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NUTRITIONAL CONTRIBUTION OF BERRIES FOR FIGHTING OXIDATIVE STRESS

Ivana Bečić¹, Ivana Polović¹, Senka Djaković¹, Jasmina Lapić¹, Jasenka Gajdoš Kljusurić^{1*}, Želimir Kurtanjek¹

¹Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

*e-mail: jgajdos@pbf.hr

Abstract

Fruits and vegetables are rich in polyphenols, which are the major source of antioxidants in diet, with a number of positive effects on human health. The balance between the production of free radicals and antioxidant intake is very important because of the stress that is present in every organism. In this study was used a standard method like determination of total phenols with Folin-Ciocalteu reagent and some methods that are rarely used in the analysis of samples which are rich in polyphenols, such as near-infrared spectroscopy and Briggs-Rauscher reaction. Wild berries offer a range of vitamins, minerals and phytochemicals, and the most of them have antioxidant properties.

The aim of this work was to examine the berries (strawberries, blackberries, raspberries, blueberries and red currants) by determining the share of total phenols (TP), the antioxidant activity using the Briggs-Rauscher's method (BRAI), and determination of the colorimetric and near infrared spectroscopy (NIRs) analysis. All the mentioned was subjected to chemometric analysis to detect the "fingerprint" of the phenolic compounds for tested samples allowing separation of these samples depending on the preparation and content of the polyphenols. The extraction was conducted using the (i) reflux method and (ii) using the ultrasound.

Colorimetric analysis showed that the darker and extracts with domination of blue-green color have more antioxidant power than the lighter and yellow-red samples. Application of near-infrared spectroscopy and Briggs-Rauscher reaction proved to be very successful in measuring the polyphenolic compounds and thus the highest value of antioxidant activity showed blackberry (BRAI = 6.03), and the smallest strawberries (BRAI = 0.49). But the application of chemometric methods that included NIRs showed the possible to predict the content of phenols and AOA just based on the absorbance spectra, what can be used in the control of berry fruit quality.

Key words: Berries, NIR spectroscopy, Oxidative stress, BR-reaction, Colorimetry, Chemometrics, classification.

1. Introduction

Studies have shown that foods rich in fibber, total phenols and antioxidants, such as fresh fruits and vegetables, legumes and whole grains, help to reduce stress in organism [1]. Antioxidants naturally present in foods are vitamin E, vitamin C, vitamin A, vitamin B₂, ions of copper, manganese, zinc, selenium and iron [2].

Nutritive composition of wild berries is of great interest regarding of its high content of phenolic compounds that have positive impact on human health [3] and are an important source of natural antioxidants [4]. Two major classes of polyphenols that are common to small fruit species are flavonoids and non-flavonoids. Of the non-flavonoid components, hydroxycinnamate esters, especially chlorogenic acid, are predominant in many small fruit species. Other non-flavonoid components include hydroxycinnamic acids and simple phenolics. The flavonoids include several major subclasses, of which anthocyanins are probably the best known since they are the pigments which impart red, purple, and blue coloration to fruit. Other small fruit flavonoids include flavonols (and their glycosides), catechins (flavan monomers) and proanthocyanidins (condensed tannins) [5]. Phenolic compounds are secondary plant metabolites that have important physiological and morphological role for growth and reproduction of plants [6] and they contribute to the colour and sensory characteristics of fruits and vegetables [7]. They contain one or more hydroxyl groups attached to the aromatic ring and they are structurally ranged from simple molecules to high polymerized phenolic compounds [6]. Polyphenols are the major source of antioxidants in a diet, so eating wild berries that are rich in phenolic compounds, contributes highly to food intake



of antioxidants. Antioxidant activity of plant foods derives from the cumulative and synergistic effects of a large number of antioxidants such as vitamin C, vitamin E and other various phenolic compounds [8]. Hence, antioxidants role is inversely proportional to free radicals that are unstable and reactive molecules which if not neutralized in the body can cause damage to proteins, lipids and carbohydrates. The consequences of such damage are atherosclerosis, diabetes, cancer and other chronic diseases. However, production of free radicals in a given concentration at the local level is essential for functions of human immune system [9]. The antioxidant capacity can be defined as the sum of the present endogenous and exogenous defence mechanisms that ensure the oxidative balance [9]. Oxidative stress can be defined as a state of imbalance between antioxidants and pro-oxidants. Pro-oxidant molecules contribute to the production of free radicals and thus increase the oxidative stress. Antioxidants are compounds that neutralize free radicals and reduce oxidative stress. Increased oxidative stress contributes to the development of the various chronic diseases [1]. A method used in the measurement of antioxidative capacity reported by Cervellati and co-workers [10] is based on the inhibitory effects of antioxidants on the oscillations of the hydrogen peroxide, acidic iodate, malonic acid, and Mn (II) catalyzde system, known as the Briggs-Rauscher (BR) reaction [11]. This method works at the pH value of the fluids in a human stomach (pH \approx 2).

Considering that fruits, vegetables and various drinks are usually consumed *per os*, it's understandable that they show their antioxidative capacity first in a stomach, thus preventing damage of the cells and tissue in that area. Therefore, the BR reaction method can give useful *in vitro* information on the antioxidant activity at acidic pH values. The BR method has many advantages because the analysis is inexpensive and rapid, reagents and apparatus are in common use in all chemical laboratories [12].

Total intake of polyphenols in the human body varies significantly and the average is about 1000 mg/day [13]. The importance of eating foods rich in polyphenols is the fact that phenolic compounds exhibit a wide range of positive physiological effects, such as anti-allergic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic and cardio protective effect [14 - 18].

A theory based on scientific research in recent years says that the intake of antioxidants from natural sources, such as polyphenols from berries, can prevent cell damage caused by free radicals. However, the same theory is questionable because of the importance of bioavailability of antioxidant compounds from food. Presence of high concentration of polyphenols in berries does not have an effect in a human body if it cannot absorb biologically active molecules which can express positive effects [19].

Understanding the absorption and bioavailability of nutrients is essential for evaluation of the antioxidant

capacity of food [20]. As mentioned, plant pigments, except of giving the colour, have an important role in the immune system of plants. They protect plants from harmful UV rays, insects and other pests and microorganisms, while entering into the human body they have a positive impact on health and vitality.

Colour changes of the analysed samples can be monitored colorimetrically. Colorimetry is a branch of the science about colours that are primarily engaged in quantitation of colour in relation to certain visual stimuli [21].

Determinations of total phenols, antioxidant activity are time-consuming, tedious and laborious for routine/ screening purposes as those required for quantitative discrimination of samples with bioactive compounds [22]. More appropriate would be a rapid technique that is able to discriminate samples with high amount of phenolic compounds and antioxidant capacity before time-consuming extraction steps take place. An appropriate method that can achieve these goals is near infrared (NIR) spectroscopy [23, 24]. One of the benefits of the NIRs is the possibility of simultaneous measurement of several constituents [25]. This technique has already been applied to the determination of phenolic compounds and antioxidant capacity in fruit and their products, such as wine [26] and blueberries [27]. The application of NIR spectroscopy has been applied to authenticate/discriminate different types of wild berries fruits [28], edible oils [29], and to predict the sensory properties of dessert wine Prošek [30].

The application of NIR spectroscopy for the determination of phenolic compounds, antioxidant capacity and/or colour of berry samples is an approach that has not been investigated yet. Therefore, the aim of this work is to evaluate the potentiality of NIR spectroscopy to determine t antioxidant capacity of different samples, their colour and total phenolic contents. The major aim is to propose a fast and clean technique as a front end procedure to qualitatively and quantitatively distinguish the fruit samples that are nutritionally rich in bioactive compounds.

2. Materials and Methods

2.1 Sample preparation

Frozen samples of berries (strawberries, blackberries, raspberries, blueberries, red currants) were defrosted, pitted and homogenized in a house blender at room temperature.

Extraction

A mass of 2.5 ± 0.001 g of the homogenized fruit was mixed with 10 mL of 96% ethanol in order to prepare the basic solution. The extraction of the prepared solution was conducted using the reflux method and using an



ultrasound. Ultrasound-assisted solvent extraction was performed in an ultrasound bath (Bandelin, Sonorex, RK 106, Germany) operated at a frequency of 35 kHz at 25 ± 3 °C. Extraction was performed in triplicate.

1) Reflux method

Refluxing involved heating of the basic solution to boiling and then returning the condensed vapours to the original flask for 10 min. The solution was culled at room temperature and the content is filtered using a Buchner funnel. The mother liquor is decanted in the flask and the remaining sample was re-poured with 12.5 mL of ethanol, and the process of refluxing was repeated. The second mother liquor is added to the flasks and refilled to 25 mL with ethanol.

2) Use of the ultrasound bath

Basic solution was placed in an ultrasonic bath for 30 min. This solution was filtered using a Buchner funnel. The mother liquor is decanted in the flask and the remaining sample was re-poured with 12.5 mL of ethanol, and the placed in an ultrasonic bath for next 15 min. The second mother liquor is added to the flasks and refilled to 25 mL with ethanol.

2.2 Total phenol concentration

Total phenolic concentration (TP) was determined by the Folin-Ciocalteu colorimetric method [31]. Gallic acid is used as a standard for the calibration curve. Total phenol content was expressed in gallic acid equivalents (GAE) (mg per 100 g fruit). Absorption at 765 nm was measured [32].

2.3 Briggs-Rauscher reaction

Antioxidant activity of extracts was determined using the Briggs-Rauscher oscillating reaction. The effect consists of an immediate interruption of oscillations, an inhibition time that linearly depends of the concentration of the antioxidant added, and following restoration of oscillations [10].

BR mixture is prepared by mixing three colourless solutions: *solution A* was 8.6 % hydrogen peroxide; second *solution (B)* was prepared by dissolving 4.3 g potassium iodate in 100 mL distilled water with addition of 0.5 mL sulphuric acid (96%); and the third *solution (C)* was a mixture of 1.5 g malonic acid, 0.4 g manganese (II) sulphate monohydrate and 0.1 g starch dissolved in 100 mL of distilled water [33]. All three solutions (A, B and C) in equal volume ratios are mixed at room temperature [10-11]. In 15 mLof BR mixture is added 1 mL of the fruit extract. BR-reaction is followed potentiometrically and the inhibition time (IT) is recorded.

2.4 NIR analysis

NIR spectra of fruit extracts were collected in the range of 904 - 1699 nm using a Control Development, Inc.,

NIR-128-1.7-USB/6.25/50 µm, with installed Control Development software Spec32. NIR spectroscopy is based on the electromagnetic absorption at the near-in-frared region but the spectral analysis has to be assisted with various chemometric techniques [34]. Each sample spectra was recorded in triplicate and the mean value was calculated. To analyse the recorded NIR spectra of samples was used program *Statistica v.10.* [35].

2.5 Colour instrumental measurement

Colour measurements were carried out with a Minolta CM-3500d (Osaka, Japan) spectrophotometer in the CIELAB space: lightness (L*), redness (a*) and yellowness (b*) [36]. Each fruit extract was analysed in 3 replicates.

2.6 Statistical analyses

One-way ANOVA was carried out for physical and chemical data. Statistical significance was set at P < 0.05. Student's t-test was used to determine whether there were differences in phenol content, antioxidant activity or colour parameters in berry-fruit extracts obtained by the use of the Reflux method or by use of the ultrasound. The results were submitted to principal component analysis (PCA) in order to interpret measured content of phenol, antioxidant activity or colour parameters and NIR spectra. All data were analysed using program Statistica v. 10. [35]. The principle component analysis (PCA) was used with the aim of screening possible outliers as well as to identify patterns in experimental data and to express the data based on their similarities and differences.

3. Results and Discussion

The method of extraction of phenolic compounds in an ultrasonic bath were obtained TP concentration ranged from 610 mg GAE/100g fruit sample strawberries to 1290 mg GAE/100 g of blueberry samples. Applying both methods of extraction of phenolic compounds berries, submitted the lowest concentration of TP in strawberries and blueberries in the highest. Pantelidis and co-workers [37] have in their study, among other things, determined the concentration of total phenols in raspberry, blackberry and red currant. The values that are given, depending on the variety and time of harvest, the raspberry range 1052-2494 mg GAE/100 g, for blackberry 1703 to 2349 mg GAE/100g and red currant 657-1193 mg GAE/100 g. Levaj et al. [38] in presented study, among other things, analyse the strawberries and blueberries and get the value of the TP concentration of 2058 mg strawberry GAE/100 mL and 1449 mg of blueberry GAE/100 mL. The results of these studies showed higher concentrations of TP for all kinds of fruits except for blueberry whose concentration TP bit more than they did the results of this work and we can conclude that the content of phenolic compounds in the fruit depends on many factors such as variety, farming area, climatic conditions, degree of maturity and method of cultivation and storage [29]. It is important to emphasize that samples analysed in this work were frozen, which could also significantly contribute to the degradation of phenolic compounds and reflect on the resulting lower concentration of total phenols in comparison with literature data. Mulinacci and co-workers [39] concluded that the processes of storage, such as freezing plant material, can result in a reduction of the present antioxidant components such as phenolic compounds which justifies our previous conjecture.

From the results can be concluded that different method of extraction therefore gave different results for the same samples, but the standard method of extraction gives us the opportunity to compare the results with other studies. Destroying of the cell structure allows multiple release cell contents resulting with better extraction of phenolic compounds that will from the vacuoles. Such a conclusion would go in favour of the method of extraction in an ultrasound bath because it does not apply the increase of bulk temperature, in contrast to the extraction of phenolic compounds by refluxing. Elevated temperatures may degrade phenolic compounds so that the resulting concentration of the extracts obtained in the TP refluxing potentially lower than the actual concentration [38]. Concentration of TP and antioxidant activity (AOA) is often proportional, especially when a Briggs-Rauscher method is applied. The inhibition time is defined as the time elapsed between the end of the addition of the antioxidant sample and appearing the oscillation again. The antioxidant activity of extracts, by applying the Briggs-Rauscher reaction, can be represented by the inhibition time or as Briggs-Rauscher antioxidant index (BRAI) calculated as Carvellati and co-workers [12] suggested.

The time of quenched BR-oscillations is obtained with the inhibition time (IT) what enables to calculate the Briggs-Rausher antioxidant index (BRAI). BRAI is the equivalent of gallic acid and shows the extent to which the antioxidant effect of phenolic molecules is stronger or weaker than the antioxidant effect of gallic acid.

The greatest value of AOA showed a pattern of blackberries which was extracted in an ultrasonic bath (BRAI = 6.03), and a minimum value of AOA was detected for the strawberry sample. Over 50% of the berry-samples has the BRAI > 1, which means that their AOA equals or is greater than the AOA of gallic acid.

In the study of Pantelidis and co-workers [37], the highest AOA value showed the blackberry sample which is consistent with the results of this work. The lowest value of AOA determined for a sample of red currant, also in line with the results of this work. The mix of berry-fruit extracts presented equal shares of each fruit (0.5 g) and this sample was examined to test the findings of Hidalgo and co-workers [40] that have concluded that flavonoids in complex systems such as food, where it has a full range interaction, in a mix thus should contribute to a greater antioxidant capacity than applying individual flavonoids. And this is correct for samples extracted with the reflux method, where individual BRAIs (of 50 mg) are lover that the AOA of the mix. Unfortunately, this statement can not be applied on samples extracted by use of ultrasound, in this paper.

Sample	phenols	IT (s)	BRAI	L*	a*	b*					
Reflux method											
Red currants (RC-R)	1048ª	291,91ª	0,51ª	23,92ª	1,39ª	2,21ª					
Blackberry (B-R)	3020ª	1328,09ª	2,82ª	17,51ª	0,77ª	0,92ª					
Strawberry (S-R)	420ª	569,00ª	1,12ª	24,27ª	1,31ª	2,66ª					
Blueberry (Blu-R)	1180ª	1377,73ª	2,93ª	18,02ª	0,22ª	0,57ª					
Raspberry (R-R)	2990 ^b	392,56ª	0,73ª	18,23ª	1,35ª	0,90ª					
Mix of all fruits (Mix-R)	2250ª	1964,15ª	4,24ª	16,17ª	0,85ª	0,98ª					
Ultrasound bath			<u>.</u>								
Red currants (RC-U)	1490 ^b	295,90ª	0,52ª	22,75ª	1,55ª	2,55ª					
Blackberry (B-U)	3030ª	2765,62 ^b	6,03 ^b	18,99ª	0,61ª	0,30 ^b					
Strawberry (S-U)	610 ^b	285,06 ^b	0,49 ^b	23,99ª	1,23ª	2,71ª					
Blueberry (Blu-U)	1290ª	1374,84ª	2,92ª	18,54ª	0,28ª	0,10 ^b					
Raspberry (R-U)	2180 ^b	413,31ª	0,78ª	18,20ª	1,65ª	1,06ª					
Mix of all fruits (Mix-U)	2500ª	1370,52 ^b	2,91 ^b	18,81ª	0,77ª	0,43 ^b					

 Table 1. Content of total phenols, antioxidant activity and colour parameters in fruit extracts for different types of extractions

Experimental average values for fruit extracts prepared using two different extraction methods: reflux or ultrasound bath. Different letters (a, b) indicate statistical significant difference (P < 0.05).

Values AOA blueberries are high as expected and consistent with a high concentration of the TP. However, the value of AOA mix berries is also high, while their concentration TP much lower than that of blueberries. From this we can conclude that the AOA mix of berries is higher because different antioxidant components from several fruits synergies and complement each other. Therefore, the synergism of the different components of the antioxidant mix berries showed high antioxidant activity in comparison with other samples of berries and their values of the concentration of total phenols [38]. High concentrations of phenolic compounds and other components of antioxidant supplements do not mean that they are equally usable in the human body. The Briggs - Rauscher method is considered as a potential in vivo antioxidant activity method [12], because the pH value of the reaction mixture is equal to 2, corresponding to the acidic conditions of the stomach in the human digestive system. Results indicate that despite the lower concentrations of TP in berry-fruits, they showed good AOA, which supports the health well-being-giving priority to a balanced diet [15].

On Figure 1 is presented the relationship of the concentration TP and AOA (expressed as value of IT). Increase in total phenols leads to increases of AOA for both extraction methods. Pantelidis and co-workers [37] determinate that the link between the TP concentration and AOA is influenced by different factors several kinds of berries, connect the obtained values and found that there is a very strong link between the AOA and the concentration of the TP. Also given the research of Lugas and co-workers [41] where the AOA individual forest fruit depends on the variety, soil type and climatic conditions and supply plants with water and nutrients, the significant deviation of the sample of raspberry extracted by use of the reflux method (Fig. 1). Results of determined BRAI values can indirectly be related to human metabolism and potential acceleration where

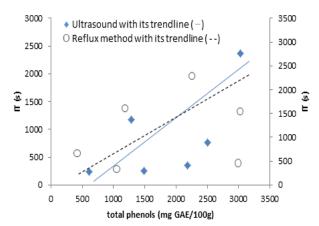


Figure 1. Relationship of total phenols and the antioxidant activity (expressed as inhibition time (IT)) for berry-fruit samples extracted with the reflux method and using the ultrasound

higher levels were determined for samples extracted under lover temperature regime (room temperature). This statement is based on the fact that metabolism speed is linked with physical activity where exercising increases oxidative stress due to increased production of free radicals during exercise [9] and more antioxidants are necessary to help the body to maintain homeostasis and oxidative stress.

The results of colour parameters for berry fruit extracts (L*, a*, b*values) are shown in Table 1. L* value ranged from 16.2 - 24, and is associated with the lightness of the samples. No significant differences were established regarding the extraction method and no relationship between the L* value and total phenols or AOA (Fig. 2) could be established. The chromatic axes ranged from 0.2 to 1.6 (a* value) and 0.3 to 2.7 (b* value). No significant differences were detected for the chromatic axis a*, but for the chromatic axis b* are confirmed differences for samples extracted using different methods; blackberry, blueberry and the mixed sample. By linking those findings with the content of total phenols and AOA (Fig. 2), obtained results show that extracts with higher b* values have less TP and lower AOA. Conversely, samples that have a lower b* value are more blue and have a higher AOA.

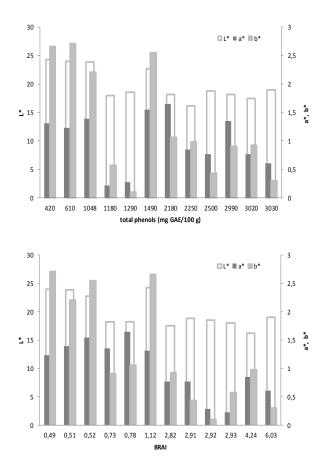


Figure 2. Content of total phenols and the antioxidant activity (BRAI) related to the colour parameters (L*, a*, b*) of berry-fruit samples



Near-infra red (NIR) spectroscopy

NIR spectra (NIRs) were recorded for the berry-fruit extracts in the range 899 - 1699 nm, what is presented on Fig. 3. According to the results, the spectra of all berry-fruit samples overlap.

NIR spectroscopy is a simple measuring method not requiring special sample preparation but is also known as a powerful tool that can be used in quantitative and qualitative discrimination of samples which requires the application of chemometric techniques.

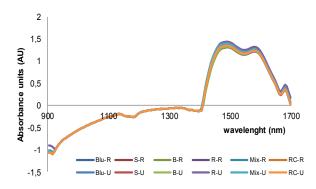


Figure 3. Raw NIR spectra of berry-fruit samples

Relationship between the content of total phenols, AOA and colour parameters

Concerning the applied NIR spectra region, it is important to dive the spectrum into regions that cover those combinations and overtones due to present phenol compounds and antioxidant activity. So the right way would be the regions that cover the O-H combinations and overtones [22]. The factor analysis indicated two main regions; from 899 to 928 nm and 1399 do 1699 nm covering the first and second O-H stretching overtone. From previous results and indication of differences between berry fruit samples extracted using different methods is prefigured but which parameter is well-marked for different fruit extracts remained incomplete. In order to analyse the whole assessment of observed variables and cases, the multivariate statistical procedure of principal component analysis (PCA) was performed using the dataset presented in table 1 and NIRs (Fig. 3). Results are shown in Figure 4.

The two principal components account 87.66% and 10.32% of the variance, respectively, (97.98% in total).

NIRs attached to the measured parameters of colour, AOA and TP are not enough to separate the samples according the extraction method, but it can be seen that the AOA parameters are positioned in the 2nd and 3rd quadrant where are also the NIR spectra wavelengths what indicates positive correlations. This correlated shows that it is possible, by use of modelling, to apply the NIR spectroscopy for the determination of the AOA (the BRAI vales) what was not been investigated yet.

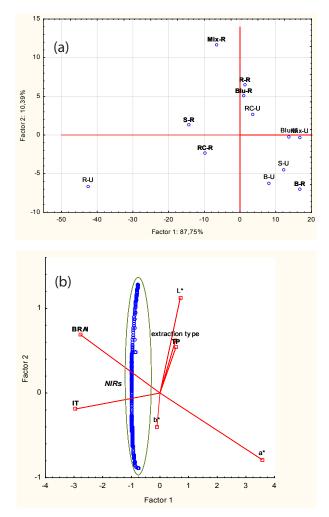


Figure 4. Plot of principal component analysis (a) with additional loadings (b) of the phenol content, antioxidant activity or colour parameters of berry-fruit extracts obtained by use of reflux method (samples R) and using ultrasound (samples U)

Following multivariate tool that was used is the partial least squares (PLS) to model the antioxidant activity (BRAI) and the content of total phenols from NIR spectra. The data set was divided according the extraction methods (2 sets) and those sets were separately analysed. Each set was divided in two sets; calibration set for development of the PLS models corresponding to 15 samples and the prediction set intended to be used as an independent test set (6 samples). The optional number of latent variables was estimated using the random subset cross-validation method [22, 42]. The following step was the calibration of the models with the calibration set using the estimated number of latent variables. The independent set is then projected onto these models yielding the prediction set results. The PLS models' accuracy was evaluated using the root mean square error of calibration (RMSEC), the root mean square error of prediction (RMSEP) and the coefficient of determination of prediction (R_{p}^{2}) , what is presented in Table 2.

Table 2. PLS calibration models' results for antioxidant activity and content of phenols base using the NIR spectra region 1399 – 1699 nm

	Ca	alibratio	n se	et	Prediction set								
	n	RMSEC		R^2_{c}	n	RMSEC	R^2_{P}						
Reflux method													
BRAI	15	0.154	0.98		6	0.507	0.99						
Total phenolic content	15	71.08	0,95		6	32.92	0.99						
Ultrasound													
BRAI	15	0.617	0.88		6	0.378	0.97						
Total phenolic content	15	32.88	C	.98	6	35.28	0.99						

The experimental vs. predicted values of AOA and TP for different extraction methods for all PLS models described in Fig. 5 are all very similar ($R^2 \ge 0.95$). The wavelengths that have a high contribution to the models are in the range 1400-1699 nm: This range is related to carbohydrates (C-H stretching in the first overtone region) [43] and to the fibre content [44] (O-H stretching & C-H stretching). The PLS models results (Fig. 5 & Table 2) showed an excellent agreement between NIR spectra predictions and AOA and very good agreement in

prediction of the content of total phenols. The best PLS models were obtained for the antioxidant activity expressed as BRAI, when the samples were extracted by use of the reflux method ($R_{p}^{2} = 0.99$). Less effective was the prediction of AOA when the extraction was conducted by use of the ultrasound ($R_{p}^{2} = 0.97$), because this set had a parameter that was an extreme - the blackberry extract. Although the content of total phenols were not the primary parameter that we wanted to predict, the results show high correlations obtained for the PLS models ($R_{p}^{2} = 0.99$), regardless which extraction method was used. The results Table 2 indicate that models are producing very accurate estimations and in general, this means that all developed PLS models could replace the time consuming and laborious chemical procedures.

If those findings are added to the nutritional data that consumption of five or more servings of fruits and vegetables a day halves the risk of developing various cancers, especially cancers of the gastrointestinal tract [45] and that dietary fibber and polyphenols from fruits improve lipid metabolism and prevent the oxidation of LDL cholesterol and prevent the development of atherosclerosis [45, 46] - the consumption of berry-fruits is a good way to keep the wellbeing of the population allowing to detect the phenols and the AOA of it on an easier way.

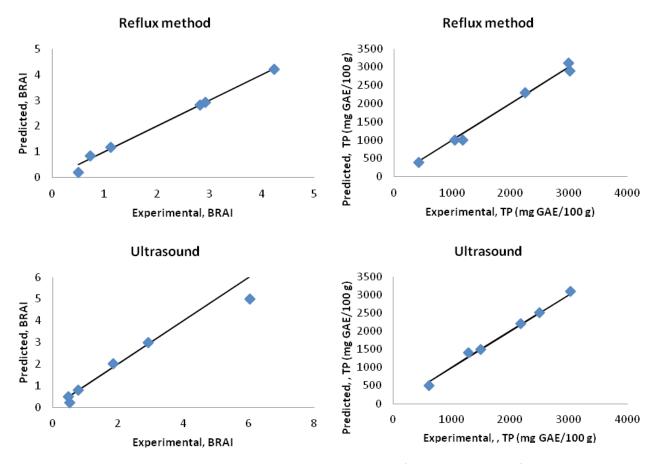


Figure 5. Experimental values vs. PLS cross-validation prediction for AOA and content of total phenols

4. Conclusions

- Results in this work showed the nutritional richness of berry-fruits when the antioxidant activity was obtained by use of the Briggs-Rauscher reaction (the majority of the samples had BRAI > 1).

- The observed concentration of total phenols, antioxidant activity and colour parameters showed significant differences regarding the method of extraction (reflux vs. ultrasound) but just for some fruits.

- NIR measurement for raw berry-fruit extracts showed overlap of the spectra but the application of multivariate analysis allows detecting the crucial spectral range that can be used for prediction of antioxidant activity and the content of total phenols in a fast and accurate way.

- Presented approach can replace time-consuming chemical methods presenting a useful tool in food composition analysis.

- Following research should be aimed to the identification and quantification of the same parameters in other fruits and their major bioactive components using NIR spectroscopy with application of chemometric techniques.

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