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Potential of Fourier transformed near-infrared (FT-NIR) spectroscopy for rapid analysis of elderberry (*Sambucus nigra* L.) fruits

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Abstract: Fruit samples were analysed to investigate the suitability of Fourier transform near infrared spectroscopy (FT-NIR) for the rapid discrimination of elderberry genotypes. Parallel analysis with classical chemical techniques and spectral measurements was performed on 11 cultivars originating from various European countries. The titratable acidity (TA) and soluble solids content (SSC) of the fruit, and the geographical origin and breeding method of the cultivar were used as reference data. Three spectrum transformation methods (standard normal variation, multiplicative scatter correction and first derivative) were applied in the calibration process. The statistical analysis and comparison of the samples was carried out using principal component analysis (PCA) and linear discriminant analysis (LDA). In all cases the analysis demonstrated a correlation between the spectra and both the chemical traits (TA and SSC) of the fruit and the other reference data, indicating that pattern recognition was not a chance occurrence. This work provides the first evidence that the NIR technique can be successfully applied to distinguish between elderberry genotypes on the basis of fruit quality, thus opening up new possibilities in breeding cultivars for food industry purposes.

Keywords: LDA; PCA; soluble solids; titratable acidity

Over the last few decades the use of elderberry (*Sambucus nigra* L.) in the food industry has made it an important berry fruit in Europe. According to Kaack and Austed (1998) a substantial quantity of pigment is formed in the fruit (361–1266 mg CGE/100 g), so the dark red juice extracted from the fruit can serve as a natural colouring agent for squash, jam and other products. Food products made from elder flowers and berries have notable antioxidant capacity (Cejpek *et al.* 2009).

Among the European countries, Austria, Czech Republic, Denmark, Germany, Hungary, Italy, and Poland are the major producers (Finn *et al.* 2008). The most widespread cultivar in Europe is cv. Haschberg. In order to overcome the unfavourable traits (uneven ripening or susceptibility to diseases), the breeding of cultivars designed for the food industry has commenced in many countries, including USA, Canada, Denmark and Hungary. For these purposes the evaluation of fruit quality is essential.

In recent years, near-infrared spectroscopy has become a widely used technique for analytical studies, particularly in the pharmaceutical and food industries. The technique is rapid, cheap and non-invasive, and offers reagent-free alternatives compared with conventional chemical analysis. A further advantage of NIR instruments is that in most cases samples can be analysed without preliminary treatment. The tech-

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nology is suitable, as an independent or alternative method, for the quantitative and qualitative analysis of ready-to-eat foodstuffs, wines, fruit and vegetables (Nicolai *et al.* 2007; Fodor *et al.* 2011; Szigedi *et al.* 2012; Martelo-Vidal & Vázquez 2014).

Large numbers of wild elderberry biotypes are available in Central Europe for breeding. Rapid analytical methods are required for the evaluation of fruit quality in breeding materials and in their progeny. Analytical techniques for green chemicals and food such as NIR spectroscopy, which have already been successfully used for other fruit species (Nicolai *et al*. 2007; Ruiz *et al.* 2009; Eisenstecken *et al*. 2015; Amodio *et al.* 2017), could be ideal for this purpose. So far the NIR technique has only been applied in elderberry to determine the antioxidant capacity of the flowers (Clara *et al.* 2016). The present work is the first attempt to investigate whether the NIR technique is suitable for distinguishing between elderberry genotypes on the basis of fruit quality. For this purpose, the results of FT-NIR measurements on a total of 11 cultivars were compared with physical and chemical reference data. In the hope of accelerating breeding and reducing costs, the work aimed to determine whether rapid spectral analysis could be relied on when selecting progeny generations on the basis of fruit quality. It was also hoped to discover whether elderberry cultivars could be discriminated on the basis of geographical origin, as reported for other species (Galtier *et al.* 2007).

MATERIAL AND METHODS

Plant material. Fruit samples were collected in 2016 in the orchard of the Soroksár Experimental Farm of Szent István University (SZIU). Eleven cultivars included in the study (Table 3). After removing the stalks, the fruit were homogenised in a food blender and then stored in a freezer (–25°C) until required.

Analysis of chemical composition. Sample preparation and chemical measurements were performed in the Department of Pomology of SZIU. The water-soluble solids content (SSC) of the fruit was analysed using an HI 96801 digital refractometer, while the titratable acid (TA) content was determined on the basis of the Hungarian standard (MSZ EN 12147:1998). Ten gram of fruit samples were made up to 100 ml with distilled water, after which they were titrated with 0.1M sodium hydroxide (NaOH) solution in the presence of dimidium bromide-disulphine blue until the colour changed. The total acid content was expressed as malic acid equivalent. The values characteristic of the cultivars were obtained in all cases as the mean of three parallel measurements for each sample and the results were given as w/w%.

Spectral measurements. Homogenised fruit samples were analysed using a Bruker MPA FT-NIR instrument (Ettlingen, Germany) in the Department of Applied Chemistry of SZIU. From each cultivar sample was measured seven subsamples, so we worked at the end with 77 data, and the mean of the results was used for further analysis. The instrument had a measurement range of 12 500–4000 cm^{-1}, with a resolution of 16 cm^{-1} and a scanning speed of 10 kHz. The spectra were recorded in transflection mode, which amalgamates the advantages of diffuse reflection and transmission. The measurements were performed with transflection unit (2 mm thick, with steel reflecting mirror). OPUS 7.2 (Bruker, Germany) software was employed to analyse the spectral images and identify characteristic light absorption regions.

Preliminary data analysis. In the course of statistical analysis the same three spectrum transformation operations were applied in all cases: (1) standard normal variation (SNV); (2) multiplicative scatter correction (MSC); (3) first derivative. The most commonly used preprocessing methods in spectroscopy are SNV, MSC and derivatives, SNV is often used on spectra where baseline and pathlength changes cause differences between otherwise identical spectra. MSC correction is achieved by regressing a measured spectrum against a reference spectrum and then correcting the measured spectrum using the slope and intercept of this linear fit. This pretreatment method has proven to be effective in minimizing baseline offsets and multiplicative effect. Derivatives are among the most common signal pre-treatments applied to spectral data. Derivatives are mainly used to resolve peak overlap (or enhance resolution) and eliminate constant and linear baseline drift between samples (Barnes *et al.* 1989; Dhanoa *et al*. 1994). The purpose of preliminary data analysis is to subject the spectral data to various mathematical function transformations in order to detect fine differences and shifts originating from the particle size.

Multivariate analysis. Statistica 8.0 (StatSoft, USA) and Unscrambler 10.4 (CAMO, Norway) software was used for the statistical evaluation of the data. The analysis and comparison of the samples was performed using the principal component analysis (PCA) and linear discriminant analysis (LDA) techniques. PCA is an unsupervised pattern recognition method. The

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basic idea is that 'latent variables' are created by the linear combination of the original variables. The principal components are ordered in such a way that the variance explained by the first principal component is the greatest; the variance explained by the second one is smaller, and so on, whereas that of the last is the smallest (Wold *et al*. 1987). Linear discriminant analysis is a supervised method, i.e., we must know the class memberships before the analysis. It is similar to principal component analysis (PCA), but here canonical variables (roots) are calculated. The algorithm is described in detail in reference (Hastie *et al*. 2009).

PCA was performed without data pre-treatment and after all three data pre-treatments (Table 1). After the first derivative data pre-treatment performed PCA resulted a poor model. Thus in the following, the first derivative data was not applied. Spectral outliers are tested at the exact confidence level based on PCA. Finally, the groups formed on the basis of reference factors were subjected to LDA analysis for pattern recognition and the discrimination of the genotypes according to various criteria.

Reference data. Four types of reference data were designated for the comparison of the spectra. Four sample groups were formed from both the SSC data and the TA data obtained in the present chemical analyses (Table 2). The classification of the cultivars on this basis is presented in the Results section. The classification of the cultivars into two groups according to geographical origin and breeding method was performed on the basis of data from the literature (Table 3). The spectra recorded using the FT-NIR instrument were compared with the data grouped as described above by means of statistical analysis (LDA).

Table 1. The multivariate analysis results

Reference data	Analysis method	Reliability (%)
	PCA + LDA*	95
Soluble solids	SNV + PCA + LDA	92.95
	MSC + PCA + LDA	92.95
	PCA + LDA	95
Titratable acid	SNV + PCA + LDA	95
	MSC + PCA + LDA	95
	PCA + LDA*	97.06
Geographical origin	SNV + PCA + LDA	97.06
	MSC + PCA + LDA	97.06
	PCA + LDA*	95.59
Breeding methods	SNV + PCA + LDA	97.06
	MSC + PCA + LDA	97.06

*without preliminary data treatment; MSC – multiplicative scatter correction; PCA – principal component analysis; LDA – linear discriminant analysis; SNV – standard normal variation; MSC – multiplicative scatter correction

Table 2. Limit values (%) for the formation of sample groups on the basis of soluble solids content (SSC) and titratable acid content (TA)

Sample groups	SSC	TA
A	11.5 >	0.4 >
B	11.5–12.5	0.4–0.5
C	12.5–13.0	0.5–0.55
D	13.0 <	0.55 <

Table 3. Classification of elderberry accessions

Code	Accession	Country	Geographical origin	Breeding method
1	Sampo	Denmark	N	H
2	Samidan	Denmark	N	H
3	Samdal	Denmark	N	H
4	Haschberg	Austria	C	S
5	Samocco	Denmark	N	H
6	Weichenstephan	Germany	C	H
7	Samyl	Denmark	N	H
8	K3	Hungary	C	S
9	Haidegg 13	Austria	C	S
10	Korsör	Denmark	N	S
11	Haidegg 17	Austria	C	S

N – North Europe; C – Central Europe; H – hybridization; S – selection of wild population

RESULTS AND DISCUSSION

Chemical composition. The soluble solids content of the cultivars (Table 4) proved to be lowest for cv. Haschberg. Among the Danish cultivars, Samyl had the highest values, confirming the results of Kaack (1989), and the Hungarian K3 also proved promising. Cv. Korsör also had high SSC, which contradicted the findings of Kaack (1997) and Lee and Finn (2007).

The lowest titratable acid content was observed for cv. Weihenstephan and the highest for cv. Sampo (Table 4). Studies conducted in Denmark by Kaack (1997) and Kaack *et al.* (2008) confirmed the present results for cv. Sampo. Cultivars Samocco, Samdal and Haidegg

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Table 4. Values recorded for soluble solids content and titratable acid content (%) (2016)

Accession	Total soluble solids	Titratable acidity
Sampo	11.10 ± 0.2	0.59 ± 0.03
Samidan	12.73 ± 0.12	0.51 ± 0.04
Samdal	11.37 ± 0.06	0.53 ± 0.04
Haschberg	10.40 ± 0.2	0.52 ± 0.02
Samocco	12.23 ± 0.4	0.56 ± 0.01
Weihenstephan	12.07 ± 0.31	0.36 ± 0.07
Samyl	13.05 ± 0.35	0.38 ± 0.03
K3	13.07 ± 1.1	0.39 ± 0.04
Haidegg 13	12.67 ± 0.4	0.44 ± 0.01
Korsör	12.67 ± 0.6	0.44 ± 0.03
Haidegg 17	11.84 ± 0.15	0.55 ± 0.06

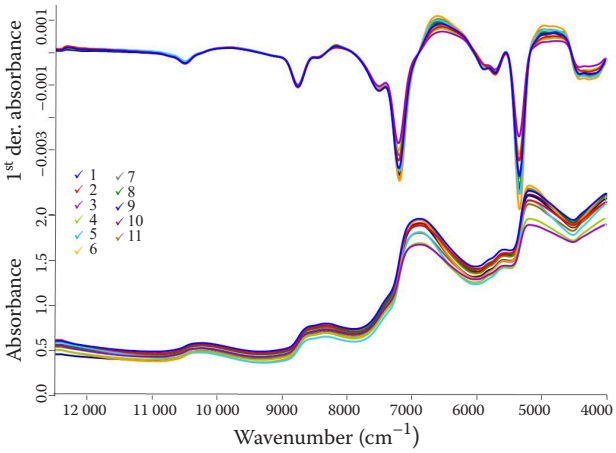

Figure 1. FT-NIR transflectance spectra of the samples and their first derivatives

17 had values similar to those of cv. Haschberg, as also reported by Szalóki-Dorkó *et al.* (2015).

Analysis of FT-NIR spectra. The raw and 1st derivate FT-NIR spectra of fruit samples from 11 elderberry cultivars are shown in Figure 1. The characteristic vibration regions of the major components are summarised in Table 5.

Soluble solids content. To obtain reference data for soluble solids content, the cultivars were divided into four groups based on the limit values given in Table 2: A – 1, 3 and 4; B – 5 and 6; C – 2, 9 and 10; D – 7 and 8. LDA analysis performed after preliminary data treatment gave a classification probability of 97.2% at the 95% confidence level. The four groups can be clearly distinguished on Figure 2A, showing that classification on the basis of soluble solids content correlates well with the recorded spectra. In this case, too, data reduction was performed prior to the analyses, and the 20 principal components obtained in this way were used for the statistical evaluation. The only error inthe LDA analysis was that one C category sample was classified in group A, while the classification of one D category sample was doubtful. Forward stepwise LDA model building method and threefold cross-validation were applied in the evaluation. Proper validation is very important; it should be tested, whether the results are artefacts or not. For this purpose, as another validation method for the model, Y-scrambling randomization test was used. The random control classification, in which the samples were put randomly in groups irrespective of their true classification, resulted in a confused picture, proving that the original pattern recognition was not accidental (Figure 2B).

Titratable acid content. Based on the available data and the limit values given in Table 2, the samples could be divided into the following groups: A = 6, 7, 8; B = 9, 10; C = 2, 3, 4; D = 1, 5, 11. In this case too, pattern recognition was carried out as described above, without preliminary data treatment, or with SNV or MSC treatment. In all three cases 20 prin-

Table 5. Identification of characteristic vibration regions based on Workman and Weyer (2012)

Component, bond and functional group	Wave number (cm^{-1})
Water	10526–10204; 6944–6803; 5208–5155
Hydrocarbons, aromatic	8754–8749
Alcohols, OH (free and with hydrogen bonding)	9386; 7300–7170; 7090–6090
Carbohydrates	6896; 4760; 4390–4283; 4000
Carboxylic acids	8260–7600; 6920; 5290; 4950–4800; 4695; 4630; 4500–4000
Fiber	8547; 7057–6944; 7092–7042
Cellulose	6897; 5618; 5495; 4785; 4405; 4283–4386; 4260–4250; 4020–4000
Protein	6756–6451; 6173–5882; 4926–4877; 4655–4545

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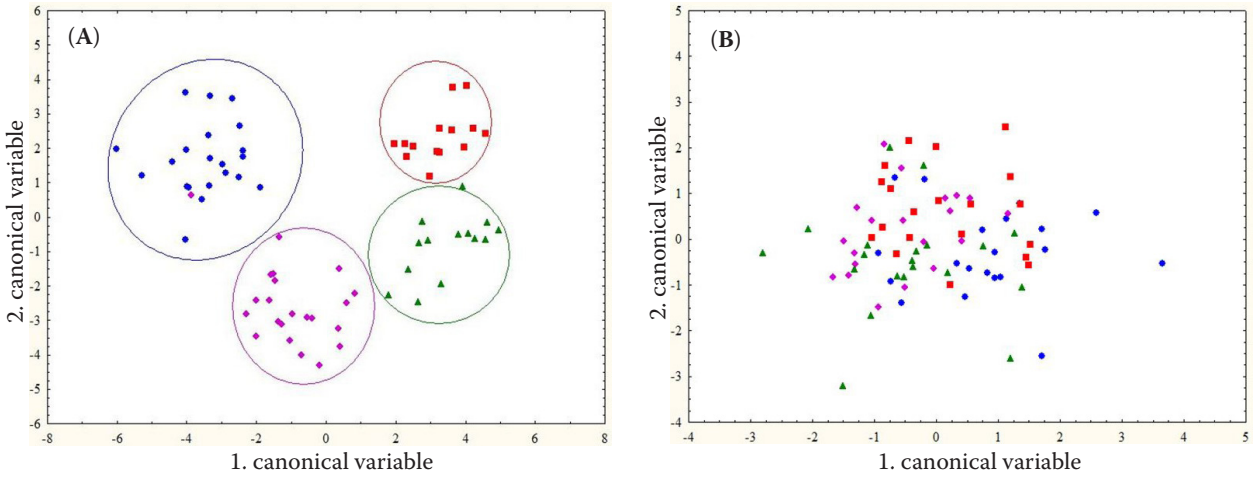

Figure 2. Pattern recognition and discrimination (**A**) on the basis of soluble solids content using LDA analysis without preliminary data treatment and (**B**) random control classification. • – 10.4–11.37; ■ – 11.84–12.23; ◊ – 12.67–12.73; ▲ – 13.05–13.0 (Brix %)

cipal components were produced from the original 1100 variables with the aid of PCA data reduction. In all three cases the discrimination of the groups was successful, as also proved by the control classifications. It should be noted that there was practically no difference in the patterns obtained with SNV and MSC preliminary treatment. In all cases the checks confirmed that the classification was not accidental. The LDA pattern recognition (Figure 3) clearly distinguished between the groups, proving the correlation between the analyses.

Geographical origin. The cultivars could be separated into two groups on the basis of geographical origin (Table 3). PCA was again used for data reduction to produce 20 principal components and, as in the previous cases. LDA was initially performed without preliminary data treatment (Figure 4). Subsequently, LDA analysis was repeated with SNV and MSC treatments as well. In all three cases one spectrum each from the same two Central European samples were erroneously classified in the North European group, No. 6 (cv. Weihenstephan) and No. 8 (K3). The former was bred in the southern part of Germany and the latter on the western border of Hungary by selection from wild-growing populations. It should be noted that several spectra were recorded for samples from the same geographical location, and the erroneous classification was only detected for one spectrum of each sample. It is possible that the erroneously classified spectra were not entirely homogeneous

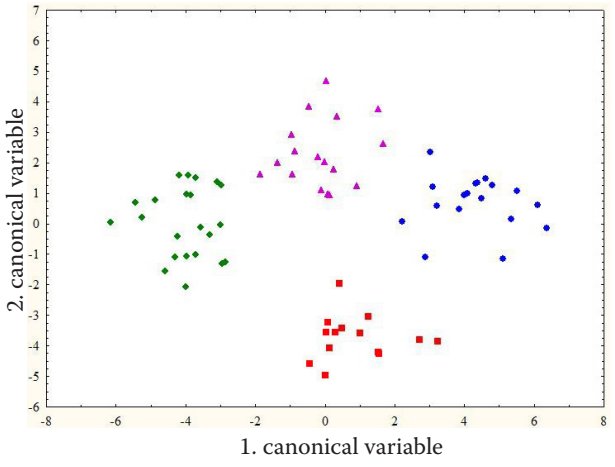

Figure 3. Pattern recognition on the basis of titratable acid content using LDA analysis without preliminary data treatment. • – 0.36–0.39; ■ – 0.44; ◊ – 0.51–0.53; ▲ – 0.55–0.59 (TA %)

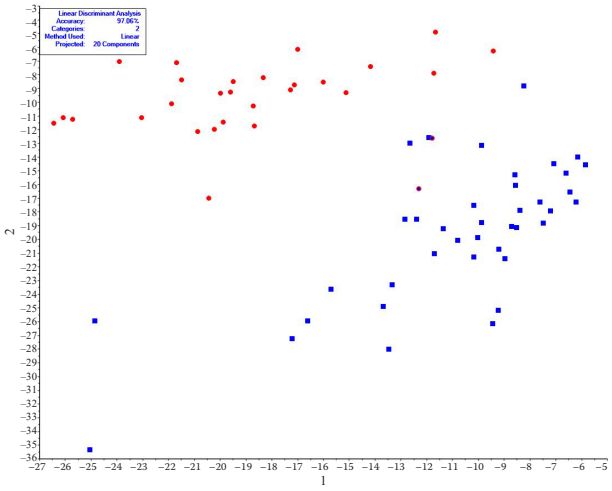

Figure 4. Pattern recognition and discrimination on the basis of geographical origin using the LDA method without preliminary data treatment. • – Central European; ■ – North European

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(e.g. they may have contained seed parts or skin), and although these were not perceived by PCA as spectral outliers, they nevertheless caused errors in LDA. In spite of this, each of the three LDA analyses separately had a reliability of 97.06% for the recognition of geographical origin. The results of the checks thus confirmed the success of pattern recognition, i.e. the classification of the cultivars according to geographical origin could be regarded as correct.

Breeding methods. Among the cultivars included in the analysis, six were hybrids produced by crossbreeding, while five were the result of selection (Table 3). In the course of the analysis, LDA was performed without preliminary data treatment and after SNV or MSC data treatment. In all cases the possibility of pattern recognition was investigated. The spectral data without preliminary data treatment were first subjected to PCA for data reduction, leading to the formation of 20 principal components. The use of these PCs allowed the two groups to be discriminated with a reliability of 97.06%, in the case of SNV preliminary data treatment (Figure 5). This was checked by means of random classification, which led to pattern recognition with a reliability of 64.7%, so the original classification was regarded as successful. The spectra demonstrated differences among cultivars developed by hybridisation or selection, so the grouping on the basis of spectral data was shown to be correct. One spectrum each of the selected cv. Haschberg (No. 4) and K3 (No. 8) were erroneously classed as hybrids, while one spectrum of the hybrid cv. Samidan (No. 2) was erroneously classed as the result of selection by the statistical program. However, in this case, too, several spectra were recorded for each sample and used in the evaluation, and only one of the parallel spectra was erroneously classified. The probable reason for this was again that errors occurred in the homogenisation stage. Therefore, although these errors occurred, the pattern recognition can be regarded as successful.

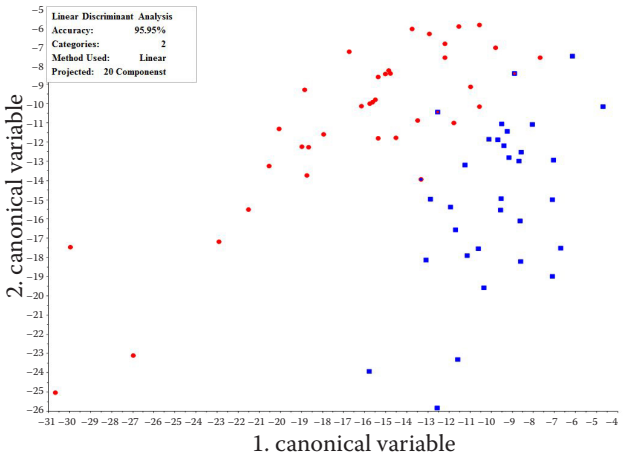

Figure 5. Discrimination of genotypes on the basis of breeding method (selection or hybridisation) using LDA analysis with SNV preliminary data treatment. • – hybrid; ■ – selected

These LDA results cannot be compared with previous research on elderberry, as, to the best of our knowledge, this is the first spectral analysis performed on the fruit of elderberry genotypes. However, previous studies on temperate zone fruit species, such as apple (Ying *et al.* 2007), pear (Ying & Liu 2008), apricot (Bureau *et al.* 2009), peach (Ying *et al.* 2005) and blueberry (Sinelli *et al.* 2008), proved that FT-NIR spectrometry was a reliable and accurate method for the non-invasive measurement of SSC and TA in fruit. The samples of pistachio have been successfully discriminated according to geographical origin by means of NIR spectroscopy (Vitale *et al.* 2013).

The technique has the further advantage that it substantially shortens the time and cost of fruit quality analysis (Sinelli *et al.* 2008). It is thus extremely useful in breeding for the positive or negative selection of large numbers of hybrids. Experience in this field has now been gained not only for cereals (Sissons *et al.* 2006), but also in fruit breeding (Bureau *et al.* 2009; Pissard *et al.* 2013). Spectral techniques have also been responsible in great part for the development of high throughput phenotyping methods (Araus & Cairns 2014), which greatly improve the efficiency of breeding.

CONCLUSIONS

The present work aimed to investigate whether the FT-NIR instrument was able to discriminate between different elderberry cultivars on the basis of either fruit quality or origin. In all cases, correlations were found between the spectral data and the categories formed, and these were confirmed during the checking process. The results indicate that the NIR technique can be successfully applied for the rapid, non-destructive comparison and analysis of elderberry cultivars. It is the first proof of the analysis of elderberry cultivars. This technique could thus be a promising approach for the evaluation and comparison of elderberry breeding materials. It is recommended that the research should be continued on hybrid breeding stocks.

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