et al.*, 2005; López-Vázquez *et al.*, 2010; Wang *et al.*, 2003). The last years, the expensive reference methods are replaced by simpler ones, non-destructive, easy to handle. The FTIR (Fourier Transform Infrared Spectroscopy) technique, in combination with chemometrics is such a fast and reproducible way to identify the authenticity and adulteration of different food and beverage products (Da Costa *et al.*, 2004; Mansor *et al.*, 2011; Rohman *et al.*, 2010). The FTIR is increasingly used for the determination of methanol in alcoholic beverages (Arzberger and Lachenmeier, 2008; Gallignani *et al.*, 2005; Lachenmeier, 2007; Mehrotra *et al.*, 2005; Nagarajan *et al.*, 2006; Pérez-Ponce *et al.*, 1998) or to verify the authenticity of different fruit juices (Leopold *et al.*, 2011). The Near-Infrared (NIR) spectroscopy was also used to estimate the ethanol content in alcoholic beverages or to verify the adulteration of different beverages (Ashok *et al.*, 2011; Pontes *et al.*, 2006).

In our previous studies we determined the ethanol and methanol content of brandies by electronic densimetry and by GC-FID techniques, respectively (Coldea *et al.*, 2011).

In this study we realized the FTIR fingerprint of distilled fruit brandies in order to verify their authenticity, to identify the specific signals for methanol and ethanol, to apply the non-targeted PCA chemometric analysis for the evidence of three types of fruit brandies, to verify the plum brandy (*țuica*) authenticity from different regions and to predict methanol concentration by applying the PLS (Partial Least Squares) regression. We also compared the results obtained by FTIR method and by GC-FID technique or densimetric method.

Materials and methods

Reagents

Highly pure methanol (Merck, Germany, 99.8% purity) and ethanol (Merck, Germany, 99.9% purity) were used.

Sampling

A total number of 26 samples of fruit brandies (plum - *țuica*, apple and pear *pălinca*) originating from different Counties of Transylvania - Maramureș (MM), Cluj (CJ), Bistrița Năsăud (BN), Alba (AB), Bihor (BH) were collected from producers. All brandies were produced by traditional method: fermentation in wood barrels and double distilled in copper alembic, kept in wood or glass barrels for maturation. They were kept in glass recipients until analysis. Tab. 1 includes the sample denominations and their region of origin.

Fourier Transform Infrared Spectroscopy

To evaluate the content of methanol, we spiked one sample (P10) with methanol in different proportions, in order to identify the spectra modifications. There were used individually pure ethanol, pure methanol, sample P10 and different concentrations of methanol added to sample P10 at ratios P10:methanol of 1:1, 10:1, 10:0.5, 10:0.25, 9:1, 8:2, 8:4, 8:6. The FTIR spectra were registered in the range 1000 to 1200 cm^{-1} , using Attenuated Transmission (ATR) and an internal reflection accessory made of Composite Zinc Selenide (ZnSe) and Diamond

crystals (Shimadzu IR Prestige-21 equipment). For each sample, spectrum was registered from 3500-750 cm^{-1} , and based on the ethanol and methanol specific wavenumbers, in all samples, the methanol and ethanol content was determined.

Statistic and chemometric analysis

Based on FTIR spectra and the frequency intensities recorded in the domain 1170-1000 cm^{-1} , the chemometric analysis was performed. By Principal Component Analysis (PCA), using the Unscrambler 10.1 Software, version 10.1 (Camo Software AS, Oslo, Norway) the variability and discrimination between samples was made. Based on peak areas, from 1170 to 1000 cm^{-1} , the concentration of methanol in samples was predicted by PLS regression. For each type of fruit brandy and each region for plum brandy a correlation analysis it was developed using Origin software (OriginLab, version 8.0).

Reference Procedures

Both, ethanol and methanol content of the same samples were previously determined. Ethanol content was analyzed in all samples by densimetric method. Methanol was evaluated by gas chromatography coupled with flame ionization detector (Coldea *et al.*, 2011).

Results and discussion

Identification of methanol and ethanol in the FTIR spectra of pure standards and a spiked sample

The identification of methanol and ethanol was done, firstly, by applying the calibration curve using pure standards, as well mixtures of methanol:ethanol (1:1; 1:9) (Fig. 1). Thus, pure methanol solution has the characteristic vibration frequency at 1020 cm^{-1} (major signal) and 1112 cm^{-1} (minor signal), while ethanol had the characteristic frequency at 1047 cm^{-1} (major signal) and 1087 cm^{-1} (minor signal). We selected the absorbances of major and minor signals specific for pure methanol and ethanol (Tab. 2). The approximate ratio of the absorbances of major/minor signals in methanol was 5.2 and for ethanol 1.5. These frequencies are specific for stretching vibrations of C-O bonds in these molecules.

Using methanol and ethanol in ratios of 1:9, or 1:1 respectively (Fig. 1) we noticed that the increase of metha-

Tab. 1. Numbering of fruit brandy samples (plum – P, apple – M, pear – PE brandies) and the provenience regions (CJ-Cluj; MM- Maramureș; BH-Bihor; BN- Bistrița Năsăud)

Sample no.	Region	Sample no.	Region	Sample no.	Region	Sample no.	Region	Sample no.	Region
P1		P5		P3		M19	BN	PE23	CJ
P2		P7		P12		M20	AB	PE24	MM
P4	CJ	P8	MM	P13	BN	M21	CJ	PE25	BN
P6		P9		P14		M22	BH	PE26	CJ
P17		P10		P15					
P18		P11		P16					

Tab. 2. The values and ratios of the absorbance intensities (A) and areas (B) of the minor and major signals specific for methanol (1020 and 1112 cm^{-1}) and ethanol (1047 and 1087 cm^{-1})

Mixture/wavenumber (cm^{-1})	1020	1112	1047	1087	1020 / 1047	1112 / 1087
A						
Methanol	2.1470	0.4125	-	-	-	-
Ethanol	-	-	1.9539	1.3448	1.1	0.3
Methanol:Ethanol 1:9	-	-	1.9484	1.2658	-	-
Methanol:Ethanol 1:1	1.9286	-	1.6314	0.9471	1.2	-
B						
Methanol	78.45	27.99	-	-	-	-
Ethanol	-	-	73.76	53.21	1.1	0.5
Methanol:Ethanol 1:9	-	-	-	-	-	-
Methanol:Ethanol 1:1	76.46	-	30.26	44.41	2.5	-

nol in the mixture, determine decreases of absorbance at 1047 and 1087 cm^{-1} and increase of absorbance at 1030-1020 cm^{-1} and 1112 cm^{-1} . Based on these observations, we consider the parameters which can identify and quantify the concentration of methanol in samples, are the ratios A_{1020}/A_{1047} and A_{1112}/A_{1087} . Therefore were selected the areas corresponding to major and minor signals specific for pure methanol and ethanol (Tab. 3).

To illustrate the effect of the presence of methanol and its limit of detection in samples of brandies, it was chosen sample P10 (Fig. 2). By a controlled adulteration of the sample, were added various concentrations of pure methanol 2.5%; 5%; 9%; 20%; 33%; 43% and 50%, and noticed the gradual increase of intensity frequencies at 1112 and 1020 cm^{-1} . The gradual evolution of methanol and ethanol signals of absorbation at 1047, 1087, 1020 and 1112 cm^{-1} is represented in Tab. 3.

Therefore, the FTIR technique was useful not only to identify and quantify the ethanol, but also to recognize the presence of methanol and to determine the ratio between ethanol and methanol.

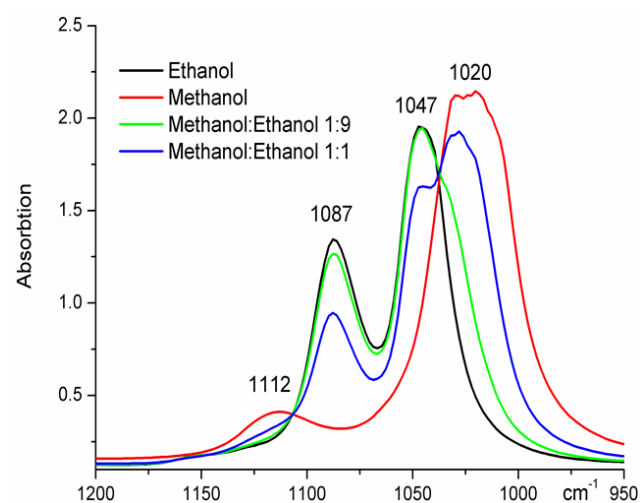


Fig. 1. The specific IR spectra for methanol and ethanol, as pure substances and in different concentration in mixture methanol:ethanol

Identification of ethanol by FTIR fingerprinting of brandy samples

FTIR spectroscopy can be used as potential means for quantitative analysis of fruit brandies, considered as a "fingerprinting tool", which means that there are no two fruit brandies samples with the same FTIR spectra, either in the number of peaks or in the absorbance of the maximum peak. Fig. 3 (up) exhibits the general FTIR spectra of 3 representative brandy samples (M21, PE25 and P5) in a region range of 3500-750 cm^{-1} , showing quite similar fingerprints of these three samples in the region 1700-900 cm^{-1} .

Fig. 3 (down) presents also the FTIR spectra in the region 1120-1000 cm^{-1} and the specific peaks and absorbances for ethanol at 1087 and 1047 cm^{-1} .

Using detailed investigation, the FTIR spectra of plum brandy samples exhibited some differences. Since ethanol has a specific frequency of vibration at 1087 and 1047 cm^{-1} , these signals were compared for samples P5, M21 and PE25 (Fig. 4). The sample P5 has higher absorbance values

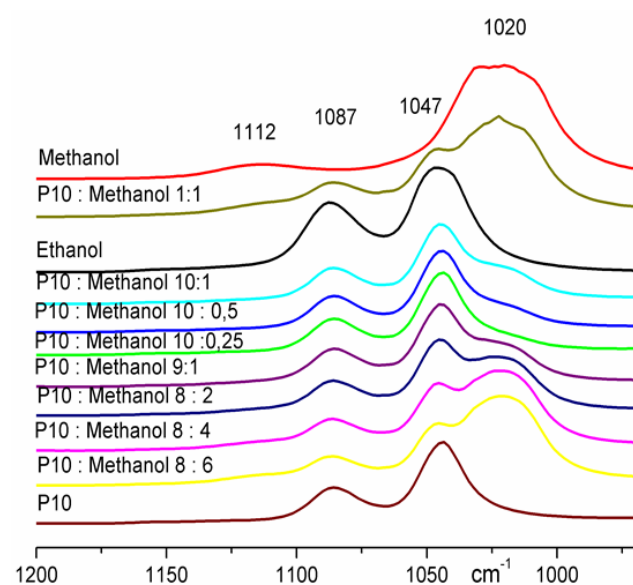


Fig. 2. Overlapped IR Spectra for methanol and ethanol in different proportion from sample P10

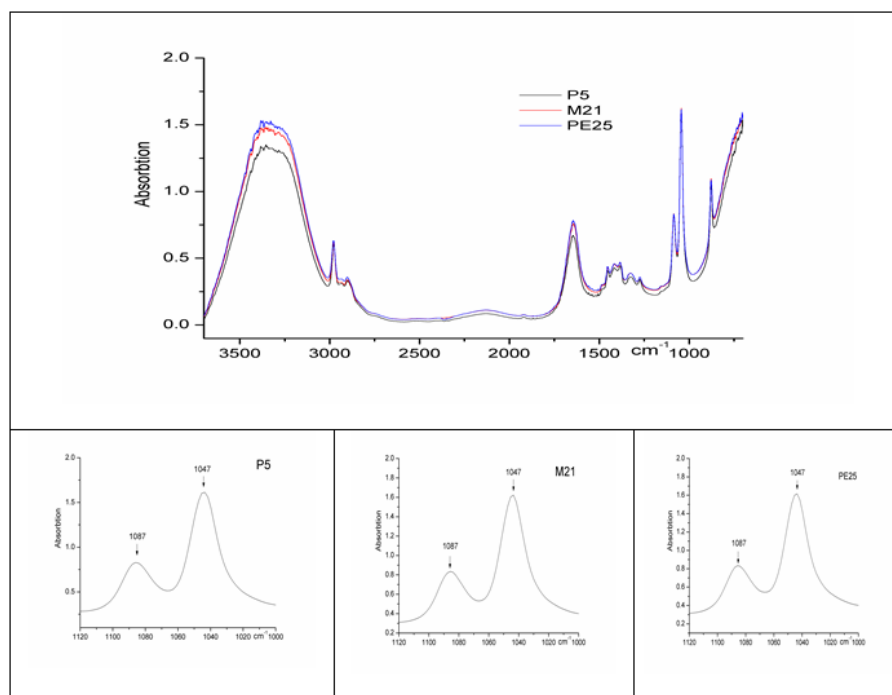


Fig. 3. Comparative IR Spectra, overlapped on domain 3500-750 cm^{-1} , for samples: P5, M21 and PE25. Specific ethanol signals (1087 and 1047 cm^{-1}) evidenced for each sample

at these frequencies followed by sample M21 and PE25, in agreement with the concentration of ethanol reported by densimetric method (Coldea *et al.*, 2011). While pear brandies had the lowest ethanol content, plum brandy sample had the highest, the peak area is a better parameter to evaluate the ethanol content.

Principal component analysis (PCA) based on FTIR data

The multivariate analysis of principal component analysis (PCA) based on the fingerprint of FTIR spectra which includes alcohols (methanol, ethanol) and some unfermented glucides within 1170-1000 cm^{-1} was carried out in order to differentiate the samples according to the biological origin and the provenience region of the tradi-

Tab. 3. The gradual evolution of methanol and ethanol signals of absorbtion at 1047, 1087, 1020 and 1112 cm^{-1}

Mixture	1047	1087	1020	1112
Ethanol	1.9539	1.3448	-	-
P10	1.6468	0.8509	-	-
P10:Methanol 10:0.25	1.6674	0.8603	-	-
P10:Methanol 10:0.5	1.6411	0.8585	-	-
P10:Methanol 10:1	1.6136	0.8487	-	-
P10:Methanol 9:1	1.6595	0.8813	-	-
P10:Methanol 8:2	1.5435	0.8232	1.2428	-
P10:Methanol 8:4	1.382	0.7526	1.5956	-
P10:Methanol 8:6	1.2761	0.6986	1.7524	-
P10:Methanol 1:1	1.6674	0.8603	-	-
Methanol	-	-	2.1470	0.4125

tional brandies. There were considered absorbances which corresponded to the peak areas within the region 1170-1000 cm^{-1} for each sample when building the PCA score plot. Fig. 5 shows the score plot of PCA for classification of the fruit brandies using the FTIR spectra values. The PCA representation was made without normalization. As shown in Fig. 6, the first two principal components (PCs) described 92% (PC-1) and 7% (PC-2) of the total variation, respectively. Therefore, it can be pointed that the variation of 99% could be described by the first two PCs. PC-1 is associated to methanol with the samples contain-

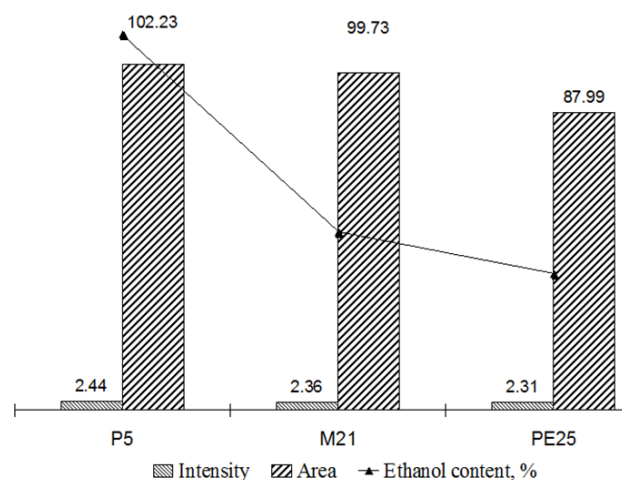


Fig. 4. Comparative values of FTIR absorption intensities and areas for signals 1047 and 1087 cm^{-1} , as obtained by FTIR and the ethanol concentration (line- %) as obtained by densimetric method (for sample P5, M21 and PE25)

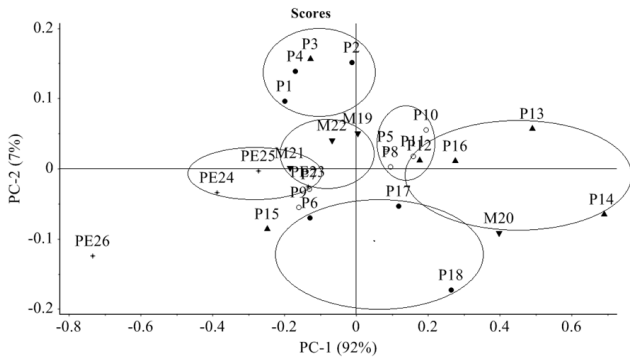


Fig. 5. Evidence of differences (scores) from analyzed beverages, depending on FTIR analysis (fingerprint zone area 1170-1000 cm⁻¹) by PCA method

ing the highest methanol level having PC-1 score. PC-2, is associated with ethanol. Although almost all samples have similar composition in alcohols, we still can distinguish six groups based on their methanol or ethanol content.

Regarding the methanol content registered by GC-FID method, sample P2 had the highest content of all samples from Cluj County. In contrast, samples M19 and P10 had the lowest values. The most evident variability have the plum brandies from BN County.

Tab. 4 includes the comparison between the FTIR area signals corresponding to ethanol and the values obtained by densimetric method. There was a significant correlation of the results obtained for ethanol by the two methods (Fig. 6).

Correlation analysis was made between results obtained by both FTIR and densimetric methods used to determine the concentration of ethanol for all the brandies. It was used two-tailed test of significance. These results have a strong correlation ($R = 0.841$) for the all samples.

Calibration and validation of PLS procedure for methanol

By applying the PLS regression for methanol calibration curve (Fig. 7 and Tab. 2) we were able to predict its content in brandies. The predicted concentrations of methanol were obtained based on FTIR absorbance at 1020 cm⁻¹ (g/100 g fruit brandy) and transformed into g/100 mg anh. alc.

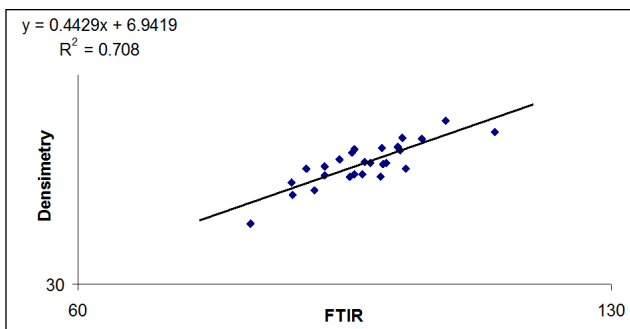


Fig. 6. Correlation curve established for the ethanol concentrations obtained by two methods (densimetry and FTIR). Correlation coefficient= 0.708 ($p < 0.05$)

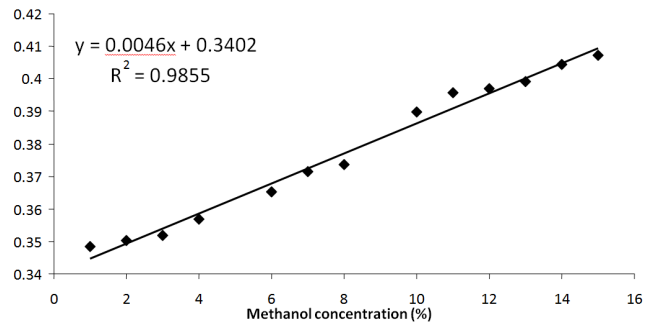


Fig. 7. Prediction of methanol concentration in fruit brandy samples, by applying the Partial Least Squares (PLS) Regression: Calibration curve with methanol at 1020 cm⁻¹ (on methanol concentration interval 1-15%)

Tab. 5 exhibits the correlations between the values for methanol content, for the set of all 26 samples, as obtained by the two methods applied, FTIR (predicted values) and GC-FID (experimental values) (Coldea *et al.*, 2011). A mean ratio of 1.1176 ± 0.2402 was obtained between the values obtained by GC-FID and FTIR, demonstrating the good prediction ($p < 0.05$) performed by FTIR technique.

Tab. 5. Correlations between the values of methanol concentrations for obtained from the two methods FTIR and GC-FID

Sample	FTIR (predicted values)	GC-FID (Actual values)	Ratio GC-FID/FTIR
P1	851.91	1077.59	1.2649
P2	818.39	1266.97	1.5481
P4	850.40	954.31	1.1221
P6	868.18	1244.35	1.4332
P17	861.54	1100.76	1.2776
P18	906.28	953.69	1.0523
P3	843.26	964.96	1.1443
P12	815.72	901.39	1.1050
P13	758.35	1073.70	1.4158
P14	819.42	913.01	1.1142
P15	926.54	765.86	0.8265
P16	795.93	953.73	1.1982
P5	803.50	857.71	1.0674
P7	841.05	504.18	0.5994
P8	804.21	999.81	1.2432
P9	875.00	1256.41	1.4358
P10	755.55	450.50	0.5962
P11	825.07	954.12	1.1564
M19	822.32	597.64	0.7267
M20	866.61	1189.29	1.3723
M21	878.57	1020.89	1.1619
M22	830.68	810.97	0.9762
PE23	881.89	973.61	1.1040
PE24	923.37	818.34	0.8862
PE25	883.67	880.69	0.9966
PE26	1045.89	1290.06	1.2334
Average ratio			1.1176
SD			0.2402

By comparison with GC-FID analysis, the obtained values for methanol, based on the forecast calibration and FTIR spectra, were of the same order, from 0 to 1.2% anh. alc. Among all samples, the minimum concentration of methanol was determined in sample P10, and the maximum in PE26, by both FTIR and GC-FID methods.

We noticed also differences among the type of brandy: the methanol from apple brandies was better correlated ($R = 0.874$), followed by the pear brandies ($R = 0.860$) and no correlations for the plum brandies ($R = 0.213$). A high correlation was in the case of MM plum brandies ($R = 0.621$). Unsignificant correlations were registered in the case of CJ plum brandies ($R = -0.565$) and BN plum brandies ($R = -0.911$). The most appropriate results for methanol obtained by the two methods were those for plum distillate from Maramureş County. The standard deviation of values obtained by FTIR method were much lower than those by GC-FID. Finally we conclude that FTIR technique applied for methanol quantification can predict reliable results and in good correlation with GC-MS technique.

Conclusions

FTIR method can be useful not only for determining the methanol and ethanol content in extract-free samples, but also to evaluate the ratio between these two volatile components in brandies. FTIR/PLS offers valuable advantages when choosing versus conventional methods. Their importance it is justified by being a rapid, efficient and non-destructive tool for screening alcoholic beverages. Besides the quantitative PLS analysis, the PCA classification of data obtained by FTIR spectra becomes important for authenticity control of the provenience region and the type of the fruit brandy.

Acknowledgements

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