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RAMAN SPECTROSCOPY IN THE CHARACTERIZATION OF AUTOCHTHONOUS SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS FROM THE BALKAN REGION

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Abstract: The quality assessment and evaluation of fruits and vegetables are crucial in their postprocessing, shelf life, and price. Most of the techniques applied to evaluate fruit and vegetable quality are invasive. However, there is a growing interest in non-invasive techniques for assessing fruit quality, which are gaining traction due to their application and operation mechanism. The present study demonstrates, for the first time, the applicability of the Raman spectroscopy for spectral signature assessment of sweet cherry (*Prunus avium* L.) cultivars ('Đuti', 'Canetova', 'Ohridska crna', and 'Dolga Šiška'). Combined with principal component analysis (PCA), Raman spectroscopy was used in assessing nutritionally similar samples, such as the studied sweet cherry cultivars. Sugars (glucose, sucrose, and fructose), anthocyanins, phenolic acids, and flavonoids, quantified by comparison to reference standards using high-performance liquid chromatography, exhibited Raman bands (at 337, 399, 455, 538, 617, 1327, and 1600 cm^{-1}, respectively) of varying intensities, indicating differences among cultivars. Compared to the other cultivars, the 'Ohridska crna' cultivar had the highest nutritional and health-promoting compounds. A correlation was found between the Raman bands and the sugar and phenolic content obtained by chemical analysis. The results indicate the applicability of chemometric modeling associated with Raman spectroscopy for rapid sweet cherry authentication.

Key words: sweet cherry, vibrational modes, multivariate analysis, HPLC analysis, anthocyanins, phenolic compounds, carbohydrates.

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Introduction

The sweet cherry tree (*Prunus avium* L.) is one of the most valuable species of stone fruit trees. Sweet cherries, typically consumed fresh and unprocessed, are considered early-season fruits (Usenik et al., 2008). Their regular consumption is associated with a balanced diet and an array of health benefits owing to the abundant presence of bioactive and nutraceutical compounds in this fruit (Faienza et al., 2020). The chemical composition of cherries is contingent on several factors, most notably the specific cultivar. Additionally, factors such as maturation age, agricultural practices, and environmental conditions can significantly influence the chemical profile of cherries. Carbohydrates, sugars (e.g., fructose, glucose, sorbitol), constitute the primary chemical compounds in cherries. Sweet cherries are esteemed as excellent sources of dietary phenolic compounds, encompassing phenolic acids (p-coumaric and chlorogenic acids) and flavonoids (anthocyanins, flavan-3-ols, and flavonols), as well as valuable sources of vitamins, particularly vitamin C, and minerals such as calcium, magnesium, and potassium (Schmitz-Eiberger and Blanke, 2012; Ferretti et al., 2010; Yıgıt et al., 2009).

Epidemiological research has revealed a link between the intake of fruits and vegetables abundant in bioactive compounds or phytochemicals and a decreased risk of developing degenerative diseases caused by oxidative stress, such as cancer (Kang et al., 2003), atherosclerosis, diabetes mellitus, and cardiovascular disease (Faienza et al., 2020). Moreover, scientific investigations suggest that the consumption of sweet cherries may have beneficial effects, including easing arthritis-related pain and inflammation (Jacob et al., 2003; Seeram et al., 2001) and protecting against neurodegenerative disorders (Filaferro et al., 2022). Kent et al. (2016) provided evidence that the consumption of sweet cherries, whether as fruit or juice, can lead to a significant decrease in both systolic and diastolic blood pressure and heart rate, especially among individuals with hypertension.

Up to this point, the majority of research endeavors have employed high-performance liquid chromatography (and/or spectrophotometric methods) as their primary method for pinpointing and measuring bioactive substances within sweet cherries (Ballistreri et al., 2013; Clodoveo et al., 2023). Nonetheless, conventional approaches to evaluate fruit quality primarily revolve around sensory assessment and chemical analysis, which are prone to external influences, lack precision, have limited detection speed, and demand significant resources. Consequently, researchers across the globe have been actively exploring and advancing novel, non-invasive detection technologies, with Raman spectroscopy being a prominent example (Xu et al., 2020).

The development of a rapid, highly accurate, non-destructive, and cost-effective fruit quality testing technology is of paramount practical importance. In addition to its inherent advantages of high efficiency and non-destructiveness,

Raman spectroscopy (RS) is unaffected by water and can be applied to aqueous solutions (Neng et al., 2020). Its strong penetration capability makes it suitable for assessing the internal quality of fruits and vegetables. Thus, Raman spectroscopy plays a crucial role in studying food chemical composition and quality control (Seidler-Lozykowska et al., 2010; Petersen et al., 2021; Nakajima et al., 2023; Xu et al., 2023). This type of spectroscopy typically provides a special structural fingerprint that is used to identify different molecules. In general, Raman spectra can provide some minor changes that allow the isolation of specific local variations of certain phytochemicals.

Raman spectroscopy, combined with chemometric data analysis, proves to be a formidable method for detecting chemical structures, even from complex matrices (Xu et al., 2020). The application of Raman spectroscopy coupled with chemometrics represents a step forward in sweet cherry fruit authentication approaches. To the best of our knowledge, this is the first study of the nutritional composition of cherry fruits using Raman spectroscopy.

The present work aims to make a comparative study of the four autochthonous sweet cherry cultivars originating from Serbia and North Macedonia. Raman spectroscopy coupled with chemometrics was applied to evaluate the differences in the nutritional profile, phenolic composition, and health qualities of cherries. In addition, high-performance liquid chromatography was also employed to quantify the individual sugars and phenolic compounds of the sweet cherry cultivars to gain a deeper understanding of the outcomes acquired through the non-invasive Raman technique. Anthocyanins, phenolic acids, and flavonoids, which are primarily responsible for the health-promoting effect of these fruits, were quantified.

Material and Methods

Five autochthonous sweet cherry cultivars originating from Serbia and North Macedonia were harvested manually in the period from May to July 2022 and used for the studies: ‘Đuti’ (May 20, Belgrade, 1), ‘Canetova’ (June 2, Čačak, 3), ‘Ohridska crna’ (June 24, Ohrid, 4), and ‘Dolga Šiška’ (June 26, Ohrid, 5) (Figure 1). The harvested fruits were transported to the laboratory, where the samples were adequately stored before further analyses.

Chemicals used as reference standards for HPLC analysis: cyanidin-3-*O* rutinoside, cyanidin-3-*O* glucoside, p-coumaric acid, glucose, fructose and sorbitol were supplied from Sigma-Aldrich (Germany), while chlorogenic acid and rutin analytical standards were purchased from Acros Organics (US). Methanol was purchased from J.T.Baker (Netherlands).

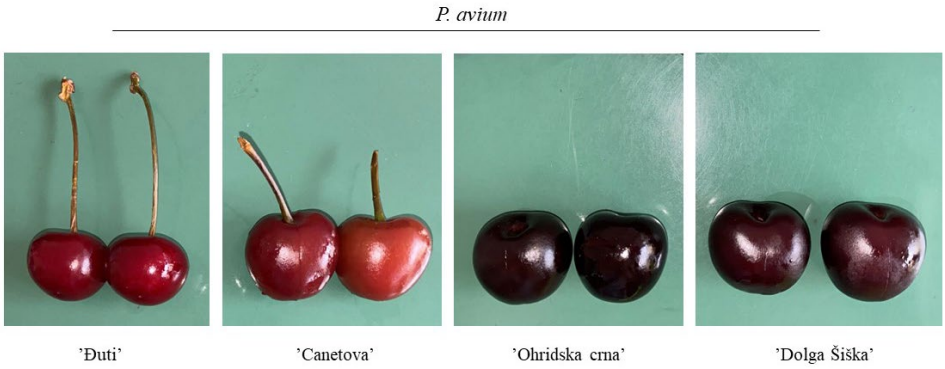

Figure 1. Sweet cherry (*Prunus avium* L.) cultivars used in the present study.

Fruit material preparation

Fresh fruits delivered to the laboratory were pitted and stored at -80°C until further analysis. For the extraction of anthocyanins and phenolic compounds, the fruits were first freeze-dried using a Beta 2-8 LD freeze-dryer (Martin Christ, Osterode, Germany) at -60°C for 48h and under a pressure of 0.012 mbar. The freeze-dried fruits were then ground in a lab mill, and the extraction was carried out following the procedure of Średnicka-Tober et al. (2019) with slight modifications. Two grams of freeze-dried fruit sample were mixed with 6 mL of methanol and 2 mL of 1M HCL in a plastic test tube. The samples were vortexed for 15 min at 30°C in a shaker and then centrifuged for 5 min at 7,000 rpm. The supernatant was filtered and cooled at 5°C. Obtained extracts were used for analyses. For the content of individual sugars, a mashed (3 g) fruit sample was dissolved with 30 mL of distilled water for 30 min at room temperature. The extracted sample was centrifuged at 7,000 rpm for 5 min (IKA centrifuge, USA). The supernatant was filtered and the obtained extracts were transferred into vials and used for analyses.

Raman spectroscopy of different fruit cherry extracts

Raman spectroscopy of fruits of *Prunus avium* L. cultivars was focused on the direct measurement of storage parenchyma cells. A Raman spectrometer system (Horiba Jobin Yvon, France) equipped with the Olympus BX 41 microscope was used. During the spectral recording, the 785 nm laser was focused onto the sample using the 50 LWD objective (Olympus, Tokyo, Japan). The spectrometer is equipped with 600 lines/mm grating, in the range from 200 to 1800 cm^{-1} in the extended mode. The measurement was conducted with a 5s integration time, with 5 spectral accumulations. The spectral resolution was approximately 3 cm^{-1} and the calibration was verified using the 520.47 cm^{-1} line of silicon. Ten spectra were

recorded per sample, making 150 spectra in total. The assignment of the bands was carried out using the literature data. The spectra were preprocessed using the Spectragryph software, version 1.2.14. (Menges, 2021), while the PC analysis was performed using the PAST software (Hammer et al., 2001). The principal components are composed of scores and loadings. When using PCA, it is possible to visualize the data while reducing the data size, allowing segregation between classes. Particularly, the scores and loadings reveal the differences between the samples.

Determination of sugars using HPLC

Samples were analyzed using a Dionex Ultimate 3000, Thermo Scientific (Waltham, MA, USA) HPLC system. The analysis was performed using deionized water as the mobile phase with an elution rate of 0.6 mL/min, on a carbohydrate column (Hi-Plex Ca^{2+},300 mm x 7.7 mm, 8 mm) incubated at 80°C. The product was detected using the RI detector (RefractoMax 520, ERC GmbH, Riemerling, Germany) preheated at 40°C. All data acquisition and processing were done using the Chromeleon 7.2 software.

Determination of anthocyanin and phenolic compounds using HPLC

The HPLC system (Dionex Ultimate 3000 Thermo Scientific, Waltham, USA) and a reverse phase column (XBridgeTM C18, 100 mm × 3 mm, particle size 3.5 μm) were used for the quantitative analysis of the samples. Solvent (A) H_2O: HCOOH = 100:0.1 % and solvent (B) MeOH were used as mobile phases. Elution was conducted in the following way: 0–5 min isocratic 0% B, 5–20 min gradient from 0 to 10% B, then 20–40 min isocratic 10% B, 40–60 min gradient from 10 to 20% B, 60–70 min isocratic 20% B, 70–95 min gradient from 20 to 50% B, 95–105 min isocratic 50% B, then 105–105.1 min gradient from 50 to 0% B and 105.1–110 min isocratic 0% B. The flow rate was 0.5 mL/min and the column was thermostated at 30°C. The injection volumes of the samples ranged from 5 to 30 μL. The products were detected by a UV detector at 310 and 520 nm. The standard curves for the analyzed compounds (cyanidin-3-O rutinoside, cyanidin-3-O glucoside, p-coumaric acid, chlorogenic acid, rutin) were constructed using different concentrations of standards and the obtained slopes were used for the calculations.

Statistical analysis

In the present study, the statistical analysis of the data obtained by HPLC was performed using analysis of variance (one-way ANOVA) followed by the Duncan's *post hoc* test within the statistical software, STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$, n=3.

Results and Discussion

Qualitative and quantitative analysis of sweet cherries using Raman and HPLC

Sweet cherry fruit analysis was performed by Raman microspectroscopy, and high-performance liquid chromatography, and the averages related to *Prunus avium* L. cultivars: 'Đuti', 'Canetova', 'Ohridska crna', and 'Dolga Šiška' are shown in Figure 2, Tables 1 and 2, respectively. The characteristic vibrational bands and the corresponding preliminary identification in the Raman spectra are listed in Table 3.

The Raman spectra of the extracted fruit samples show an increase or decrease in the intensity of the bands and the appearance of new bands (Figure 2) depending on the cultivar. An increase in the intensity of the bands in the carbohydrate region ranged from 200 to 500 cm^{-1}, especially at 455 cm^{-1}. The appearance and increase in the intensity of the band at 1327 cm^{-1} probably indicate an increase in the phenol concentration or anthocyanins in the fruit samples (Edwards et al., 1997; Zaffino et al., 2015; Farber et al., 2020), especially in 'Ohridska crna', while this band was not clearly observed in the 'Đuti' cultivar. A correlation was found between the mentioned Raman bands and the sugar and phenolic contents obtained by HPLC quantitative analysis (Table 2).

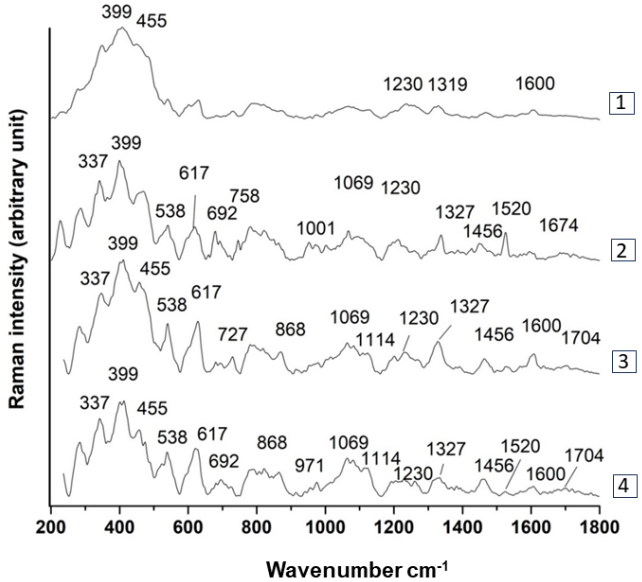

Figure 2. Averages of normalized Raman spectra of four *Prunus avium* cultivars ('Đuti' – 1, 'Canetova' – 2, 'Ohridska crna' – 3, 'Dolga Šiška' – 4) extracted fruit samples, recorded in the spectral range from 200 to 1800 cm^{-1}.

More precisely, significant bands of higher intensity were observed in the Raman spectra (Figure 2) at 337, 399, and 455 cm^{-1}, which are associated with the essential components of the fruits and assigned to the glucosidic ring vibrations, fructose, cellulose, and pectic acid (Boyaci et al., 2015; da Silva et al., 2008; Camerlingo et al., 2017; Zeise et al., 2018). These bands can be assigned to the C-C-C and C-O-C bending vibrations, indicating the presence of carbohydrates as major components of the storage parenchyma cells of cherries as the main tissue of the pericarp. The fruit samples were also analyzed for the content of individual sugars (glucose, fructose, and sorbitol) using HPLC analysis. Generally, glucose was found to have the highest content, ranging from 46.44 to 80.93 g/100g FW (fresh weight). The content of fructose varied from 39.93 g/100g FW ('Canetova') to 64.03 g/100g FW ('Ohridska crna'), and the sorbitol concentration was in the range 7.53–31.56 g/100g FW. The highest sum of sugars was found in 'Ohridska crna' and 'Dolga Šiška' from North Macedonia (Table 1). The results obtained in our work confirm the results of Usenik et al. (2008).

Table 1. Mean sugar content in g/100g FW ± standard deviation of the different sweet cherry cultivars.

	Glucose	Fructose	Sorbitol
'Đuti'	47.59±1.96^{a}	41.85±3.17^{a}	7.53±0.42^{a}
'Canetova'	46.44±0.73^{a}	39.93±1.55^{a}	8.17±0.37^{b}
'Ohridska crna'	80.93±3.44^{b}	64.03±3.05^{b}	31.56±1.23^{c}
'Dolga Šiška'	74.73±2.12^{c}	62.31±2.98^{b}	24.75±0.93^{d}

Different letters indicate significantly different values at $p<0.05$.

The medium intensity band positioned at 1327 cm^{-1} could indicate significant differences between 'Ohridska crna' and other cultivars, and this band could be associated with phenylpropanoids, anthocyanins, or cellulose (Zaffino et al., 2015; Farber et al., 2020). Furthermore, medium-intensity bands at 538 and 617 cm^{-1} are associated with polygalacturonic (pectic) acid and cyanidins, respectively (Edwards et al., 1997; Boyaci et al., 2015; Camerlingo et al., 2017). According to these bands, the only difference between the samples was observed in the band at 617 cm^{-1} associated with anthocyanidin content (Zaffino et al., 2015); for the 'Đuti' cultivar (lower intensity band) and the 'Ohridska crna' cultivar (higher intensity band) (Figure 2).

Two different anthocyanins and three phenolic compounds have been identified and quantified after applying the extraction process and the chromatographic method described previously. Variations in the anthocyanin concentrations were found between the cultivars. The most abundant anthocyanin was cyanidin 3-*O*-rutinoside (ranging between 4.175 and 289.275 mg/100g FW), followed by cyanidin 3-*O*-glucoside (0.453-49.625 mg/100g FW) in all cultivars.

As can be observed in Table 2, the sweet cherry cultivar showing the highest level of anthocyanin belonged to 'Ohridska crna'.

Very weak bands have been identified in the range from 650 to 1300 cm^{-1} and from 1440 to 1800 cm^{-1}, tentatively attributed to polygalacturonase (pectic acid), pectin, and the lower amounts of carotenes and phenols (Synytsya et al., 2003; Agarwal, 2006; da Silva et al., 2008; Boyaci et al., 2015; Kang et al., 2016; Farber et al., 2020). A band at ~1600 cm^{-1} could indicate the C=O ring vibration of phenylpropanoids, flavonoids, and chlorogenic acid (Maiti et al., 2013; Zaffino et al., 2015; Krysa et al., 2022), and this band was the most intense in 'Ohridska crna' (Figure 2). Three phenolic compounds were detected and identified in sweet cherry cultivars by comparison with reference standards: p-coumaric acid (phenolic acid), chlorogenic acid (phenolic acid) and rutin (flavonoid). 'Ohridska crna' showed the highest levels of phenolic compounds (26.26 mg/100g FW as the sum of the three identified compounds), while the 'Đuti' cultivar showed the lowest amount of phenolics (3.93 mg/100g FW as the sum of the three identified compounds). In all cultivars, rutin was the most abundant, except in 'Canetova', with the highest amount of chlorogenic acid. The results obtained for phenolic compounds are similar to those reported by González-Gómez et al. (2010) and higher than those reported by Usenik et al. (2008), probably because of the differences in geographical origin of the cultivars (Balkans, Spain, and Slovenia, respectively). The results of this work show a correlation between the Raman band intensities and the concentrations of the studied compounds.

Table 2. Anthocyanin and phenolic content of the different sweet cherry cultivars in mg/100g FW ± standard deviation.

	Cyanidin-3-*O* Rutinoside	Cyanidin-3-*O* Glucoside	p-coumaric acid	Chlorogenic acid	Rutin
'Đuti'	4.17 ± 0.03^{a}	0.45 ± 0.01^{a}	0.95 ± 0.08^{a}	0.66 ± 0.01^{a}	2.32 ± 0.09^{a}
'Canetova'	18.44 ± 0.92^{b}	4.48 ± 0.03^{b}	n.d.	3.34 ± 0.10^{b}	1.91 ± 0.04^{b}
'Ohridska crna'	289.27 ± 7.99^{c}	49.63 ± 2.22^{c}	7.07 ± 0.21^{b}	4.46 ± 0.12^{c}	14.73 ± 0.76^{c}
'Dolga Šiška'	35.14 ± 1.35^{d}	1.68 ± 0.02^{d}	1.31 ± 0.04^{c}	1.82 ± 0.02^{d}	3.38 ± 0.15^{d}

n.d. not detected, different letters indicate significantly different values at $p<0.05$.

Principal component analysis (PCA)

PCA (Fig. 3A) was applied to the data obtained from the Raman spectra in the 200–1800 cm^{-1} range to obtain the criteria for distinguishing the cherry cultivar samples. The first PCA model for the cherry samples resulted in two principal components that explained 65.77% of the total data variance. The first principal component (PC1) explained 48.30% of the total data variance, while the second (PC2) accounted for 17.47%. The mutual projections of the factor scores and their loadings for the first two PCs are shown in Figure 3.

The loading plot of the PC1 (Figure 3B) shows the positive loadings responsible for the separation between the 'Canetova' and 'Dolga Šiška' cultivars from the other cultivars. Examination of the PC1 shows many medium-positive contributions at 538, 677, and 617 cm^{-1}, which could be attributed to pectic acid and anthocyanins (Boyaci et al., 2015; Zaffino et al., 2015). In the higher range of differentiation, the bands of influence were at 1065, 1108, 1450, 1526, and 1336, 1593 cm^{-1}, which are likely from carbohydrates, especially cellulose, methyl and acetyl ester groups in pectin and phenylpropanoids, chlorogenic acid or flavonoids, respectively (da Silva et al., 2008; Pompeu et al. 2018; Farber et al., 2020).

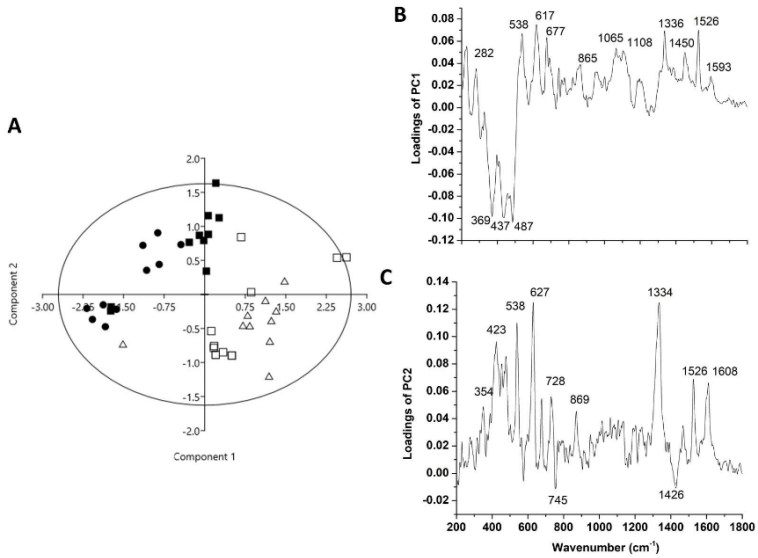

Figure 3. PCA applied to the Raman spectra data of extracted cherry samples: (A) score plot, (B, C) loading plots (symbols on the fruit samples: 'Đuti'– cycle, 'Canetova' – open square, 'Ohridska crna' – closed square, 'Dolga Šiška' – open triangle).

According to the PC2, the key differentiation between the 'Ohridska crna' cultivar and all other cultivars is related to the higher intensity of positive loadings at 423, 538, 627 cm^{-1} involving the C-C-O bending vibration of α-glucose and pectic acid (Boyaci et al., 2015) and the highest intensity of positive loading at 1334 cm^{-1} (Figure 3C), which could be Ziska related to CH_2 or $=C(CH_3)_2$ bending vibrations of plant fibers or flavonoids (Edwards et al., 1997; Maiti et al., 2013; Pompeu et al., 2018). Furthermore, these cultivars differed to a higher extent by loadings at 1526 and 1608 cm^{-1} (Figure 3C), and these differences might be related to phenolic compounds, e.g. chlorogenic acid (da Silva et al., 2008; Maiti et al.,

2013; Farber et al., 2020; Pompeu et al. 2018). In the lower extent of the separation, the negative loadings on PC2 at 245 and 1426 cm^{-1} probably indicate the glucosidic ring vibration (Synytsya et al., 2003; da Silva et al., 2008), or 1426 cm^{-1} could indicate chlorogenic acid (Eravuchira et al., 2012).

From a practical standpoint, the combination of Raman spectroscopy with principal component analysis (PCA) holds promise for distinguishing between the same fruit cultivars. The selectivity of the method is crucial for further investigation related to the quality of sweet cherry fruits. The Raman technique offers a more efficient and selective approach to the investigation of nutritionally similar samples than HPLC. The evolution of various Raman spectroscopy techniques has expanded its utility in identifying raw materials, pushing the boundaries of fruit investigation. Our further investigation will be focused on the application of the Raman method to the non-processed (fresh) cherry fruit, as there is no interference from water molecules compared to other methods.

Table 3. Assignment of vibrational bands observed in the spectra collected from the extracted cherry fruit samples.

Extract samples	Literature data	Vibrational mode	Chemical moiety	Reference
108		δ(C-C-C)	Glucosidic ring	da Silva et al., 2008
308		δ(C-C-C)	Glucosidic ring	da Silva et al., 2008
337	344, 348	δ(C-C-C), δ(C-O-C)	Glucosidic ring, fructose	da Silva et al., 2008, Camerlingo et al., 2017, Maiti et al., 2013
399	400	δ(C2-C1-O1) bending	α-Glucose	Boyaci et al., 2015
410	415 (pure compound)	δ(C2-C1-O1) bending	α-Glucose	Boyaci et al., 2015
455	441 449	C–O–C Phenyl ring	Polygalacturonic (pectic) acid Chlorogenic acid	Synytsya at al., 2003, Maiti et al., 2013
-	518-527	C-O-C, C-C-O	Glucosidic ring, cellulose	Edwards et al., 1997, da Silva et al., 2008, Camerlingo et al., 2017, Nekvapil et al., 2018
538	537		Polygalacturonic (pectic) acid	Boyaci et al., 2015
617	614		cyanidin	Zaffino et al., 2015
692, 677	686 677	Low frequency vibrations of pyranoid ring, Phenolic group	Polygalacturonic (pectic) acid, Chlorogenic acid	Boyaci et al., 2015, Eravuchira et al., 2012
758	747	γ(C–O-H) of COOH	Pectin	Synytsya et al., 2003, Eravuchira et al., 2012; Boyaci et al., 2015, Farber et al., 2020

Continuation Table 3.

Extract samples	Literature data	Vibrational mode	Chemical moiety	Reference
850	849–853	(C6–C5–O5–C1–O1)	Pectin	Farber et al., 2020
868, 865	870	CH and CH_2, C-C	Furanose	Boyaci et al., 2015, Camerlingo et al., 2017, Nekvapil et al., 2018
971	974	$\rho(CH_2)$, ν(C-O-H)	Glucosidic link stretch	da Silva et al., 2008, Eravuchira et al., 2012
1001	1000-1008	ν(C-C), CH_3	Carotene	Schulz et al., 2005, Schulz and Baranska, 2007, da Silva et al., 2008, Boyaci et al., 2015, Farber et al., 2020
1065	1056	υ(C-O-C), υ(C-C)	Carotenoids, carbohydrates	Edwards et al., 1997, Schulz et al., 2005, Wiercigroch et al., 2017, Farber et al., 2020
1108	1107-1122	ν(C-O-C) ν (C-O-C)	Cellulose	Baranski et al., 2005, da Silva et al., 2008, Farber et al., 2020,
1230		δ(C-C-H)	Carotenoids, xylan	Yu et al., 2007, Flores et al., 2008
1319, 1327	1325-1341	$\delta(CH_2)$ bending	Aliphatics, cellulose, Polygalacturonic (pectic) acid phenylpropanoids Anthocyanins	Edwards et al., 1997, Boyaci et al., 2015, Farber et al., 2020, Zaffino et al., 2015
1334-1337	1337-1340	ν(C-O-C) CH_2 $=C(CH_3)_2$ phenyl group	Cellulose phenylpropanoids, chlorogenic acid flavonoids	Zeise et al., 2018, Edwards et al., 1997, Pompeu et al. 2018, Eravuchira et al., 2012, Maiti et al., 2013
1426	1440-1444	$\delta(CH_2)$ phenyl group	Lipids and glucosidic signal chlorogenic acid	Da Silva et al., 2008, Eravuchira et al., 2012
1456 1450	1456 1444	$\delta(CH_2) + \delta(CH_3)$, δ(COH) Phenyl ring	Methyl and acetyl ester groups in pectins, fructose chlorogenic acid	Boyaci et al., 2015, Maiti et al., 2013
1520, 1526	1525 1518	-C=C- ν(C=C) benzopyrilium phenyl ring stretch	Carotenoids chlorogenic acid anthocyanins	Farber et al., 2020, Maiti et al., 2013 ,Zaffino et al., 2015
1600, 1593	1590-1608	ν(C=C), υ(C-C) ring + δ(CH) carbonyl group	Lignin, henylpropanoids chlorogenic acid anthocyanins rutin	Agarwal, 2006, da Silva et al., 2008; Eravuchira et al., 2012; Kang et al., 2016; Farber et al., 2020; Maiti et al., 2013; Zaffino et al., 2015; Krysa et al., 2022
1674	1680	COOH conjugated C=C and C=O modes	Carboxylic acids coniferyl alcohol and conifer aldehyde, chlorogenic acid	Farber et al., 2020; Zhu et al. 2018; Eravuchira et al., 2012

Conclusion

Although phenolic composition and sugar contents are significant factors in determining the potential of cherries, no rapid analytical method has been established so far for the authentication of these fruits. In this work, Raman spectroscopy allows the selective observation of nutritional and bioactive compounds from fruit samples by presenting a successfully performed authentication of sweet cherries. According to RS, the cherry samples are rich in carbohydrates, glucose, fructose, cellulose, and pectic acid, which are stored in the parenchyma tissue of the pericarp. Phenols, anthocyanins, and flavonoids are also present in lower concentrations. According to the PCA, 'Ohridska crna' differs from the other cultivars mainly in glucose, pectic acid, flavonoids, and phenolic compounds. The differences observed among samples through Raman and chemometric analysis correlate well with the results obtained using reference standards, indicating the reliability of the method. The potential demonstrated by Raman spectroscopy in this study can be extended to the analysis of fresh fruits, offering even greater efficiency. The capability of Raman spectroscopy to identify raw materials without interference from water molecules makes it particularly advantageous in this regard.

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PRIMENA RAMANOVE SPEKTROSKOPIJE ZA KARAKTERIZACIJU AUTOHTONIH SORTI TREŠNJE (*PRUNUS AVIUM* L.) POREKLOM SA BALKANA

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R e z i m e

Procena kvaliteta voća i povrća je od ključne važnosti za njihovu dalju obradu, predviđanje roka trajanja i formiranje cene. Većina tehnika koje se primenjuju za analizu kvaliteta ovih namirnica su invazivne. Međutim, sve veće interesovanje se javlja za neinvazivnim tehnikama za ocenjivanje kvaliteta voća, koje dobijaju na značaju zbog svoje jednostavnije primene i mehanizma rada. Ova studija po prvi put demonstrira primenljivost Ramanove spektroskopije za merenje spektralnih karakteristika različitih sorti trešnje (*Prunus avium* L.) (‘đuti’, ‘canetova’, ‘ohridska crna’ i ‘dolga šiška’). U kombinaciji sa analizom glavnih komponenti (engl. *principal component analysis* – PCA), Ramanova spektroskopija je korišćena za procenu uzoraka nutritivno sličnog sastava, kao što su proučavane sorte trešnje. Šećeri (glukoza, saharoza i fruktoza), antocijanini, fenolne kiseline i flavonoidi, kvantifikovani poređenjem sa referentnim standardima, korišćenjem tečne hromatografije visokih performansi (engl. *high-performance liquid chromatography* – HPLC), pokazali su Ramanove pikove (na 337, 399, 455, 538, 617, 1327 odnosno 1600 cm^{-1}) različitih intenziteta, što ukazuje na razlike između sorti. Sorta ‘ohridska crna’, u poređenju sa drugim sortama, sadrži najveću količinu nutritivnih i bioaktivnih jedinjenja. Nađena je korelacija između Ramanovih pikova i rezultata sadržaja šećera i fenola dobijenih hemijskom analizom. Ostvareni rezultati su ukazali na primenljivost hemometrijskog modelovanja povezanog sa Ramanovom spektroskopijom za brzu autentifikaciju trešnje.

Ključne reči: trešnja, vibracioni režimi, multivarijantna analiza, HPLC analiza, antocijanini, fenolna jedinjenja, ugljeni hidrati.

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