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DETERMINATION OF ETHANOL CONTENT OF FERMENTED ALCOHOLIC BEVERAGES WITH FTIR SPECTROSCOPY AND MULTIVARIATE CALIBRATION

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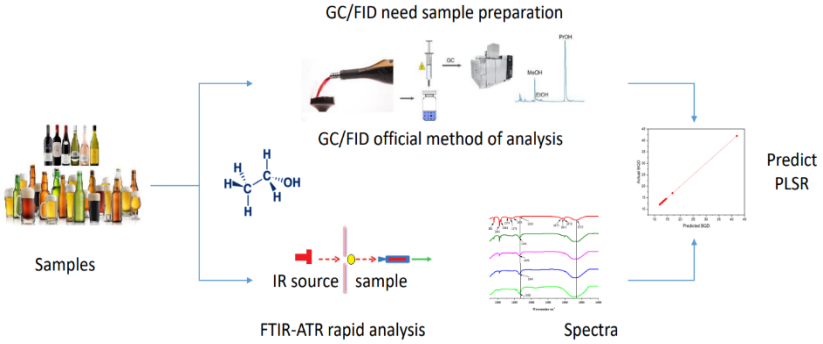
FTIR spectra of beverages with known content of ethanol (from 4 to 42%) and that of pure ethanol were acquired to reveal differences in the absorption values in the wavenumber range of 1000–1500 cm^{-1}. As a routine analysis, the ethanol determination was conducted by GC-FID. FTIR-ATR coupled with chemometric analysis were conducted to detect and quantify the content of ethanol in fermented beverages. Ethanol content in alcoholic beverage was predicted in the spectral range of 1220–1520 cm^{-1}. Partial least squares regression (PLSR) was used to create models from the 2nd derivative spectra acquired in the 1220–1520 cm^{-1} region and with some pre-treatments such as Sawitzky-Golay smoothing second polynomial filter and 10 point of windows. A coefficient of determination of 0.999 was determined for calibration and the same high value was determined and for validation, respectively. The developed method is not expensive, does not require any sample preparation, is considered a green method of analysis, is very fast and highly precise. By this mean, we experimentally verified and calculated the lowest concentration of compound in unknown sample that could have been discriminated from the concentration obtained from the pure sample (LOD) and the limit of quantification (LOQ), and for the selected optimized method, a LOD of 0.02% (v/v) and LOQ of 0.07% (v/v), were determined.



INTRODUCTION

The old and traditional fermented alcoholic beverages such as wines, spirits and beers are mixtures of compounds such as ethanol, water and sugars, which represent the major components,[1] while the minor components are other alcohols, organic acids, carbonyl compounds, esters, aldehydes, lactones, sulphuric compounds.[2,3] During manufacturing of any alcoholic beverage and the alcoholic fermentations, ethanol levels are permanently monitored by the producers usually through density measurements, to ensure standardised production patterns, to perform quality

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control during manufacturing, to check compliance with standards and with legal regulations. Many countries have determined minimum ethanol level requirements for alcoholic beverages and correlated them with other implications. For example, for wine a special tax tied to its ethanol percent level was established through national legislation.[4] Thus, monitoring the ethanol content of alcoholic beverages, but also the determination of its chemical characterization is an important point of interest. A common method for alcoholic strength determination is the density determination, a method with less accuracy, which is often not satisfactory, because some interferences which might occur due to the other organic compounds such as sugars and acids and other alcohols esters from the composition of the drinks.[5] On the other side, GC-FID is a powerful technique, which is recommended for ethanol and methanol analysis.[6] Anyway, GC-FID is time consuming and the analysis including sample preparation, expensive equipment and higher cost of analysis, usage of a lot of toxic and hazardous chemicals. For this reason, GC-FID is not very suitable to be applied in routine analysis conducted in the traditional winery industries and, if it used on long term, could become harmful for the laboratories staff.

FTIR spectroscopy equipped with ATR allows the direct sample analysis. The analysis in combination with the specialized multivariable software is gaining attention for its non-destructive, rapid, easy operation and green technology approach.[7,8] Despite that infrared vibrational spectroscopy provides unique details about molecular structures and their fingerprint regions, the obtained signals could be very complex and could conduct to difficulties in interpretation, for this purpose being necessary to apply multivariable tools available in software, to overcome such obstacles.

FTIR spectroscopy was already evaluated as a predictive method and was suitable for the analysis of ethanol in fermented alcoholic beverages such as wine and beer. Ethanol determination by FTIR has been reported at different possible infrared regions, of different signal intensities, and was studied in relationship with the ratio of different bands, using chemometrics, for the full selected spectrum and with some different spectra pre-treatment.[9–12]

To our knowledge, for the ethanol determination, FTIR spectroscopy still does not have a PLSR (Partial least squares regression) optimized method, acquired by applying suitable pre-treatments, or a well-established best possible infrared region, where compounds of the matrix do not interfere and the detection of the pure ethanol is possible. Therefore, the objective of the study was to explore the potential of FTIR-ATR spectroscopy and multivariable parameters for the detection and quantification of the ethanol in fermented alcoholic beverages.

RESULTS AND DISCUSSION

1. Preliminary spectral analysis

FTIR spectra of pure ethanol and alcoholic beverage are shown in Fig. 1. Peaks of various intensities appeared in the MIR fingerprint regions, in the wave number range of 4000−400 cm^{-1}.

Major spectral peaks at 3355 cm^{-1} are due to the stretch vibration of the OH group and at 1641 cm^{-1} due to bending vibration of the H-O-H angle, both being characteristic vibrations of a water molecule. Identified peaks at wavenumbers 1276 cm^{-1}, 1088 cm^{-1} presented also OH bending vibrations groups from ethanol molecules and that at 1045 cm^{-1}, which is connected with the C-O bonding of the ethanol molecule. Other identified bands at 2976 cm^{-1}, 2931 cm^{-1} and 2875 cm^{-1} were present due to the symmetric and asymmetric stretching vibration of C–H bonds of aliphatic CH_3 and CH_2 group probably from ethanol. Also at 1453 cm^{-1} and 1378 cm^{-1} peaks were registered because of the C-H bending of CH_3 and CH_2 from the ethanol composition.

A major band that was identified in the pure ethanol was the peak at 881 cm^{-1} which present the vibration from C-C stretch, but this peak overlapped in the spectra of the real samples, where ethanol is in lower level, thus interference with other components in alcoholic beverages occurred. Fig. 1 allows selecting the fingerprint region which showed a clear variation, detecting the presence of added ethanol in alcoholic beverage. The variation occurred in the 1000−1500 cm^{-1} regions. Those characteristic peaks were not present in the sample with low level of ethanol. As ethanol concentrations increased in the samples, the intensity of this band which corresponds to ethanol (OH and CH_2 bending vibrations) increased (Table 1).

Other interesting spectral changes observed, were those of the shift of the OH stretch group from the water molecule, which bands around 1638 cm^{-1} and were identified in the sample with low level of ethanol respectively in that with high level of water. A similar shift was observed for the peak occurring at 3355 cm^{-1}. Both observed shift changes were corresponding to changes in hydrogen bonding in correlation with changes in the water/ethanol ratio.[15]

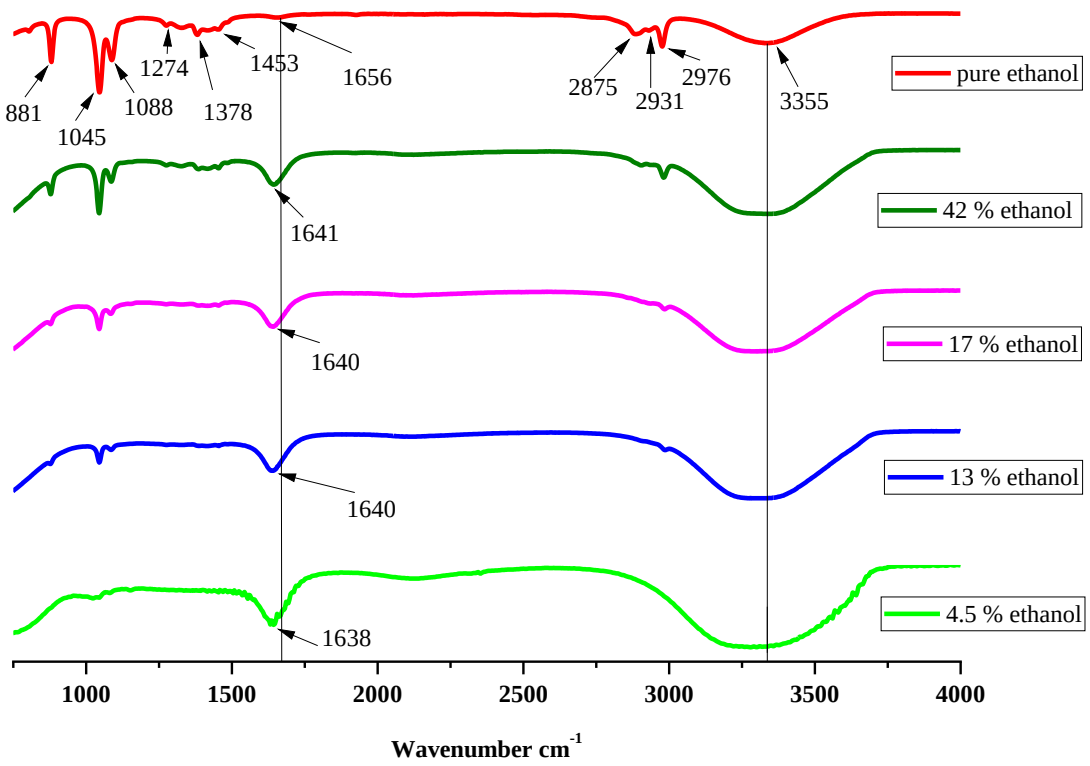

Fig. 1 – Spectra of pure *ethanol* and several alcoholic beverage samples.

Table 1

Analytical evaluation of infrared spectra of ethanol in alcoholic beverage samples.

Wavenumbers (cm^{-1})	Definition of the spectral assignment
3355	O-H stretch. vibration group of water[15]
2931	Asymmetric stretching vibration of C–H bonds of aliphatic CH_2 group[14]
2976 and 2875	Symmetric and asymmetric stretching vibration of C–H bonds of aliphatic CH_3 group[14]
1641	H-O-H bending vibration angle
1453	C-H bend of α-CH_2 and β-CH_3[15]
1378	Bending symmetric vibration of C–H bonds of CH_2 group[16]
1274	O-H bending[15]
1088	O-H bend and C-O stretch[15]
1045	O-H bend and C-O stretch[15]
881	C-C stretch [15]

A more detailed representation for the variation of FTIR spectra was obtained by the principal component analysis (PCA), loadings and scores being shown in Fig. 2. Two principal component (PC) were found to explain the major variation (99.3%) of the FTIR spectra with PC1 and PC2 made up 97.2% and 2.1%, respectively, of the total variation. The scores of PC1 separated beverages with different ethanol concentration, indicating difference in ethanol level. On the other hand, the scores of PC2 separated the profiles of beverage with ethanol level 17% and 42% from other samples.

The spectra loadings of the PC1 did not show any negative correlation, but the loading of PC2 showed a strong negative correlation at regions of 852–900 cm^{-1}, 1000–1120 cm^{-1} and at 1220–1520 cm^{-1}, which were brought together by the presence of oxygenated compounds especially alcohol functional groups (Fig. 3). Selected regions were applied for further create PLSR method for the ethanol determination in alcoholic beverage except the region 852–900 cm^{-1} which are avoided due to interference from other components.

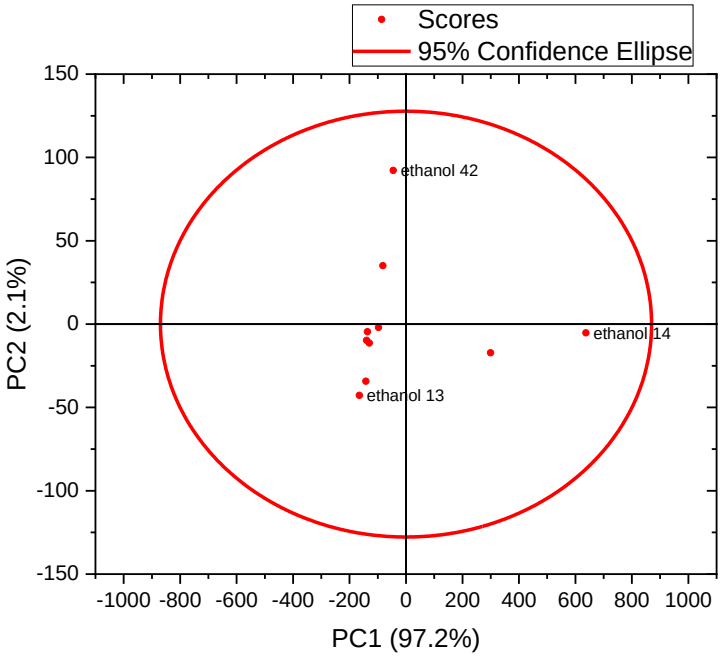

Fig. 2 – Principal component analysis (PCA) of the raw FTIR spectra of some popular Balkan traditional alcoholic beverages with alcohol content ranging from 4–42%.

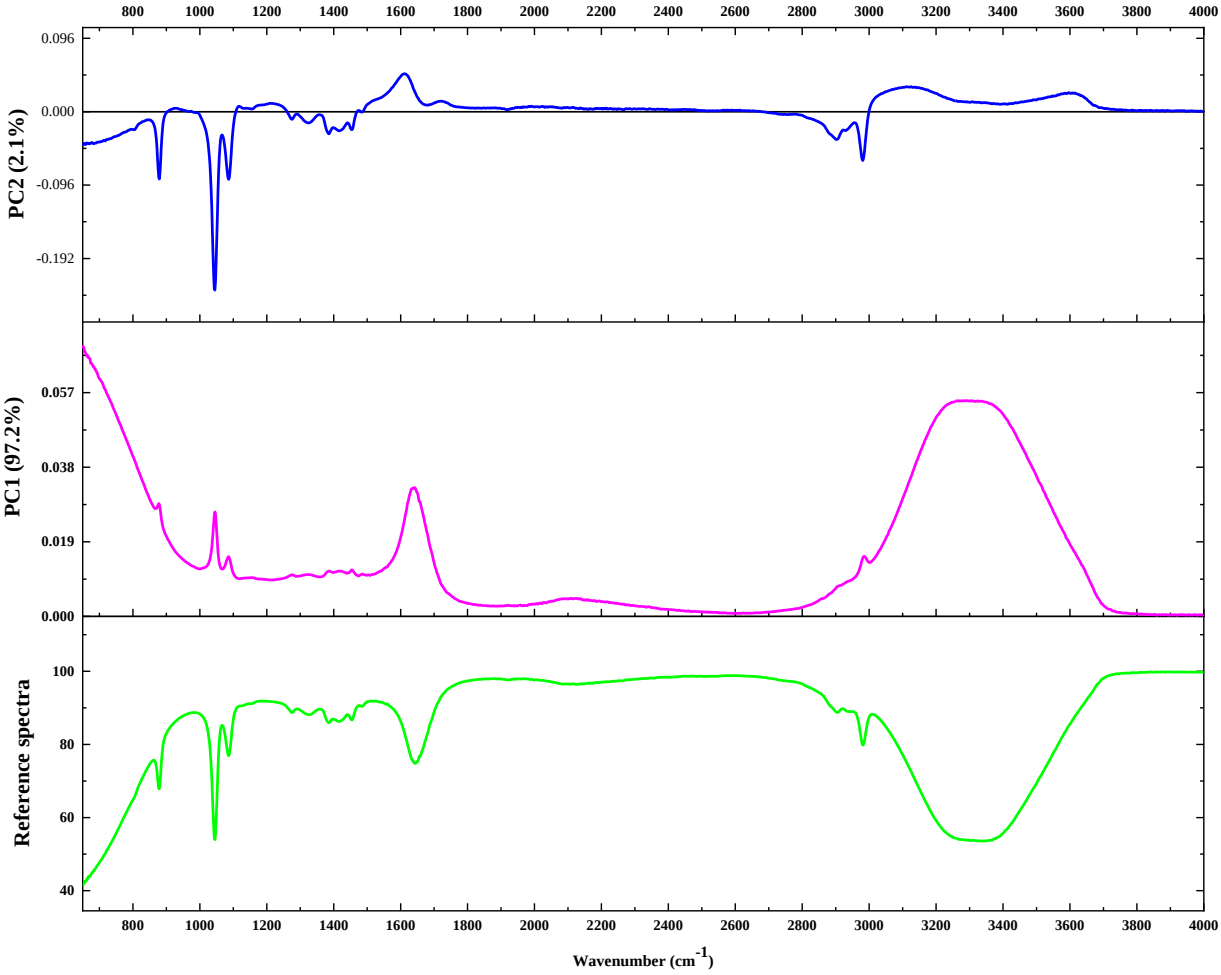

Fig. 3 – Loadings of principal component 1 (PC1), principal component 2 (PC2) and reference spectra of pure ethanol.

2. Quantitative Prediction of ethanol by PLSR

The regions of the infrared spectrum defined as important for predicting the ethanol content (selected variables) were firstly selected by PCA spectra loading comparing the spectra of real samples with known concentration of ethanol and the spectra of the pure compound (Fig. 3).

A spectral region which was already validated for the ethanol determination by earlier works [10] was 850–1200 cm^{-1}, but this region was not suitable in our case because of the strong matrix interferences, especially those occurring at 850–1000 cm^{-1}. Based on this point of view we proposed two other regions, namely 1000–1120 cm^{-1} and 1220–1520 cm^{-1} where peaks corresponding to ethanol vibrational characteristics, without matrix interferences, were formed. To envisage different level of ethanol in alcoholic beverage samples, chemometric models for ethanol quantification were formulated (Table 2).

Table 2

Effect of different spectral windows on spectral data modelling using PLSR for the selected range of wavenumber

Spectral treatment	Factors	Wavenumber region	Calibration		Prediction		LOD (%)	LOQ (%)
			RMSEC	R^2	RMSEP	R^2		
Raw spectra	7	1000–1120	0.067	0.999	0.261	0.999	0.15	0.45
1-st deriv.	5	1000–1120	0.122	0.999	0.421	0.997	0.27	0.84
2-st deriv	5	1000–1120	0.023	0.999	0.072	0.998	0.21	0.63
2-st deriv SG-10	4	1000–1120	0.242	0.999	0.986	0.977	0.55	1.66
2-st deriv SG-20	6	1000–1120	0.112	0.999	0.511	0.991	0.25	0.77
2-st deriv SG-35	6	1000–1120	0.025	0.999	0.109	0.999	0.12	0.62
Raw spectra	5	1220–1520	1.047	0.986	3.122	0.978	2.38	7.23
1-st deriv.	3	1220–1520	1.394	0.976	4.808	0.952	3.18	9.66
2-st deriv	3	1220–1520	1.334	0.978	4.521	0.952	3.04	9.22
2-st deriv SG-10	8	1220–1520	0.011	0.999	0.051	0.999	0.02	0.07
2-st deriv SG-20	7	1220–1520	0.043	0.999	0.212	0.999	0.09	0.29
2-st deriv SG-35	4	1220–1520	1.257	0.981	3.212	0.963	2.87	8.69

All of the optimized methods at different wavenumbers regions and the spectral treatments had a coefficient of determination R^2 higher than 0.95. The best performance of models was chosen based on the lowest root-mean-square error of calibration (RMSEC) and validation (RMSEP), the highest coefficient of determination (R^2). Four out of ten selected spectral windows had R^2 values higher than 0.998 with very low RMSEC and RMSEP values. Maximum R^2 values for calibration and validation were found to be 0.999, for the spectral region 1000–1120 cm^{-1} while the other spectral region, 1220–1520 cm^{-1}**,** had the highest coefficient of determination R^2 0.999, (Table 2). PLSR calibration curve was obtained (Fig. 4) for the data in the 1220–1520 cm^{-1} region with maximum number factors (8 factors), low RMSEC and RMSEP, the application of 2nd derivative for the spectra and Sawitzky-Golay smoothing, second polynomial filter and 10 points of window.

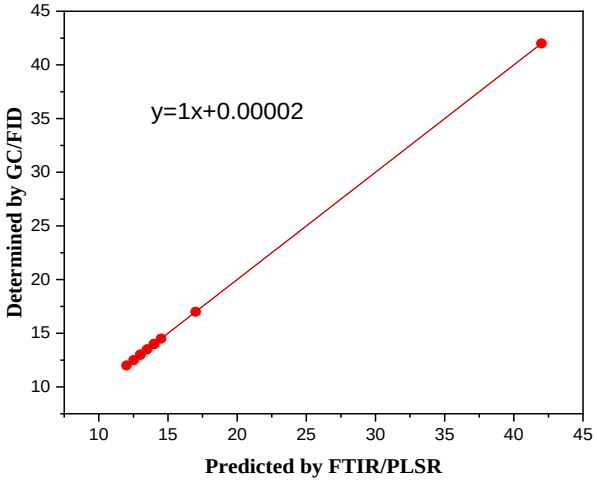

Fig. 4 – Plots of ethanol result obtained by GC/FID (actual value) and FTIR method (obtained by PLSR).

3. Comparative study between FTIR-PLSR and GC/FID

FTIR-ATR in combination with multivariate PLSR chemometric approach was compared with the reference chromatographic method, GC-FID in regard to the obtained ethanol level and the strong correlation between both methods presented in their ratio. At 95% confidence level, both of the methods did not present any significant difference about the ethanol percentage (Table 3). This is the confirmation that our proposed calibration model with derivative spectral treatment and Sawitzky-Golay method, smoothing and polynomial filtration, was excellent to predict the ethanol content in different alcoholic beverages.

Table 3

Comparison FTIR-PLSR and GC-FID on prediction of ethanol in beverage samples ($p < 0.05$)

	Wine	Wine	Wine	Wine	Wine	Wine	Wine	Wine	Wine	Spirit	Beer
GC/FID	12	13	14	14.5	17	14	12.5	13	13.5	42	4.8
FTIR/PLSR	12.02	13	13.99	14.52	16.99	13.98	12.52	13.05	13.48	42.13	4.81
Ratio GC-FID/FTIR	0.998	0.9998	1.000	0.998	1.000	1.001	0.998	0.996	1.001	0.996	0.997

4. LOD and LOQ determination

The lowest concentration of a compound from an unknown sample, which can be reliable detected and distinguished from interferences or noise, is defined as the limit of detection (LOD), while the amount of compound that can be quantified in sample is known as limit of quantification (LOQ). Calculations for both parameters were conducted $LOD = 3.3\sigma/S$ and $LOD = 10\sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve, respectively.[17] Results for the selected optimized method with 8 number of factors were LOD 0.02% (v/v) and LOQ 0.07% (v/v), respectively.

EXPERIMENTAL

1. Alcoholic Beverage Samples

Some of the most popular Balkan traditional alcoholic beverages such as "Cabernet Sauvignon Reserve", Cabernet Sauvignon', "Rose wines", spirits and beers produced in Kosovo by local producers following traditional processes, were taken in consideration. Fifty different commercially available samples were purchased from local markets in Kosovo. Ten samples with different ethanol levels were selected to create the calibration model and the other samples were used for the method validation.

2. Chemicals and Equipment

Pure ethanol and n-propanol (both from Merck, Darmstadt, Germany, 99.9% purity) were used for the determination of the ethanol in alcoholic beverage with the calibration curve (0–45% v/v). FTIR spectrophotometer (Shimadzu –IRAffinity-1) equipped with ATR diamond and Deuterated Triglycine Sulfate (DTGS) detector, connected to the "IR-Solution software" was employed to acquire the spectra of beverages samples which were also analysed in terms of alcohol content with GC-FID, as a reference method. GC (7890B GC System, Agilent Technologies) integrated with automatic liquid and headspace samplers (7697A Headspace Sampler, Agilent Technologies), equi pped with flame ionisation detector (FID) employed as an analytical method for determination of ethanol contents and this was possible by using DB-WAX-UI analytical column (30 m x 0.250 mm x0.25 mm, Agilent Technologies). The carrier gas was high purity helium.

3. Ethanol determination by GC-FID

The first step was the sample filtration through a 0.45 μm Millipore filter and at the same time, it was mixed with 5% n-propanol as an internal standard compounds. The ethanol was determined by GC-FID. The working conditions used for GC were 3 μL injection volume operating in split mode (ratio1:12), the temperature of injection was set to 180 ˚C. The temperature of the oven was initially at 75 °C (for 2 min), then it was increased at rate of 1 °C/min. to 80 °C, at which was held for 1 min. The gas flow rates were maintained as follows: carrier gas (helium, 10 mL/min), hydrogen (30 mL/min), and air (300 mL/min) and detector was set at 260 ˚C and silica capillary column of polyethylene glycol (DB-WAX, JW Scientific, Folsom, CA, USA) 60 m in length, 0.32 mm i.d.,

and with 0.25 μm film thickness were also used. Calibration curve was obtained for the ethanol quantification, based on the peak area ratio of the mixture ethanol: n-propanol versus ethanol concentration in percent.

4. FTIR spectra acquisition

Spectra of all collected samples were recorded at an ambient temperature of 20 ± 2 ˚C and measured in the wave number range of 4000–400 cm^{-1}, with 4 cm^{-1} applied resolution, and 32 scans at a scan speed 0.2 cm s^{-1}. For each sample, the spectra was acquired for three times and these spectra were subtracted against the air spectrum, which was also collected as the background and were recorded in the reflectance mode. Subsequent to every single scan, the ATR crystal plate surface was carefully cleaned with acetone after scanning each sample to remove traces of preceding sample. Treatments applied to experimental spectral data and mathematical calibration models were made using OriginPro-2021 software.

5. Data Analysis

A PLSR model was developed using OriginPro software, to correlate spectral data with ethanol concentrations in alcoholic beverage samples. The model was calibrated using 10 standard solutions (ethanol: 0–45 % v/v) and pre-processed.

The wavenumbers region was optimized for ensuring a good model, capable of predicting the levels of the ethanol content accurately and precisely. Wave numbers regions were to provide the highest coefficient determination (R^2) and the lowest values of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).[13] Quantitative models were generated with multivariate calibration PLSR using the IR spectra (independent variable) and reference ethanol concentrations from GC/FID (dependent variable).

Raw spectra and spectra pre-processed using first and second derivative, Savitzky-Golay second derivative (SG second deriv.), with second polynomial filter, and different point of windows exactly 10, 20 and 35 and necessary transformations were applied to develop the best calibration models.

Full spectra of selected regions and not individual peaks were chosen to construct a model that could lead to the most efficient estimation of the ethanol content in alcoholic beverage samples. This is because FTIR spectral data contains subtle information in the IR spectral region characteristic for molecular vibrational intensities, which are not visible as individual peaks.

CONCLUSION

The present investigation evaluated the utility of FTIR spectroscopy with PLSR detection and quantification of ethanol in different fermented beverage samples. For ethanol we determined two specific spectral regions; 1000–1120 cm^{-1} and 1220–1520 cm^{-1}. Among these regions, that which did not presented interfering effects and conducted to comparable result with the GC-FID was 1220–1520 cm^{-1}. A relative study based on PLSR model (normal, 1^{st} and 2^{nd} derivative spectra in combination with Sawitzky-Golay spectra smoothing and different points of window) procedures for spectral data pre-treatment were performed. The PLSR model for 2^{nd} derivative spectra in combination with Sawitzky-Golay spectra smoothing, second polynomial filter and 10 points of window gave the best prediction results among all the models. R^2 for prediction of ethanol by PLSR was found to be 0.999 for calibration and for validation with RMSEC and RMSEP values of 0.011% v/v and 0.051% v/v respectively. LOD and LOQ are very low which revealed high precision and accuracy of the developed model. Hence it can be concluded that the FTIR spectroscopy is a simple, non-destructive, rapid and reliable tool that can be used with accuracy for the characterization and quantification of ethanol fermented beverage with minimal sample preparation and data treatment. This approach constitutes a powerful alternative for the rapid ethanol determination in alcoholic beverages.

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