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Near-infrared spectroscopy as a tool for rapid estimation of the antioxidant capacity of red wines

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The present investigation evaluates the feasibility of using near-infrared (NIR) spectroscopy as an accurate, fast and non-destructive analytical tool for estimation of the antioxidant properties of red wines. The evaluation of the antioxidant properties was conducted using ABTS and DPPH assays for total antioxidant activity determination. NIR measurements were performed by NIRQuest 512 spectrophotometer in the region 900-1700 nm using transmittance mode. Partial least-square regression with internal cross-validation was used for calibration models development for determination of tested parameters and SIMCA for creation of model for classification on the base of the spectral data. All the investigated wines have demonstrated better antioxidant properties in the ABTS model system compared to the DPPH one. Although the observed activity highly varied, the wines containing varieties Syrah and Malbec presented better antioxidant effect in both model systems. The Merlot wines produced from the vineyard situated on the southern slopes of Sakar Mountain in the time interval of 2012 – 2016 denoted a tendency of decreasing SV_{50} values from year to year. In both model systems Merlot 2016 demonstrated the best antioxidant effect correlating to the lowest SV_{50} values. The determination of the antioxidant activity of the tested wines on the basis of their spectra in the NIR region revealed a high degree of accuracy of estimation. This indicates that NIR spectroscopy could be a promising technique in quantitative determination of antioxidant activity and building classification models for discrimination of wines according to their antioxidant properties.

Keywords: Wine, Antioxidant activity, ABTS, DPPH, NIR spectroscopy

INTRODUCTION

During the years the customers' taste and preferences have changed in response to health concerns. The fact that the increased consumption of products rich of natural polyphenols like grapes, cherries and berries is associated with diminished rick of cardiovascular disease has attached particular attention to red wine products due to the abundant content of biologically active compounds in them [1, 2].

The phenolic compounds (anthocyanidins, flavanols, flavones, flavanones, cynnamic acids and stilbenes) which have important impact on the organoleptic characteristics of the wine product are some of its most important quality parameters. There are literature data revealing their multiple biological activities associated with anti-carcinogenic, antiviral, antibacterial and anti-inflammatory effects mainly attributed to their antiradical activity and powerful antioxidant properties [3, 4].

The customers' desire for quality products possessing health benefits and the increasing number of available new wines on the market has created the necessity of the development of a fast and easy way to operate, accurate analytical tool for the quantitative determination of these bioactive compounds.

During the years several methods have been used for screening of the chemical composition and food quality determination and prediction – high-performance liquid chromatography (HPLC), near-infrared spectroscopy (NIR), mass spectrometry (MS), capillary electrophoresis in combination with different electrochemical (EC) detectors [5]. From all of them NIR spectroscopy has proven itself as a perfect technology for routine analysis in the food industry due to the speed of the performed analysis and its low cost [6]. It is a non-destructive method, requiring minimal or no sample preparation which has shown good prediction abilities in the determination of the content of flavanols, anthocyanins and other phenolic compounds [7-9].

The objective of the present investigation was to prove the applicability of the NIR spectroscopy as an analytical tool for estimation of the antioxidant properties of red wines and to verify its effectiveness in building classification models for

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discrimination of wine according to their antioxidant properties.

EXPERIMENTAL

Wine samples

The analysis was carried out using a total of 35 wine samples – 33 of them were Bulgarian red wines. All samples were from commercially available wines produced from a wide range of different varieties. Details for the origin and the principal grape variety are presented in Table 1. Ten wines (numbered from 1 to 10) were supplied from a winery vineyard situated on the southern slopes of Sakar Mountain – all of them produced using the same wine making technology. Five of the samples (from 1 to 5) were selected to present variation in the age (years ranging from 2012 to 2016) and six of them – grape varieties (from 5 to 10).

Spectrophotometric model systems

To determine the antioxidant properties of the wine samples we have used test systems containing stable ABTS radical cations and DPPH radicals. Despite the fact that both radicals are foreign to the living organisms the combination of these methods is the most commonly used approach for *in vitro* assessment of the antioxidant activity (AOA) of complex samples. The methods are fast, reliable and reproducible. The scavenge of ABTS radical cation and DPPH radical has a different mechanism (SET for $ABTS^{\bullet+}$ and HAT for $DPPH^{\bullet}$). The simultaneous use of both methods provides more accurate evaluation and interpretation of the character of the observed antioxidant properties.

Both methods are based on the ability of the tested compounds to scavenge the pre-formed $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ radicals, which is resulting in decolorization (decrease of the absorbance) of the sample solution proportional to the extent of the radical reduction. The latter is used as a determinant of the antioxidant activity. Stronger antioxidant properties of the tested compounds are associated with higher extent of suppression of the absorbance of the solution.

ABTS method – the experimental part was accomplished according to Re *et al.* [10]. The assay utilizes the free mono-cation radical of 2'2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), generated by the oxidation of ABTS with potassium persulfate. The working solution was obtained by diluting the preformed $ABTS^{\bullet+}$ in buffer solution - K_2HPO_4/KH_2PO_4, pH 7.4 to produce a final solution with absorbance of 0.700 ± 0.002 at 734 nm. The reduction of the absorbance of 2 ml of the $ABTS^{\bullet+}$ by different volumes of a 2% solution of the bottled wine product was measured at 734 nm after 1 hour incubation. Fresh radical solution was prepared for each experiment.

DPPH method – the procedure was performed according to Goupy *et al.* [11]. A working purple colored solution of the DPPH radical in ethanol with absorbance of 0.900 ± 0.003 at 517 nm was prepared. Different volumes of a 2% solution of the bottled wine product were mixed with 2 ml of it. After 60 min incubation the absorbance of the probes was measured at 517 nm.

All analyses were performed in triplicate. The obtained results were presented as means ± S.D. The AOA of the wine samples was estimated using the equation:

$$AOA\% = \frac{A_{control} - A_{sample}}{A_{control}} \bullet 100\%$$

Considering the observed results for AOA, the volume of bottled wine product, which reduces the quantity of free radicals in the final reaction volume of the system by 50% was calculated – SV_{50} (scavenge volume 50) The smaller the SV_{50} the higher is the antioxidant potential of the tested wine.

NIR spectral measurements and data analysis

The near-infrared analysis was performed using NIRQuest 512 spectrometer (Ocean Optics, Inc) in the region of 900-1700 nm using transmittance mode and 10 mm cuvette.

A commercial software program – Pirouette Version 4.5 (Infometrix, Woodinville, WA) was used to process the obtained data. PLS regression was used for quantitative determination of SV_{50} on the base of spectral data. The calibration equations for each parameter were developed and validated with leave-one-out cross validation. Leave-one-out method is recommended when a few samples are used to build the calibration equations. The leave-one-out cross-validation routine works by omitting one observation once a time, recalculating the equation using the remaining data, and then predicting the omitted observation. This routine is repeated until each observation in the dataset is used once as validation data. Finally, any validation errors generated are combined into a standard error of cross-validation SECV.

The prediction capacity of each calibration equation was evaluated using R – multiple correlation coefficients between reference values and NIR spectra, SEC – standard error of calibration, SECV – standard errors of cross validation and the ratio performance deviation

(RPD) parameter, which can be defined as the relationship between the standard deviation of the chemical method (SD) and the standard error of cross-validation SECV in the NIR model. The RPD evaluated the prediction errors in light of the standard deviation of the reference data and thus enables comparison between models for constituents with different variation ranges. The RPD values showed levels of prediction accuracy as follows: RPD between 2.0 and 2.5 indicates good prediction; RPD between 2.5 and 3.0 indicates very good prediction; and RPD > 3 indicates excellent prediction.

Soft independent modeling of class analogy (SIMCA) was performed to classify samples according to their antioxidant capacity. SIMCA is a supervised classification method and develops models for each initially determined group of samples (class) based on principal components analysis (PCA). SIMCA first centers and then compresses raw data by means of PCA, and a multidimensional space is constructed containing the scores corresponding to each class. Each class model treats new samples separately, and an assessment of cluster membership is made on the basis of the distance of any given sample to the center of the cluster.

Samples were divided into two classes with low and high antioxidant capacity according to SV_{50} values, estimated by DPPT method. The threshold value was set to be 3.0 μl.

RESULTS AND DISCUSSION

The radical scavenging activity of the red wine samples was tested in model systems containing stable free radicals – ABTS and DPPH. The results from the performed experiments and additional information for the wines (brand, varieties and vintage) are presented in Table 1. The examined wines showed antioxidant activity in both model systems.

Comparing the results from both model systems presented in Table 1 it is evident that all samples exhibit significant differences in their reduction activities against the ABTS and DPPH radicals. The wines demonstrate changeable antioxidant activity in each model system, which is due to the different wine varieties, vintages and differences in the production technologies. All wine samples denote almost twice higher efficiency against the ABTS radical (SV_{50} from 0.919 μl to 2.154 μl) in comparison to the DPPH radical (SV_{50} from 2.170 μl to 4.850 μl). Although the observed activity highly varies the wines containing varieties Syrah and Malbec presented better antioxidant effect in both model systems.

Due to the fact that the different wine making techniques are an important factor affecting the phenolic levels of the obtained final product and respectively its antioxidant properties we have included in our investigation eleven red Bulgarian wines purchased from winery which vineyard is situated on the southern slopes of Sakar Mountain. The fact that all wines were produced using the same wine making technology will give us the possibility to compare the antioxidant properties of wines produced from diverse varieties and vintages from this region.

The comparison of the data from the Merlot wines produced from the same vineyard in the time interval of 2012 – 2016 denoted a tendency of decreasing SV_{50} values from year to year (Table 1 lines 1-5). In both model systems Merlot 2016 demonstrated the best antioxidant effect corresponding to the lowest SV_{50} value.

The NIR spectra of the tested red wine samples are presented in Figure 1a. The NIR spectrum of wine in the 900-1700 nm region is dominated by a large absorption band around 1400 nm that corresponds to OH bonds of water.

Second derivatives of the spectra of measured wine samples are presented in Figure 1b. The second derivative technique is often used approach in processing NIR data. It gives the possibility to separate the overlapping absorbance bands, to remove the baseline shifts and to increase the apparent spectral resolution. The broad absorption maximum around 1400 nm is composed from two separate maxima at 1349 and 1395 nm, connected with C-H and O-H bonds. The other intensive maxima at 976 and 1196 nm are connected with second overtone of O-H and C-H bonds, respectively. The biggest variations in the spectral data are observed between 1300 and 1400 nm.

Statistical parameters from the calibration procedure for NIRS evaluation of antioxidant activity of examined red wine samples are presented in Table 2.

Table 1. Details of red wines analyzed for antioxidant properties and SV_{50} values

	Year	Origin	Principal grapes	SV_{50} [μl] ABTS	SV_{50} [μl] DPPH
1	2012	Harmanly	Merlot	1.11	2.71
2	2013	Harmanly	Merlot	1.10	2.84
3	2014	Harmanly	Merlot	1.07	2.71
4	2015	Harmanly	Merlot	1.01	2.51
5	2016	Harmanly	Merlot	1.01	2.33
6	2016	Harmanly	Cabernet franc	1.01	2.84
7	2016	Harmanly	Cabernet sauvignon	0.92	2.17
8	2016	Harmanly	Syrah	1.00	2.30
9	2016	Harmanly	Malbec	0.96	2.28
10	2016	Harmanly	Cabernet sauvignon, Merlot, Syrah, Pamid	1.16	2.54
11	2014	Suhindol	Cabernet sauvignon	1.30	3.03
12	2013	Stara Zagora Thracian Plain	Cabernet sauvignon	1.08	2.45
13	2013	Stara Zagora Thracian Plain	Cabernet sauvignon, Merlot & Syrah,	1.05	2.45
14	2013	Pleven	Merlot	1.37	2.95
15	2013	Thracian Plain	Merlot	1.22	2.76
16	2013	Vidin	Cabernet sauvignon & Syrah	1.35	3.04
17	2013	Yambol	Merlot	1.32	3.55
18	2014	Thracian Plain	Merlot & Maller	1.19	3.07
19	2013	Asenovgrad	Merlot	1.52	3.72
20	2013	Stara Zagora	Merlot	1.45	3.59
21	2013	South Africa	Merlot	1.54	3.37
22	2014	Chilean Central Valley	Merlot	1.18	3.25
23	2013	Sakar Mountain	Cabernet sauvignon & Syrah	1.12	2.83
24	2014	Svilengrad	Merlot & Malbec	1.11	3.10
25	2015	Thracian Plain	Beaujolais	1.30	3.92
26	2013	Thracian Plain	Cabernet sauvignon	1.43	3.26
27	2013	Montana	Cabernet sauvignon	2.15	4.58
28	2013	Montana	Cabernet sauvignon & Merlot	1.30	2.97
29	2013	Veliki Preslav	Merlot	1.33	3.82
30	2013	Thracian Plain	Cabernet sauvignon	1.19	3.42
31	2013	Stara Zagora	Cabernet franc	1.26	3.49
32	2013	Thracian Plain	Cabernet sauvignon	1.15	3.01
33	2013	Danube plain	Merlot	1.21	3.47
34	2013	Italy	Barbera d'Asti	1.78	4.85
35	2013	Sakar Mountain	Cabernet sauvignon, Merlot & Syrah	0.99	2.50

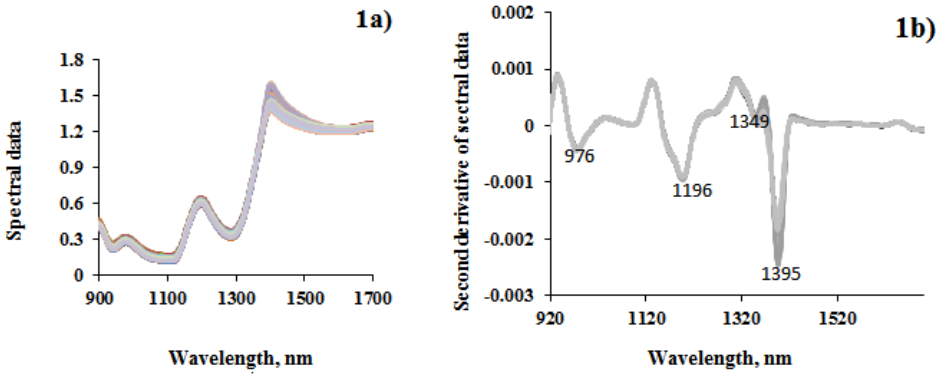

Figure 1. Near-IR spectra of wine samples (1a) and their second derivative transformation (1b).

Table 2. Statistical data of the calibration equations for NIRS determination of AOA of the red wine samples.

Method	SECV	Rcv	SEC	Rcal	RPD
DPPH	0.154	0.97	0.152	0.97	4.08
ABTS	0.079	0.94	0.077	0.95	3.06

R – multiple correlation coefficients, SEC – standard error of calibration, SECV – standard errors of cross validation, RPD – relationship between the standard deviation of the chemical method (SD) and the standard error of cross-validation SECV in the NIR model.

The obtained multiple correlation coefficients R between reference values and NIR spectra were bigger than 0.94 and the ratio performance deviation RPD parameter bigger than 3, which showed excellent prediction abilities. Graphical illustration of the accuracy of determination of tested parameters is presented in Figure 2.

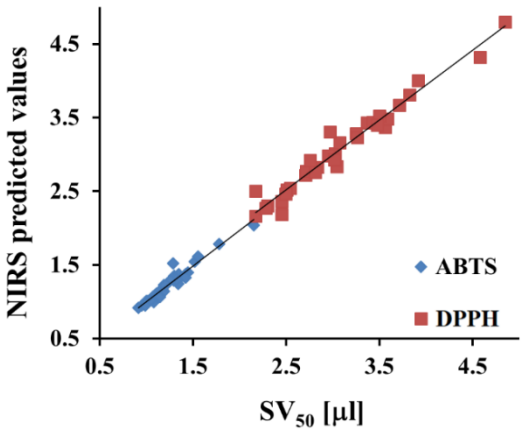

Figure 2. Scatter plots of laboratory measured and NIRS predicted values of SV_{50} determined using the ABTS and the DPPH model systems.

The SIMCA models for discrimination of wine samples with low and high antioxidant capacity were developed. The SIMCA models were compared in terms of class distance values and number of misclassified samples. The best models were obtained using smoothing and second-derivative transformation of spectral data, which correctly classified 100 % of the samples from the calibration set (Figure 3). The value of the parameter „Interclass distance“, which describes the distance from the centre of the classes, was 4.26. Large class distances imply well-separated classes. A distance of more than 3 is an indication of good SIMCA class separation and that the models are really different. The obtained value of interclass distance in our investigation indicates very good possibilities of NIRS for classification of the tested wines in different classes according to their antioxidant activity.

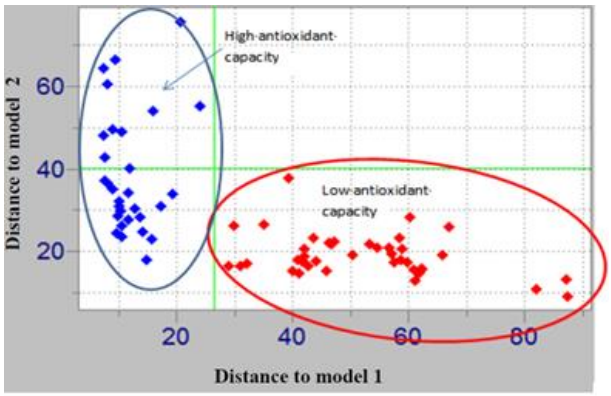

Figure 3. SIMCA classification of wine samples according to their antioxidant capacity**.**

CONCLUSION

All the investigated wines demonstrated better antioxidant properties in the ABTS model system compared to the DPPH one. The comparison of the results obtained for the wines purchased from the vineyards situated on the southern slopes of Sakar Mountain revealed that for the Merlot wines produced in the time range of 2012 – 2016 denote a tendency of decreasing SV_{50} values from year to

year. According to the data obtained from both methods Merlot 2016 demonstrated the best antioxidant effect correlating to the lowest SV_{50} values. The evaluation of the antioxidant activity of all tested wines on the basis of their spectra in the near-infrared region revealed the high degree of accuracy of estimation. According to the obtained results NIR spectroscopy could be a promising technique in quantitative determination of antioxidant activity and building classification models for discrimination of red wines according to their antioxidant properties.

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СПЕКТРАЛЕН АНАЛИЗ В БЛИЗКАТА ИНФРАЧЕРВЕНА ОБЛАСТ КАТО СПОСОБ ЗА БЪРЗО ОПРЕДЕЛЯНЕ НА АНТИОКСИДАНТНАТА АКТИВНОСТ НА ЧЕРВЕНИ ВИНА

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(Резюме)

Целта на настоящото изследване е да се оцени възможността за прилагане на спектралния анализ в близката инфрачервена област (NIR спектроскопия) като способ за бързо, точно и недеструктивно определяне на антиоксидантните свойства на червени вина. За оценка на антиоксидантните свойства са използвани моделни системи, съдържащи стабилни свободни радикали (ABTS и DPPH), позволяващи определяне на тоталната антиоксидантна активност. Спектралното измерване е проведено посредством измерване на пропускливостта на пробите вино чрез спектралния апарат NIRQuest 512, работещ в диапазона 900-1700 nm. За количествен анализ е използвана Partial least-square regression (PLS). За класификация на пробите на базата на техните спектри е използван методът SIMCA (Soft Independent Modeling of Class Analogy). Всички изследвани вина проявиха по-добър антиоксидантен потенциал в моделната система съдържаща ABTS радикал в сравнение с тази с DPPH. Въпреки че е отчетено значително вариране в изследваните свойства между отделните тествани продукти, и в двете моделни системи винята съдържащи винен сорт Сира и Малбек показаха по-добър антиоксидантен ефект. При пробите вино, произведени от един и същи лозов масив от сорта Мерло от винарната, разположена на южните склонове на Сакар планина в периода 2012-2016 г., се наблюдава тенденция за нарастване на стойността на SV_{50} със стареенето на виното. И при двата изследвани радикала стойностите са най-ниски за виното от 2016 г. Точността на определяне на антиоксидантната активност на изследваните вина на базата на спектрите им в близката инфрачервена област е много добра. Това показва, че NIR спектроскопията е подходящ метод за количествен анализ на антиоксидантния потенциал и информацията от спектрите на винята позволява създаването на модели за класификация им в зависимост от антиоксидантния им капацитет.