



## ORIGINAL SCIENTIFIC PAPER

# Application of NIR spectroscopy and chemometrics in quality control of wild berry fruit extracts during storage

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## Summary

Extracts of wild berry fruits (Bilberry, Cranberry, Raspberry and Strawberry) were analysed using the near infrared spectroscopy (NIRS). NIRS can be used quantitatively and qualitatively to detect, identify, and qualify raw materials like berry fruits and to control their quality. The spectra of fruit products were measured by diffuse trans-reflectance and raw spectra were used for chemometric analysis. The range of NIR used in this research was 904–1699 nm with the aim to use the potential of near-infrared spectroscopy to detect differences between berry-fruits themselves, differences between the sample based on the preparation procedure as well if it is possible to detect the staleness of the samples (fresh and one month old). NIR is a fast and non-destructive analytical method that in association with chemometric modelling becomes a powerful tool for application in food industry. The chemometric methods applied to the recorded NIR data were classification analysis based on principal component (PC) scores and partial least squared regression (PLSR) model. Analyses of the spectrums reveals changes in specific wavelengths of 904-1181 nm that are related to the 3rd CH overtone and 2nd overtone of the OH stretch of H<sub>2</sub>O as well as in the range of 1434- 1635 nm which correspond to C–H combinations and the 1st overtone of the bonds. The simple PLS regression showed that the influence of the storage on the NIR spectra of samples is high ( $R^2=0.79$ ). This study showed that near infrared spectroscopy has potential to distinguish extracts based on unprocessed fresh wild berry fruit extracts, sample preparation procedure and their quality decrease affected by the storage. All findings indicate the advisability of use of NIR for on-line monitoring of product quality and selection.

**Keywords:** berry fruits, storage, NIRs; chemometrics

## Sažetak

Ekstrakti šumskih plodova (borovnica, brusnica, malina i jagoda) analizirani su primjenom blisko-infracrvene spektroskopije (NIRS) koja se može koristiti kao kvantitativna i kvalitativna metoda za detekciju, identifikaciju i razvrstavanje sirovina poput bobičastog voća i nadzirati njihovu kvalitetu. Spektri voća mjereni su difuznom trans-reflektancijom i ti spektri su korišteni u kemometrijskoj analizi. Raspon NIR instrumenta korištenog u ovom istraživanju bio je od 904nm do 1699 nm. Cilj rada bio je odrediti potencijal primjene infracrvene spektroskopije u međusobnom razlikovanju samog voća, razlikovanja uzoraka prema načinu pripreme uzorka i svježini istih (svježi ili stari 1 mjesec).

NIR je brza analitička metoda za koju nije potrebno tretirati uzorak na bilo koji način te se primjenom kemometrije pokazala kao moćan alat koji nalazi svoju sve češću primjenu u prehrambenoj industriji. Kemometrijske metode koje su primijenjene na NIR spektrima bile su klasifikacija analiza koja se temelji se na analizi glavnih komponenata (PC) i parcijalne regresije najmanjeg kvadrata (PLSR). Analize spektara ukazale su na promjene u određenim valnim dužinama; 904-1181 nm koji se odnose na treći C-H overton i drugi O-H overton za H<sub>2</sub>O te također pri rasponu od 1434 do 1635 nm koji odgovaraju C-H kombinacijama i prvom overtonu navedene veze. Jednostavan PLS model pokazao je značajnost utjecaja skladištenja na NIR spektre ( $R^2=0.79$ ), a time i na kvalitetu uzoraka koja opada ovisno o trajanju skladištenja. Ovo istraživanje je pokazalo kako NIR spektroskopija ima potencijal kao metoda koja može razlikovati; ekstrakte šumskog voća, uzorke prema načinu pripreme i vremenu skladištenja. Svi rezultati pokazuju svrhovitost korištenja NIR za on-line praćenje kvalitete proizvoda i njihove selekcije.

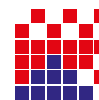
**Ključne riječi:** bobičasto voće, skladištenje, NIR; kemometrija

## Introduction

Berry fruits are consumed as raw food or in the production of beverages, juice, pulps and jams. Berries are also used in traditional medicine where many studies have reported that the consumption of fruits and vegetables can reduce the risk of chronic diseases such as cancer, diabetes and cardiovascular diseases (Egan et al., 2001; Franzini et al., 2012; Krishnaiah et al., 2011; Wang et al., 2011). Over recent years, consumers have become increasingly interested of the consumed uptake on human and environmental health (Forbes et al., 2009) and

the authenticity of foods (Lesschaeve and Noble, 2005). And use of conventional analytical techniques is very time-consuming and labour intensive (Huang et al., 2008).

To insure the quality and safety of the products for the consumer, new methods are developed, among them is also application of near infrared spectroscopy (Williams et al., 1988; Ozaki et al., 2007; Parpinello et al., 2009). Among the methods that require pre-treatments of samples and also must include highly-skilled personnel, near infrared spectroscopy has proven to be a successful analytical method that does not requires pre-treatment of samples and is used for analysis of a variety of



food products and beverages (Nicolai et al., 2007; Sirisomboon et al., 2007). NIR spectroscopy is based on the electromagnetic absorption at the near-infrared region but the spectral analysis has to be assisted with various chemometric techniques (Ding and Xu, 1999; Alishahi et al., 2010). In the food industry, NIR measurements are used in measurement (Hernández Gómez et al., 2006; Liu et al., 2010) and evaluation of quality (Sirisomboon et al., 2007), quality prediction (Cayuela and Weiland, 2010), and differentiation of samples (Reid et al., 2005). NIR region (750 – 2500 nm) is very useful spectral fingerprint of food samples in which vibration and overtone combinations of basic bonds (O–H, C–H and N–H) are the main recordable phenomena (Williams et al., 2001).

Studies have shown that NIR is a suitable technique for: sugar, acidity content measurement in various fruits (Beckles, 2012; Wedding et al., 2013; Li et al., 2013); detection of the right harvest time to avoiding bruising of fruits (Pholpho et al., 2011; Sánchez et al., 2013). For berries are the studies oriented on the use of NIR spectroscopy in the quality and authenticity control of fruit purées, fruit preparations and jams (Fügel et al., 2005) and quantification of sugars (glucose, fructose and sucrose) in juices (Xie et al., 2009).

Application of the NIR method on berries is quite unexplored and therefore the subject of this study was to examine, for the first time, (i) the ability of NIRS in monitoring and identification of different wild berry-fruits extracts (two Bilberry samples, Cranberry, Raspberry and Strawberry), (ii) detect the possibility of sample differentiation based on the stage of sample preparation (filtered or not) and (iii) to examine the possibility to classify the samples according the effect of storage (fresh extract and old 1 month) that should decrease the fruit extracts quality. To present the inter-relationships between

changed spectral properties of the samples (Bueno et al., 2010; Parpinello et al., 2009), chemometrics will be used.

## Materials and methods

### Sample preparation

Bilberry (*Vaccinium myrtillus* L.) fruits, samples Bilberry V and R (table 1), were hand-picked from their natural habitats in the western and south-western part of the Rhodope Mountains (Bulgaria), respectively. Wild raspberry (*Rubus idaeus* L.) and strawberry (*Fragaria vesca* L.) fruits were also from the south-western part, while wild cranberry (*Vaccinium vitis-idea* L.) sample – from the western part of the Rhodope Mountains. After sorting to eliminate unripe, overripe or damaged fruits, berries were placed in polyethylene bags (~ 200 g) and frozen stored (–20 °C, 3 months) until lyophilized. Berry fruit extracts were obtained by mixing 10 g of the finely ground lyophilized fruits with 100 ml of distilled water. After left overnight, aliquots of the extraction mixtures were filtered using a paper filter.

### Storage

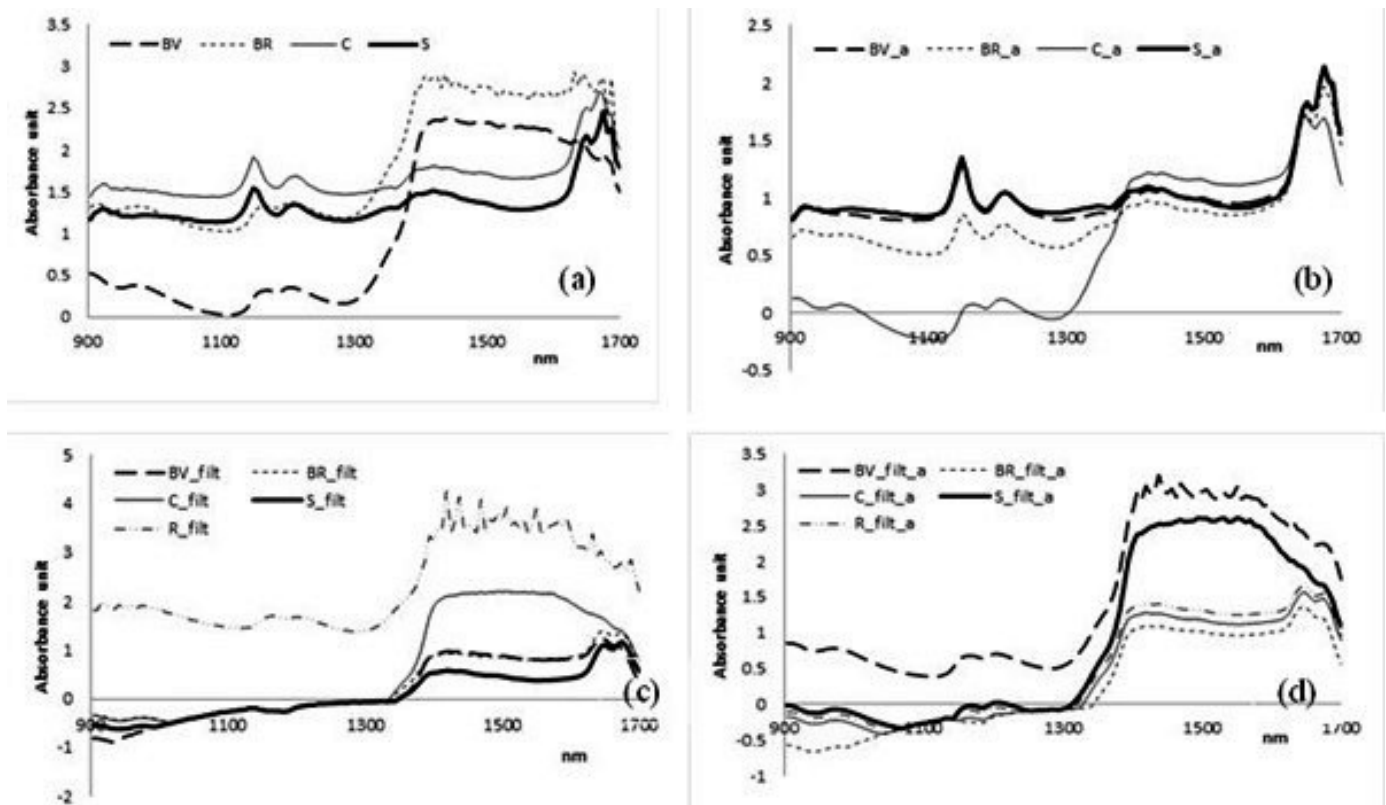
All extracts were placed in the fridge with the average temperature +6°C, without exposure to light and additional moisture. The samples were stored for 30 days.

### NIR analysis

The NIR spectrometer Control Development, Inc., NIR-128-1.7-USB/6.25/50µm, with installed Control Development software Spec32 was used as a non-destructive technique in

**Table 1.** Abbreviations used for different samples of berry-fruit extracts

Berry fruits	sample	abbreviation
<i>Vaccinium myrtillus</i> L. Bilberry R (Rhodope Mountains)	Non-filtered, fresh	BR
	Non-filtered, 1 month	BR_a
	Filtered, fresh	BR_filt
	Filtered, 1 month	BR_filt_a
<i>Vaccinium myrtillus</i> L. Bilberry V (west Rhodope Mountains)	Non-filtered, fresh	BV
	Non-filtered, 1 month	BV_a
	Filtered, fresh	BV_filt
	Filtered, 1 month	BV_filt_a
<i>Vaccinium vitis-idea</i> L. Cranberry	Non-filtered, fresh	C
	Non-filtered, 1 month	C_a
	Filtered, fresh	C_filt
	Filtered, 1 month	C_filt_a
<i>Rubus idaeus</i> L. Raspberry	Non-filtered, fresh	R
	Non-filtered, 1 month	R_a
	Filtered, fresh	R_filt
	Filtered, 1 month	R_filt_a
<i>Fragaria vesca</i> L. Strawberry	Non-filtered, fresh	S
	Non-filtered, 1 month	S_a
	Filtered, fresh	S_filt
	Filtered, 1 month	S_filt_a



**Figure 1.** NIR spectra of 4 non-filtered fruit extracts, fresh (a) and stored for one month (b), and 5 filtered fruit extracts, fresh (c) and stored for one month (d)

the near-infrared range from 904 - 1699 nm, in which the radiation reflected by the berry-fruit extracts, was conducted. Each sample spectra was recorded in triplicate and the mean value was calculated. The spectra were measured by diffuse trans-reflectance and raw spectra were used.

### Statistical Analysis

In the data analysis was used program *Statistica v. 10*. NIR spectroscopy combined with multivariate analysis (chemometrics) is a powerful tool used to identify patterns in experimental data (Budić-Leto et al., 2011). Used were the principal component (PC) and clustering analysis in order to divide the fruit samples according their type, stage of preparation and storage conditions. The program *Statistica v. 10.*, was used to conduct the principle component analysis in order to identify patterns in experimental data and to express the data based on their similarities and differences. Partial least squares regression was conducted, in order to find out what is the impact of the time of storage. No pre-treatments were applied to the spectral data before calibration development where the storage times represent the latent variable. The model was calibrated on non-filtered fruit extracts and tested on the filtered one.

Berry-fruit extracts as Bilberries, Cranberry, Raspberry and Strawberry were analysed as fresh extracts as well as extracts stored for one month. Their abbreviations used in the chemometric analysis are given in table 1.

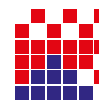
### Results and Discussion

Using NIR process analyser, each spectrum of different fruit extracts (filtered and non-filtered extracts of: bilberries,

cranberry, raspberry and strawberry) for different storage time, were collected in triplicate. In Fig. 1 are presented mean samples of NIR spectrums (absorbency).

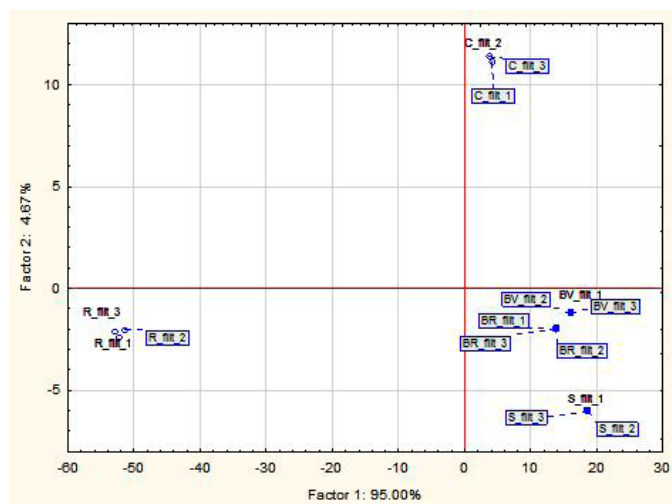
As can be seen, the extract of Raspberry was difficult to record when the sample was filtered (Fig. 1. c and d). This is the reason why this sample is not presented as non-filtered sample - because no useful spectrum was obtained. This is often a problem when the sample presents an emulsion (Wihodo and Moraru, 2013). Analysing presented spectres in Fig. 1., it seems that most parts of the recorded spectra's are similar, with an exception in the ranges 905 - 950 nm and 1100-1600 nm. This is the reason why in the following chemometrics the spectrums in the range of 950-1100 nm is not observed. According fig 1., after 1400 nm can be observed slight differences what is expected for fruit products as wine and orange fruits (Nicolăi, et al., 2007; Liu et al., 2010; Budić-Leto, et al, 2011). The used NIR instrument range is significantly narrower then the instruments usually used with the range from 400 nm to 2500 nm (Ozaki et al, 2007), but to ensure the data differentiation between the examined samples the range of the used NIR instrument is sufficient (Xie et al., 2009).

Since water is the most important chemical constituent in fruits and vegetables, their near infrared spectrum is dominated by water absorbance (Nicolăi, et al., 2007). The complex chemical composition of a typical fruit or vegetable will result with a large set of overtones and band combinations, which can be observed on the near infrared spectrum. This shows that it is difficult to assign specific absorption bands to specific functional groups and multivariate statistical techniques are therefore required to extract the information about quality attributes which is buried in the NIR spectrum (Ozaki et al., 2007). This



fact justifies the use of chemometrics (multivariate statistical techniques) in order to detect similarities or differences for the studied samples (Bueno et al, 2010; Moret et al., 1994).

The differentiation based on chemometrics is presented with Figures 2 to 4 where, as statistical separation tool, was used the principal component and classification analysis as multivariate exploratory technique.

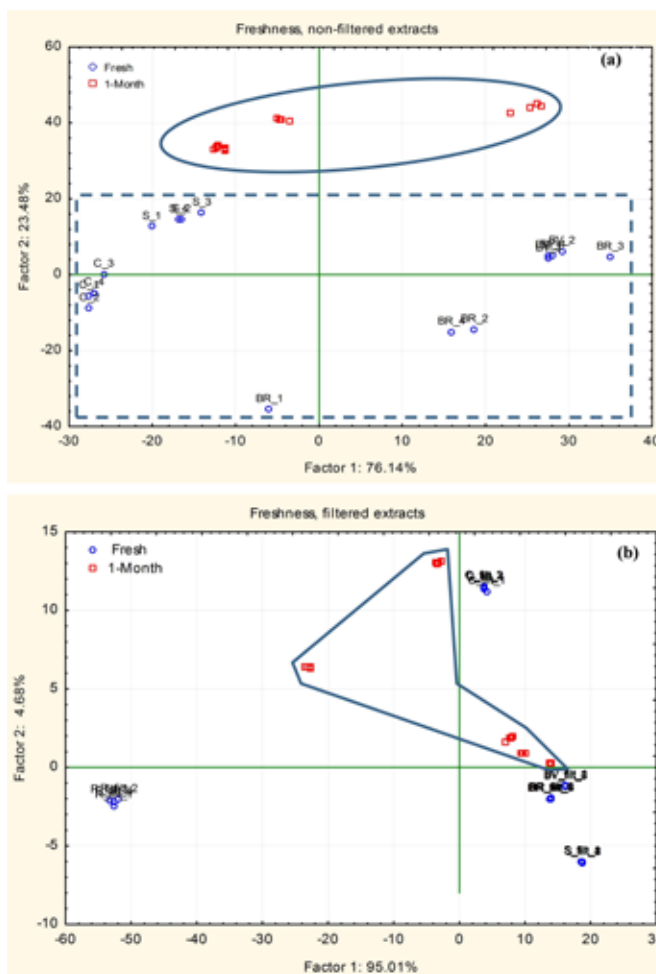


**Figure 2.** Classification analysis used for differentiation of samples based on absorbance data of filtered, fresh fruit extracts

The aim was to distinguish berry-fruits samples mutually where the classification is based just on near-infrared spectroscopy data. The approach was a chemometric procedure consisting of stages that include such methods as the factor and cluster analysis (Wihodo and Moraru, 2013; Roggo et al., 2007). Results of the classification analysis show that the samples can be distinguished and even the Bilberry samples originating from 2 different picking regions (R and V) can be divided and separated just using absorbance units of the sample (Fig. 2). This result is important because fruit classification is an important task for quality control and identification of foods considering the possibility of food product adulteration. With results presented on Fig. 2, it was shown that the procedure presented can be recommended as very helpful for qualitative estimation of the effects examined.

The next step was use of multivariate techniques in detection of separation of samples based on the sample preparation procedure.

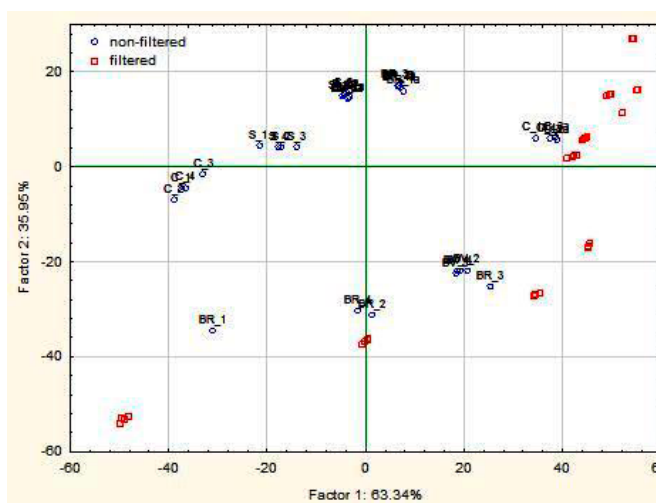
From results presented in Figs. 3a & 3b., can be concluded that the chemometric approach using principal component analysis (PCA) was effective because the data have split into 2 sets and the sets correspond to the storage stage of the fruit extracts. Regardless of whether the samples were filtered (Fig. 3b) or not (Fig. 3a), the fresh samples have been separated from the samples that have been stored for one month. The values of the factors show the influence of the freshness of filtered (PC2 to approximately 5%) and non-filtered samples (PC2 = 23.5%), what leads to the conclusion that the effect of the freshness has much more influence on the samples that are not filtered. NIRs advantage as a measuring method should be the non-destructiveness of the samples (Williams et al., 1988; Reid et al., 2005). Therefore, this justifies the use of PCA scores,



**Figure 3.** Plot for the first two PC factors, based on spectral data used in differentiation of fresh fruit extracts and those that are stored for one month of (a) non-filtered and (b) filtered samples.

especially using the first two PCA scores, where the first two components (PC1 and PC2) that gave a high level of classification of berry-fruit extracts (99%) stored for one month.

Different scores were obtained for filtered and non-filtered samples and in order to test the hypothesis that the samples can



**Figure 4.** Plot for the first two PC factors, based on spectral data used in differentiation of berry-fruit extracts according to the sample preparation procedure

also be separated due to the absorbance spectra regarding the sample preparation, the PCA scores plot was obtained from the NIR analysis of all recorded samples prepared as filtered or samples without additional preparation (filtration) what is presented in Fig. 4.

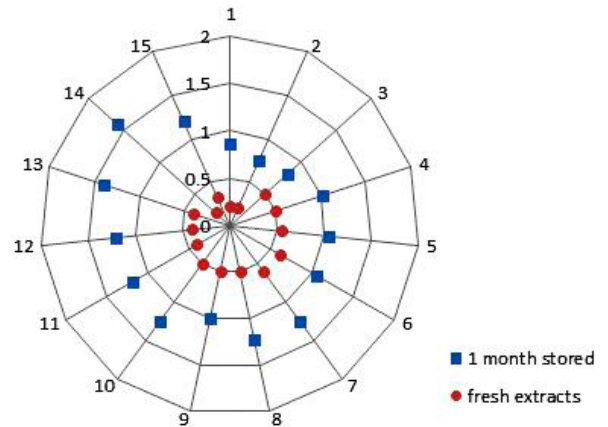
In the analysis, 3 absorbance spectrums for each fruit extract were analysed. As shown, the differentiation was possible and also effective as the differentiation of the berry-fruit extracts freshness shown on previous figure. In Fig 4., the first two components (PC1 and PC2) gave the highest level of fruit extract classification (99.29%) based on different extract preparation, what implies that using the NIR spectra and multivariate exploratory technique as the principal component analysis as a classification analysis is a helpful tool used to divide different fruits, detect their freshness as well as to differ the samples based on the preparation procedure.

Presented results and analyses of the spectrums indicated changes in specific wavelengths of 1434 and 1635 nm which corresponds to the C–H combinations and the 1<sup>st</sup> overtone of the bonds as well as in the range 904-1181 nm which is related to the 3<sup>rd</sup> CH overtone and 2<sup>nd</sup> overtone of the OH stretch of H<sub>2</sub>O and a combination of stretch and determination of the OH group in H<sub>2</sub>O can be observed (Alishahi et al., 2010; Charters and Pettigrew, 2007), respectively. That indicates the advisability of use of NIR for on-line monitoring for berry-fruit monitoring because the use in product quality and selection (Alishahi et al., 2010; Liu et al., 2010; Nicolai et al., 2007) or to use it in order to select harvested fruit (Hernández Gómez et al., 2006) or to differentiate juice samples as Reid and his co-workers (2005) have already been conducted. This study shows that also other approaches using NIR absorbance spectra in product and fruit cultivars can be made.

Multivariate data analytical methods can be used to simplify methods and reduce analytical times for many analyses (Cozzolino et al., 2011). Such multivariate method is partial least squares regression (PLSR) that presents the generalisation of multiple linear regression (Cozzolino et al., 2011). PLS regression can analyse data with strongly collinear (correlated), noisy and redundant variables (X variables or wavelengths) and also model several characteristics (Y values) at the same time. In PLS regression an orthogonal basis of latent variables is constructed one by one in such a way that they are oriented along the directions of maximal covariance between the spectral matrix and the response vector (Wold et al., 2001).

This method performs particularly well when the various X-variables express common information, for example, when there is a large amount of correlation, or even co-linearity, which is the case for spectral data of biological materials such as fruits. The required number of latent variables is typically smaller than that in a PCR calibration model for a similar model performance. PLS regression can be easily extended to simultaneously predict several qualities attribute (Cozzolino et al., 2011). The latent variable (time of storage) was used to simplify a complex model where the NIR spectrums could be used to classify the samples as fresh ones or not. Calibration statistics included the standard error of calibration (SEC), the coefficient of determination in calibration and the standard error in cross validation (SECV). The optimum calibrations were selected based on minimizing the SECV. The efficiency of the PLSR model can be observed in figure 5 that presents

the samples that are fresh (which predicted value ranged between 0 and 0.6) and those that have been stored for one month (range of predicted values is over 0.75). The true values for fresh samples were 0 and for the stored sample, 1. The correlation between the experimental results and those obtained with the PLSR model was estimated with the determination coefficient using Chaddocks' scale ( $R^2 = 0.79$ ) what after the scale presents a high relationship between observed variables (experimental and model data).



**Figure 5.** Radar of predicted values based on the PLSR model

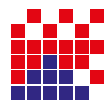
Those results indicate the advantage of using NIR spectroscopy in the data analysis because any new pattern of wild berries can be qualified by its type (Fig.2), according to the method of sample preparation (Fig. 3) or by its freshness (Fig.5).

The results clearly show the potential for the application of near-infrared spectroscopy for a non-destructive detection of different cultivars and especially in detection of the preparation of the samples as well as the spoilage during prolonged storage. The aim for our following study is to examine further the influence of the storage time on the decrease in quality of the extracts with wider range of time and temperature and to develop a PLS model that could predict the decrease just based on the NIR spectrum.

## Conclusion

Fruit classification is an important task for quality control and identification of food product adulteration. NIR technique, as a widely accepted and the most promising on/in-line process control technique, was used and from the absorbance spectrums revealed changes in specific wavelengths 904-1181 nm and 1434 and 1635 nm. Presented results and analyses of the spectrums indicated changes in specific wavelengths of which correspond to the C–H combinations and the O–H stretch of H<sub>2</sub>O.

The results showed that near infrared spectroscopy has potential to distinguish fruit samples regarding the cultivar, to divide them according the way the sample was prepared and the samples were qualitatively divided based on the storage time. All findings are based on the used chemometric methods what shows that NIR measurement, in combination with chemometrics, is a useful tool for quality control and on-line appli-



cations. In this paper the chemometrical methods have shown its potential being used as cost-effective, both quantitative and qualitative methods combined with modern analytical tools.

This paper has proven that NIRS can be used qualitatively to detect, to identify, and to qualify raw materials. With further studies it could potentially be utilized as a protocol to detect different berry cultivars and their origin as well as to establish the quality based on the storage.

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