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Identification of phenolic and alcoholic compounds in wine spirits and their classification by use of multivariate analysis

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Abstract: During the ageing period wine spirits are changing their color, chemical composition and sensory characteristics. These changes should be simply monitored. The aim of this study was to develop partial least squares regression (PLS) models for higher alcohols and phenols in wine spirits as well as to show the feasibility of the NIR spectroscopy combined with chemometric tools to distinguish wine spirits and brandies with different ageing degree. To get the reference values, the usual methods for the analysis of spirits drinks were used. Ethanol, esters, acids, methanol and higher alcohols were studied. Wine spirits and brandies phenol composition was determined by liquid chromatography. Principal component analysis (PCA) was used to classify the wine spirits and brandies according to their phenolic and higher alcohols composition. Moreover, the Partial least squares regression (PLS regression) was used to calibrate and predict expected contents of higher alcohols and phenols in the wine spirits. Success of the classification of samples by ageing based on individual alcohols was 93.8 %, while success of the classification based on individual phenols raised to 100 %. This efficiency of the prediction was evaluated by use of linear discriminator analysis (LDA).

Keywords: distillate; ageing; NIR spectroscopy; principal component analysis.

INTRODUCTION

Wine spirits (a spirit drink produced exclusively by the distillation of a wine at less than 86 vol.%) are commonly used for the brandy production. Brandy is a spirit drink produced from wine spirit, whether or not wine distillate has been added, distilled at less than 94.8 vol.% and matured for at least one year in oak receptacles, or for at least six months in oak casks with a capacity of less than

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1000 L, to obtain characteristic taste, flavor and attractive color. No addition of ethyl alcohol of agricultural origin or distillates of agricultural origin is permitted. Brandy may only contain added caramel as a means to adapt color and should not be flavored. The first stage in the brandy production is the selection of wine spirits for further processing, mostly based on classical sensory analysis. After selection, wine spirits used in brandy production are subjected to ageing process in wooden barrels for certain time, depending on traditional practices and legislative.[1] During the ageing period, chemical composition of wine spirits is altered, due to extraction of wood compounds (mostly phenols and sugars) and oxidation process, contributing to spirit aroma, taste and colour.[2,3] Since these reactions are slow, ageing of high-quality brandies takes several years, resulting in the low production efficiency and high cost. For economic reasons, producers sometimes use caramel and vanillin to imitate color and aroma equal to the aged brandies or apply methods for accelerating distillate ageing (ageing in a wooden barrel exposed to warmth and light or ageing in a wooden barrel with wooden chips). Although legislative allows this way of production, these products are fraud to consumers and are example of unfair market competition. Another complex group of compounds that are primarily produced during fermentation and distillation, but also during ageing of spirits, are volatiles (higher alcohols, esters, aldehydes). The volatile compounds are responsible for the quality and safety of spirits, depending on their composition, concentration and sensorial properties, essential for customer's acceptance.[4]

Phenolic compounds and volatiles that give specific aroma to spirit drinks can be used to classify these drinks by type, origin, country and even provenience region.[4] The widely used method for the phenolic and volatile compounds analysis is chromatography.[5] Prior to chromatographic analysis which are time-consuming, relatively expensive and require skilled personnel, various extraction methods have been widely used, such as liquid-liquid extraction.

From aforementioned, there is a need to establish a rapid method to validate the authenticity and quality of these alcoholic beverages. Such an analytical method should accomplish a fast data acquisition, carry out data treatment accurately with relatively low costs and measure sample as intact, without any additional preparation. Spectroscopic techniques combined with chemometric data analysis are non-destructive methods that provide relative rapid and low cost alternative to traditional chemical composition and sensory analysis.[5,6] Welke *et al.* used HS-GC×GC/TOFMS associated with multivariate analysis (Fisher ratio, PCA and LDA) to investigate the volatile composition of wines.[7] This proved to be an interesting approach to differentiate wines according to their original grape cultivars and also to find potential markers of these grape cultivars. NIR technique has been used for determination of food and beverage quality.[8] NIR and chemometrics were proposed to classify 69 samples of distilled spirits

with respect to type (whiskey, brandy, rum and vodka) and presence/absence of adulterants.[9] UV–Vis and near infrared (NIR) spectroscopy demonstrated the possibility of grouping single-malt whiskies according to their geographic area of production.[10] A distinguishing of commercial samples of Slovak, Belgian, German, Czech and British juniper-flavored spirit drinks based on spectroscopic methods combined with the principal component analysis (PCA), followed by the PCA-linear discriminant analysis (PCA-LDA) were presented by Sádecká *et al.*[11] The same authors geographically classified Czech, Hungarian and Slovak plum spirit drinks by synchronous fluorescence spectroscopy.[12] Jakubikova *et al.* classified 67 fruit spirits (apple, apricot, pear and plum spirits) by type using NIR combined with multivariate analysis.[13] Although there are numerous reports on the use of NIR spectroscopy in food and beverage analysis,[9,10,12–15] the potential of NIR spectroscopy in the wine spirit/brandy ageing degree estimation have not been studied.

Thus, the aim of this study was to develop partial least squares regression (PLS) models for higher alcohols and phenols in wine spirits as well as to show the feasibility of the NIR spectroscopy combined with chemometric tools to distinguish wine spirits and brandies with different ageing degree. The multivariate analysis of obtained spectroscopic and chromatographic data was performed. Principal component analysis (PCA) was used to classify the wine spirits and brandies as spirit drinks produced from the wine spirits based on their phenolic and higher alcohols composition. Moreover, the partial least squares regression (PLS regression) was used to calibrate and predict expected contents of higher alcohols in the wine spirits and their ageing. This strategy can be used as a screening analysis for rapid classification of spirit drinks and could become an effective authentication tool when coupled to chemometrics.

EXPERIMENTAL

Samples

In these study, 13 brands of wine spirits with different ageing period (WS1-WS13) acquired from the small scale manufacturer and 3 commercial brandies (Brandy 1–3, colored) produced in Croatia were analyzed. Manufacturer defined samples as: WS 1, 3, 11, 12, 13 not--aged, colorless wine spirit; WS 4 and 7 colored samples briefly aged (2–3 years) and WS 2, 5, 6, 8, 9, 10 aged, colored samples. Wine spirits aged in lightly charred oak barrels whose volumes were 225, 300 and 330 L. All samples were characterized by ethanol content, total acidity, total esters and higher alcohols composition. According to color, only the colored samples were submitted to phenolic composition determination by HPLC, while NIR spectrum was obtained for all samples. Samples were stored in dark at room temperature until analysis.

Determination of wine spirits physicochemical parameters

Determination of alcoholic content of samples is based on density determination by pycnometer method.[16] Total acidity and total esters were determined in accordance with Regulation.[17]

Determination of wine spirits volatile and polyphenol compounds

Major volatile compounds were analyzed by gas chromatography (GC)[18] while qualitative and quantitative analyses of polyphenol composition of wine spirits and brandies were performed by HPLC.[19] A detailed methods description is given as Supplementary material to this paper.

Near infrared spectroscopy (NIR spectroscopy)

The NIR spectrophotometer, Control Development, Inc., NIR-128-1.7-USB/6.25/50 μm, with installed Control Development software Spec32 with halogen light source (HL-2000) was used. The ranges of this spectrophotometer are 11062–5885 cm^{-1} or 904–1699 nm with a spectral resolution of 6.25 cm^{-1}. Ten different spectral measurements were conducted for each wine spirit and brandy. As spectral data pre-processing method the Savitzky–Golay smoothing was used.[20]

Multivariate analysis

All statistical analyses were carried out using software (Statistica, v. 8.1, StatSoft Inc., USA). Analysis of variance (ANOVA) with use of Tukey's test ($P < 0.05$) was applied to determine the significance of differences among the wine spirits and brandies.

All conducted results, as NIR spectroscopy (796 data per scan) and contents of observed alcohols (10 parameters) as well as the polyphenolic content (9 parameters) of wine spirits and brandies, were submitted to principal component analysis (PCA) in order to interpret measured content of the observed different wine spirits and brandies and changes in the observed alcohols as well as the polyphenolic content vs. associated NIR spectra.

Multivariate tools as PCA are aimed to derive a small number of independent linear combinations (principal components, PCs) for the observed set of variables, retaining as much as possible information. Scatter plot of the 1st principal component *versus* the 2nd principal component was used to present the results of this study. The PC loadings explain which parameters were responsible for such separation of the observed samples.[21]

Partial least regression models (PLS) were developed based on the NIR spectroscopy of wine spirits and brandies. The data matrix used in the modeling consisted of 160 rows (10 spectra for each WS and brandy) and 796 columns (NIR spectra wavelengths, pace 1 nm). For each sample, a set of 6 spectra was chosen to serve as a training dataset (96 spectra in total) and the rest of 4 spectra were used for the testing dataset (64 spectra in total). All PLS models were evaluated on the values of coefficient of determination (R^2), root mean square errors of cross calibration and validation (*RMSEC* and *RMSEV*, respectively), ratio of performance to deviation (*RPD*) and ratio of error range (*RER*).

In the PLS models were used NIR spectra to calibrate and validate the expected contents of higher alcohols and phenolic compounds in wine spirits. Desirable parameters used for estimation of PLS model efficiency are lower root mean square errors with higher R^2, *RPD* and *RER*.[22] A good model will range from 0.83 to 0.9 for the R^2; 5–6.4 for the *RPD* value and the preferable *RER* is over 10.[23]

Applied linear discriminant analysis (LDA) was used to evaluate the efficiency of sample separation based on type (wine spirit or brandy). LDA is a supervised pattern recognition technique with the task of inferring a function from labelled training data. The training data were randomized wine spirits and brandies, and their phenolic components and alcohols.

RESULTS AND DISCUSSION

Wine spirits chemical analysis

Wine spirits chemical composition depends on the compounds present in wine being sufficiently volatile to distill. The most abundant volatile components in distillate are derived from yeast metabolism (esters, higher alcohols, aldehydes and acids) and grapes volatile which give distillates fruity and floral notes. There are two main categories of flavor-active esters in fermented beverages: acetate esters as well as medium-chain fatty acid ethyl esters. Fusel alcohols produced during fermentation and contribute to essential aroma and flavors include propan-1-ol, 3-methylbutan-1-ol (isoamyl alcohol), 2-methylpropan-1-ol (isobutanol), 2-methylbutan-1-ol (active amyl alcohol), 2-phenylethan-1-ol.[24] Terpenes like geraniol, nerol, linalool, citronellol, nerolidol, β-damascenone and vitispiranes are key odorant compounds in distilled wine spirit derived from grapes.[25] During the ageing process, the interaction between distillate components, oxygen and the substances derived from wood occurs, causing many changes of the chemical and sensory characteristic of the distillate. Physicochemical parameters and volatile compounds identified by gas chromatography in the tested fresh and aged distillate and brandies are listed in Table I.

TABLE I. ANOVA results and mean values of content of ethanol, esters and acids observed in all wine spirits and brandies; different letters in the same column indicate significant differences according to the Tukey's test ($P < 0.05$); a.a

Sample	Content of ethanol vol%	Content, g/(100 L absolute alcohol)								
		Total esters	Total acids	Meth-anol	Higher alcohols	3-Meth-ylbutan-1-ol	2-Meth-ylpropan-1-ol	Propan-1-ol	Butan-2-ol	Butan-1-ol
WS1	76.6^{a}	40.4^{a}	11.8^{a}	104.6^{a}	112.5^{a}	81.6^{a}	20.3^{a}	10.2^{a}	0.2^{a}	0.2^{a}
WS3	81.8^{a}	7.0^{b}	7.7^{a}	26.5^{b}	176.8^{c}	122.6^{b}	29.3^{a}	22.8^{c}	1.1^{c}	1.0^{c}
WS11	77.1^{a}	15.3^{b}	37.2^{b}	53.9^{b}	212^{b}	166.4^{b}	21.9^{a}	21.6^{c}	1.7^{c}	0.4^{a}
WS12	75.2^{a}	17^{b}	83.0^{c}	49.5^{b}	284.2^{b}	211.4^{c}	54.8^{b}	14.9^{a}	1.9^{c}	1.2^{c}
WS13	77.0^{a}	26.7^{a}	19.4^{a}	56^{b}	212.1^{b}	156^{b}	32.4^{a}	21.7^{c}	1.3^{c}	0.7^{c}
WS4	67.0^{b}	10.7^{b}	9.0^{a}	0.2^{c}	245.1^{b}	187.2^{b}	34.1^{a}	22.5^{c}	0.0^{a}	1.3^{c}
WS7	64.7^{b}	63.1^{c}	52.7^{b}	112.2^{a}	243^{b}	169.7^{b}	47.1^{b}	25.3^{b}	0.0^{a}	0.9^{c}
WS2	71.9a	28.5^{a}	36.7^{b}	140.8^{a}	268.7^{b}	180.5^{b}	50.7^{b}	28.5^{b}	4.3^{b}	4.7^{b}
WS5	73.6^{a}	144.2^{c}	134.5^{c}	42.6^{b}	375.9^{d}	242.3^{c}	59.9^{b}	36.1^{b}	32.5^{d}	5.1^{b}
WS6	67.2^{b}	77.6^{d}	26.2^{b}	46.7^{b}	378.8^{d}	286.3^{c}	67.4^{b}	19.4^{c}	3.9^{b}	1.8^{c}
WS8	70.0^{a}	117.6^{c}	81.4^{c}	79.4^{c}	370.2^{d}	243.5^{c}	60.4^{b}	37.0^{b}	26.4^{d}	2.9^{b}
WS9	68.5^{a}	4.5^{b}	54.0^{b}	13.8^{b}	385.5^{d}	279.8^{c}	66.9^{b}	24.1^{b}	10.8^{b}	3.9^{b}
WS10	39.9^{c}	69.8^{d}	38.0^{b}	106.4^{a}	259^{b}	181.9^{b}	50.3^{b}	25.9^{b}	0.0^{a}	0.9^{c}
Brandy 1	41.3^{c}	33.2^{a}	5.8^{a}	187^{e}	165.9^{c}	108.4^{b}	28.0^{a}	29.1^{b}	0.0^{a}	0.4^{a}
Brandy 2	39.2^{c}	38.6^{a}	163.3^{c}	79.7^{c}	259.7^{b}	171.8^{b}	46.6^{b}	32.4^{b}	7.6^{b}	1.3^{c}
Brandy 3	41.1^{c}	87.7^{d}	217.7^{d}	70.4^{c}	316.6^{d}	224.7^{c}	52.9^{b}	22.8^{c}	14.8^{b}	1.4^{c}

The ethanol concentration of the tested samples varied from about 65 to 80 vol% in accordance with the industry practice to be placed on ageing distillate with a high ethanol degree. Higher ethanol degree provides better lignin degrad-

ation and better extraction of compounds from the wood. The ethanol concentration differences between the samples originate from different ethanol concentration achieved by distillation, but oscillations in the ethanol content of the distillate may occur also due to evaporation of alcohol and water, depending on the cellar temperature and humidity, as well as ethanol oxidation to ethanal and acetic acid.[26]

In the samples analyzed, total esters (ethyl acetate, ethyl lactate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) varied from 4.5–144 g/(100 L absolute alcohol (a.a.)). Using univariate statistics, such as ANOVA approaches, differences ($P > 0.05$) in the total esters were not observed between not-aged and aged samples. However, some differences can be noticed. In aged samples, esters show a mean content of ≈ 74.5 g/(100 L a.a.), higher than the mean values obtained for the not-aged samples (≈ 21 g/(100 L a.a.)). During the ageing process the content of esters changes; some of esters are formed and others change their amount.[27] Total ester content increases mostly due to ethanol oxidation and acetic acid formation. Ethyl acetate is the most abundant acetate in the distillates derived from the yeast metabolism, as well as esterification process during the ageing. Additionally, distillates usually contain other odorous esters, especially fatty acid ethyl esters (ethyl butanoate, ethyl hexanoate; ethyl octanoate) as well as acetates of higher alcohols, mostly 3-methylbutyl acetate (isoamyl acetate). Except for the ageing process, the presence and concentration of esters depend on other variables like grape variety, soil type, climate conditions, yeast strain and must fermentation conditions, as well as type of distillation and presence of yeast cells in the wine at the time of distillation.[27]

Literature data indicate that the amount of acid during ageing increases[26] due to ethyl alcohol oxidation stimulating the wood hydrolysis of components and liberation of phenolic acids. Regard to tested samples there was no difference ($P > 0.05$) in total acid content with respect to the ageing process (Table I). Difference can be explained by different initial amount of acids in wine and in distillate before ageing which is characteristic of the quality of raw materials as well as grapes varietal characteristics. Similar results were obtained for the methanol. Methanol concentration depends on quality of raw materials, type of distillation and proper fractions separation. In distillate, methanol is found in concentrations ranging from 30–70 g/(100 L a.a.) while the total acid concentration ranges from 20–100 g/(100 L a.a.).[28]

Higher molecular weight alcohols present in distillates in optimal concentration contribute to the sensory quality of the distillate. They are products of yeast amino acids metabolism and because of that yeast species, fermentation conditions and distillation process are affecting their content in the not-aged distillate. According to the results given in the Table I, there was statistically significant difference in higher alcohol concentration in the not-aged (^{a,b,c}) and

aged samples ([d]). Concentrations of all measured alcohols, especially isoamyl alcohol, were higher in aged samples. Concentration of higher alcohol in aged distillates was approximately 375 g/(100 L a.a.). According to Tsakiris *et al.*[28] concentrations of higher alcohols in brandy is usually in the range of 250–500 g/(100 L a.a.).

The relationship between phenolic compounds and the ageing period is well--known.[29,30] During the ageing process distillate is enriching with extractable phenolic compounds that are released from the wooden cask. Duration of ageing period, as well as type of wood, are among the most important factors that determine phenolic composition and sensory characteristics of the resulting aged distillate.[31] The phenolic compounds identified and quantified by HPLC in the analyzed wine spirits and brandies are listed in Table II. Since phenolics originate exclusively from wood, and are not present in fresh distillate, they could be used as wood ageing markers. Based on phenolic compounds, there is a significant difference ($P > 0.05$) between wine spirits aged in wooden cask for a longer period (WS 2, 5, 6, 8, 9) and wine spirits aged for a shorter period (WS 4, 7) or not-aged spirits/adulterated (WS 10). These differences result from the physical and chemical changes (extraction and oxidation processes) during wine distillate ageing, but there is also a strong influence of the chemical composition of the wood itself and its heat treatment/toasting level.[32] The phenolic aldehydes are produced by thermodegradation of the terminal monomer units of lignin: the cinammic aldehydes convert to benzoic aldehydes, and then they are oxidized to corresponding phenolic acids.[33]

TABLE II. ANOVA results and mean values (mg/(100 mL) of phenolic compounds HPLC analysis) observed in different wine spirits and brandies; different letters in the same column indicate significant differences according to the Tukey's test ($P < 0.05$); ND – not detected; GA – gallic acid; dGA – gallic acid derivate; VA – vanillic acid; Syr – syringic acid; V – vanillin; SYAL – syringaldehyde; CoAL – coniferaldehyde; EA – ellagic acid ; dEA – ellagic acid derivate

Sample	Content of phenolic compounds, mg/(100 mL)								
	GA	dGA	VA	Syr	V	SYAL	CoAL	EA	dEA
WS4	ND	ND	ND	ND	0.02^{a}	ND	ND	0.20^{b}	ND
WS7	0.02^{b}	ND	0.01^{a}	0.05^{a}	0.03^{a}	0.15^{a}	0.51^{b}	1.08^{d}	ND
WS2	0.13^{a}	ND	0.04^{a}	0.05^{a}	0.02^{a}	0.08^{a}	ND	0.95^{a}	ND
WS5	0.79^{c}	ND	0.36^{b}	0.45^{b}	0.44^{b}	0.78^{c}	0.54^{b}	6.61^{c}	0.97^{b}
WS6	0.58^{c}	ND	0.28^{b}	0.30^{b}	0.27^{c}	0.63^{c}	0.46^{b}	5.07^{c}	0.86^{b}
WS8	0.62^{c}	0.06^{a}	0.48^{b}	0.58^{c}	0.53^{b}	1.33^{d}	0.19^{c}	5.03^{c}	ND
WS9	0.30^{a}	0.18^{b}	ND	0.08^{a}	ND	0.21^{a}	0.26^{c}	2.05^{d}	ND
WS10	ND	0.15^{b}	ND	ND	ND	ND	ND	ND	ND
Brandy 1	ND	ND	0.17^{b}	0.06^{a}	0.35^{b}	0.02^{b}	0.06^{a}	0.13^{b}	ND
Brandy 2	ND	ND	ND	ND	0.09^{a}	ND	ND	ND	ND
Brandy 3	0.38^{c}	ND	0.48^{b}	0.20^{b}	1.10^{d}	0.43^{c}	0.62^{b}	1.99^{d}	ND

Wine spirits aged in wooden cask for a longer period are richer in phenolic acids than wine spirits aged for a shorter period (Table II). In the former, all the phenolic acids are present in the higher concentrations: ellagic acid (EA) is the most abundant compound, followed by gallic acid (GA), syringic acid (Syr) and finally, vanillic acid (VA). Although ellagic acid was found in the almost all analyzed samples, higher concentrations were observed in wine spirits 5, 6 and 8 followed by wine spirits 9 and 7 and brandy 3. The content of gallic acid in analyzed wine spirits was significantly higher in wine spirits 5, 6 and 8 and brandy 3. In contrast, in wine spirits 4 and 10 and brandies 1 and 2 gallic acid was not found. Vanillic and syringic acid were present in significant concentrations also in wine spirits 5, 6, 8 and brandy 3. Vanillin is the phenolic aldehyde which greatly influences the aroma of spirits because of its low threshold value (320 μg L^{-1}) and adds positive vanilla notes.[34] Consequently, vanillin could be added afterwards by the producers in order to adapt brandies aroma. This was the case with brandy 2 where only vanillin was detected. Because of the absence of other phenolics it could be concluded that this sample is not-aged. Moreover, ratio of syringaldehyde to vanillin above 1 indicates spirits produced by ageing in oak barrels over a long period as in sample WS5, WS6 and WS8 (ratio ≈ 1.7–2.5, Fig. 1). Brandy 1–3 do not meet this criterion, which indicates the manufacturer's habit to add extra vanillin quantities to their products. Also, gallic acid/vanillin ratio indicates quality of spirits and it is the largest for same samples WS5, WS6 and WS8.

Ellagitannin degradation during the heat treatment of the wood and ellagitannin hydrolysis during the ageing process are the major sources of ellagic acid in brandy.[32] The ellagic acid content is particularly important for the evaluation of brandy authenticity, as wood ageing marker, the differentiation of brandies according to the botanical species and the geographical origin of wood[29] as well as for the taste, flavor and color of the final products. Gallic acid concentration in aged spirits depends on hydrolysis of wood digallic acid and the toast level of casks, since gallic acid is degraded at high temperatures. Consequently, this compound is more abundant in spirits aged in casks with light or medium toast level.[29,32] Furthermore, concentration of vanillic acid also decreased with higher temperatures during the toasting process. Vanillic acid can be directly extracted from oak wood or be formed by oxidation of vanillin during the ageing period, whereas syringic acid is formed during toasting by the oxidetion of the corresponding aldehyde.[30] Syringaldehyde is predominant, probably due to higher accumulation in the wood as a consequence of higher thermal stability than the vanillin and coniferaldehyde. According to Panossian *et al.*[35] adulterated, not-aged spirits/brandies are easy to recognize by the absence of sinapaldehyde, syringaldehyde and coniferaldehyde.

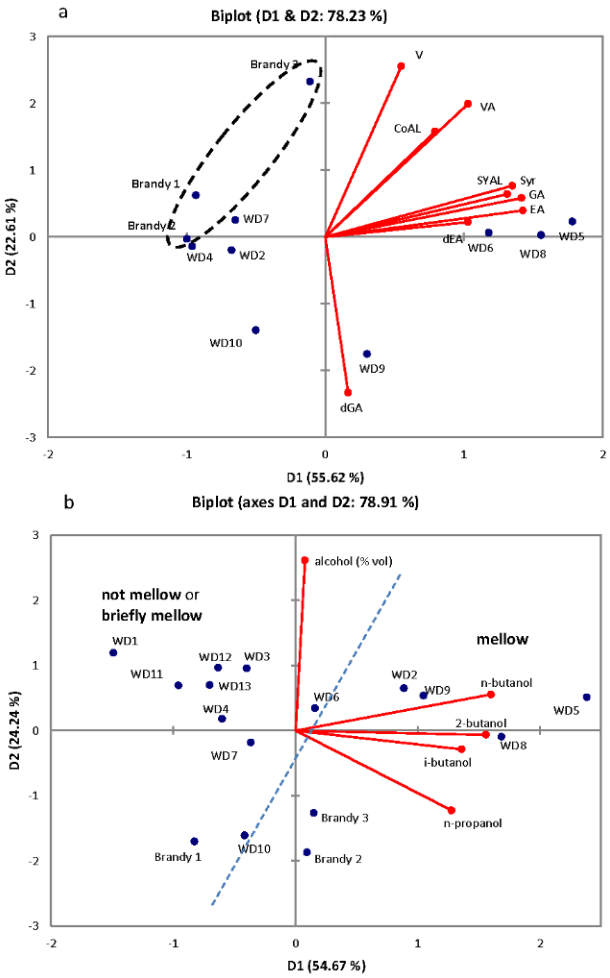

Fig. 1. Principal component analysis of clustering of brandies from the wine spirits (WS) based on the phenolic composition (a) and on higher molecular weight alcohols (b).

NIR spectra

Although all tested samples had similar NIR spectra differences in the intensity of some peaks were observed (Supplementary material, Fig. S-1). Significant differences were observed in two different NIR spectra regions, first: 904 to 935 nm and the second identified specific region from 1400 to 1699 nm. The vibrations in in the first specific range of the NIR spectra are related to the third CH and ROH overtone, second overtone, and a combination of stretch and determination of the OH stretch of H_2O,[15] which are the bands and groups expected in phenolic and aromatic compounds. Spectral differences based on vibration differences of observed samples; in the range from 1400 to 1699 nm present the vib-

ration of the C–H and O–H bonds corresponding to the water and phenolic and aromatic absorbance.[5] Those spectra performance is used in the PLS modelling of alcohols and phenols in the wine spirits as well as to detect and predict the ageing of spirits and brandies.

Chemometric data analysis

In the current work, the performance of three different multivariate statistical techniques was assessed: PCA, PLS and LDA.

Principal component analysis (PCA) allowed identification of significant parameters in the total data matrix which consisted of 42 rows (samples were measured in triplicate, and the average presented the fourth value) and 815 columns, number of columns was reduced identifying 336 significant NIR data (absorbance units at wavelengths from 904–935 and 1400–1699 nm), 5 physico-chemical parameters and 9 phenolic compounds per each row). The clustering of the samples was investigated by visualization of discriminating the analyzed wine spirits and brandies based on the phenolic composition (Fig. 1a) and higher alcohols (Fig. 1b). Each component of a PCA model is characterized by two complementary sets of attributes: loadings and scores. Loadings describe the data structure in terms of variable correlations. The scores describe the properties of the samples (differences or similarities). When the phenolic compounds are observed, explained are 78.3 % of all variances in the observed data matrix and the brandies separated from the wine spirits in the second quadrant.

When the content of total and partial alcohols is observed, the plot of scores on PC1 against PC2 with the cumulative contribution over 78 % was the first step to visualize the main trends in the sample set. The aged spirits are dominant on the right side of the chart while the briefly and non-mellowed spirits are positioned in the second and third quadrant. The grouping is mostly caused by the alcohol content (ethanol, butan-2-ol and butan-1-ol) with the PC2 = 24.2 %, and 2-methylpropan-1-ol and propan-1-ol with the dominant contribution in the PC1 (54.6 %).

In order to study applicability of NIR spectroscopy in calibration and validation of alcohol and phenol content as well as aging process, partial least square models method was used (Supplementary material, Table S-I).

The PLS modelling was performed on the entire NIR range (904–1699 nm) to observe the absorption bands related to alcohol(s) and phenolic compounds (C–H stretch; $C–H_3$ stretch or compounds containing C–H aromatic groups; the O–H overtone of water and related R–OH). Row NIR spectra *vs.* content of individual phenols and alcohols in wine spirits, as well as the information of ageing of wine spirits and brandies were included in the modeling. The spectral data undergo spectral pre-processing, the smoothing by Savitzky–Golay. The final

input data matrix for PLS models consists of 64 rows and 811 columns. Based on developed PSL models 2/3 of data were used in calibration and 1/3 for validation.

To evaluate the efficacy of the models, the root mean square errors of calibration (*RMSEC*) and cross validation (*RMSECV*) as well as the relative errors of prediction (*REP*) were calculated.[36]

The R^2 values in the calibration models ranged from 0.82–0.98. Fearn[38] explained that mentioned parameters (*RMSECV*, *RPD* and *RER*) are useful indices of the model efficiency and they are most commonly used parameters in assessing the applicability of the model in process control and prediction of parameters.[15] Fragoso *et al.*[39] reported FT-MIR spectroscopy and chemometrics as a rapid method to quantify phenolic compounds all during the red winemaking process. The calibration models yield good calibration statistics for the different parameters evaluated ($R^2 > 0.95$ and $RPD > 4.0$ for *TPC*; $R^2 > 0.90$ and $RPD > 3.0$ for TA; $R^2 < 0.8$ and $RPD < 3.0$ for CT). It was concluded by the authors that FT--MIR spectroscopy together with multivariate calibration could be a rapid and valuable tool for wineries to carry out the monitoring of phenolic compound extraction during winemaking. R^2, for individual alcohols calibration and validation (prediction) are higher than for individual phenols as well as *RPD* and *RER*, respectively. The highest values are achieved for aging ($R^2 = 0.99$; $RPD \approx 6.8$ and $RER \approx 15$) with preferable values showing the potential to use NIR spectra in quantitative prediction of this parameter (ageing). With successful prediction of ageing process it should be possible to detect potential fraud. The price of spirits proportionally increases with ageing. De Villiers and co-workers[40] were successful in classification of wines according to the grape variety using discriminant analysis (DA). Evaluation of the classification performance regarding the examined samples (wine spirits and brandies) was provided by use of LDA (Fig. 2), containing 16 variables (ethanol; esters; acids; 2-methylpropan-1-ol; propan-1-ol; butan-2-ol; butan-1-ol; gallic acid; gallic acid derivate; ellagic acid; ellagic acid derivate; vanillic acid; syringic acid; vanillin; syringaldehyde; coniferaldehyde).

First two roots (F1+F2) for both classifications (based on individual alcohols and phenols) were found to explain 100 % of the properties of different wine spirits and brandies (Fig. 2). The classification based on individual phenols in spirits and brandies are totally successful, while the classification based on individual alcohols was successful for 93.8 % with the less effectiveness for samples that are briefly aged (66 %). Phenols are reliable parameters that should be used in identification of aged samples. Thus, applied LDA showed to be a suitable and simple supervised statistical approach to assess the ageing process of spirits and brandies based just on NIR spectra.

Multivariate tools or chemometrics proved to be a useful tool in classifications, even of complex samples as wine spirits and spirits that differ in their phenolic composition and alcohol composition.

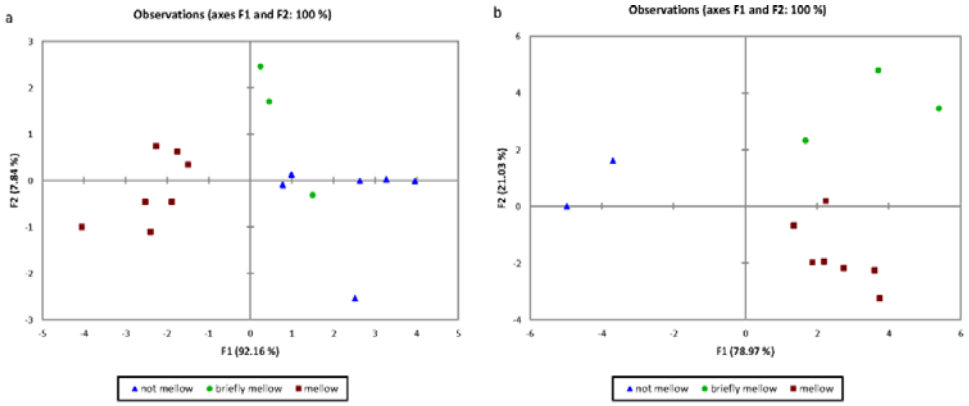

Fig. 2. Linear discriminator analysis classification of wine spirits (WS) and brandies based on aging using individual alcohols (a) and phenols (b).

CONCLUSIONS

NIR spectroscopy was used in present study to analyse raw wine spirit and wine spirit and brandy with different ageing times. The principal component analysis based on the phenolic composition and higher molecular weight alcohols has been proven as a good method for classification of brandies of different ages. Chemometrics showed to be a powerful mathematical technique when it is related to spectroscopic techniques for the analysis of phenolic compounds and alcohols in wine spirits and brandy. Such modelling allows valuable information from large data sets to be obtained, underpinning the application of methods based on NIR spectroscopy that is a fast and easy-to-operate techniques. The *RER* values (ranged from 4.3 to 20.8) suggest that NIR spectroscopy could be an operative method for the measurement of important parameters as alcohols in wine spirits/brandies and for quantitative prediction of those parameters.

SUPPLEMENTARY MATERIAL

Additional data are available electronically from http:// www.shd.org.rs/JSCS/, or from the corresponding author upon request.

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ИЗВОД

ИДЕНТИФИКАЦИЈА ФЕНОЛНИХ И АЛКОХОЛНИХ ЈЕДИЊЕЊА У ВИНСКИМ ПИЋИМА И ЊИХОВА КЛАСИФИКАЦИЈА ПРИМЕНОМ МУЛТИВАРИЈАНТНЕ АНАЛИЗЕ

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Винска пића мењају своју боју, хемијски састав и укус током сазревања, а промене би требало лако пратити. Циљ ове студије је био да развије парцијални регресиони модел најмањих квадрата (PLS) за више алкохоле и феноле у винским пићима и ракијама, као и да докаже применљивост NIR спектроскопије комбиноване са хемометријским приступом за препознавање пића различите старости. Референтне вредности су добијене коришћењем уобичајених метода за анализу алкохолних пића. Одређивани су етанол, естри, киселине, метанол и виши алкохоли. Садржај фенолних једињења је одређен течном хроматографијом. Класификација пића је урађена на основу садржаја фенолних једињења и виших алкохола користећи методу анализе главних компонената (PCA). PLS регресиони модел је послужио за калибрацију и предвиђање очекиваног садржаја ових једињења у винским пићима. Успешност класификације узорака по основу старења је била 93,8 %, мерена према садржају појединачних алкохола, а 100 % мерена према садржају фенола. Ефикасност предвиђања је процењена користећи линеарну дискриминантну анализу (LDA).

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