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Fresh sweet cherry fruit characterization and differentiation by Raman spectroscopy coupled with PCA

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A B S T R A C T

This study has shown that Raman spectroscopy can be used to analyze the unique patterns of sweet cherry cultivars ('G-2', 'Burlat', 'Canetova', 'Ohridska Crna', and 'Dolga Šiška'). As the minor scattering effect of water molecules guarantees no water interference, Raman spectroscopy can be applied to unprocessed, fresh fruits compared to other methods. The obtained Raman spectra indicate that using the above method can effectively confirm the authenticity of different cherry varieties. By combining Raman spectroscopy with principal component analysis (PCA), samples nutritionally similar to studied cherry cultivars can be distinguished.

Keywords: Raman spectroscopy, non-destructive testing, sweet cherry authentication, multivariate analysis, bioactive compounds

И З В О Д

Овим истраживањем је показано да се Раманова спектроскопија може користити за анализу различитих сорти трешања (Г-2, Бурлат, Цанетова, Охридска црна и Долга шишка). С обзиром на занемарљив ефекат расејања молекула воде, ова неинвазивна метода се може применити на свежим узорцима воћа, што је издваја у односу на друге методе за анализу овог типа узорака. Добијени спектри указују да се коришћењем наведене методе може ефикасно потврдити аутентичност различитих сорти трешања. Комбиновањем Раманове спектроскопије са хемометријском анализом могу се разликовати узорци сличног нутритивног састава.

Кључне речи: Раманова спектроскопија, недеструктивно тестирање, аутентификација трешања, мултиваријациона анализа, биоактивна једињења

1. Introduction

Sweet cherries are recognized for their abundance of dietary phenolic compounds, which offer numerous health benefits and play a significant role in preventing various chronic diseases. Being among the earliest fresh fruits available, sweet cherries are primarily consumed in their unprocessed form. Sweet cherries contain a moderate level of carbohydrates, particularly simple sugars like glucose, fructose, sucrose, and sorbitol, which contribute to their sweetness. In addition to sugars, cherries stand out as a nutrient-rich food with a relatively low caloric value, packed with essential nutrients and bioactive compounds such as fiber, carotenoids, vitamin C, and potassium (McCune et al., 2011). Moreover, they are abundant in dietary phenolic compounds such as phenolic acids and flavonoids like anthocyanins, flavan-3-ols, and flavonols (McCune et al., 2011; Commisso et al., 2017). Due to the significant presence of these bioactive compounds, it is unsurprising that consuming cherries is associated with health benefits. Findings from both animal and human studies indicate that including cherries in diet may lower the risk of various chronic inflammatory conditions such as arthritis, cardiovascular disease, diabetes, and cancer (Bell et al., 2014). As living standards rise, consumers increasingly prioritize healthy diets and pay more attention to the quality of fruits and vegetables. Consequently, there is a growing interest in enhancing research efforts focused on quickly detecting the quality of sweet cherries' chemical composition, which can depend on different factors (cultivar, environment, phytopathogens, etc.) (Iličić et al., 2019).

Until now, only invasive methods have been used in the characterization of cherry fruits, but these methods include fruit pre-processing, they are time-consuming and expensive. Besides its common benefits of high efficiency and non-destructive properties, Raman spectroscopy offers the unique advantage of being unaffected by water, making it capable of detecting substances even in aqueous solutions (Jones

et al., 2019). This technique is proficient in identifying various types and characteristics of fruits and vegetables, making it suitable for internal and external quality assessments. By analyzing the characteristic vibration frequencies of molecules, Raman spectroscopy quickly evaluates the quality and nutritional composition of produce, while also providing insights into their material composition and structure (Weng et al., 2019). Moreover, it effectively identifies hazardous substances and surface pesticide residues on fruits and vegetables. Given its promising potential for further development and application, Raman spectroscopy emerges as a valuable solution for assessing the quality and safety of fruits and vegetables.

In this work, Raman spectroscopy was used, for the first time, in the characterization and differentiation of five fresh sweet cherry cultivars from Serbia and North Macedonia.

2. Materials and methods

2.1. Sample material

Five autochthonous sweet cherry varieties originating from the Balkan region were harvested in the same ripening stage in the period May–July 2022 and used for the studies: G-2 (May 20th, Belgrade, 1), Burlat (May 30th, Trbušani, 2), Canetova (June 2nd, Čačak, 3), Ohridska Crna (June 24th, Ohrid, 4), and Dolga Šiška (June 26th, Ohrid, 5) (Figure 1).

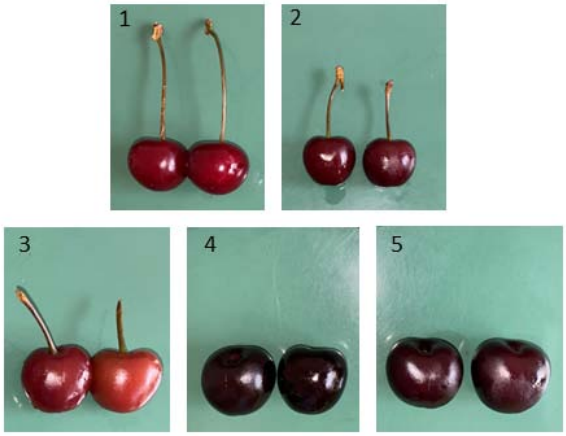

Figure 1. Sweet cherry varieties used in the present work: 1) G-2, 2) Burlat, 3) Canetova, 4) Ohridska Crna, 5) Dolga Šiška

Biological material, such as fruit organs, is characterized by a complex structure and varied chemical composition. The cherry fruits without the seeds (endocarp composed of sclerified cells) are mainly structured as very complex parenchyma storage tissues (mesocarp), which are composed of round parenchyma cells of different sizes as well as of tiny transversally elongated cells rich in anthocyanins (called exocarp). These cells have cell walls and internal cell content, which could include changes in the content of polysaccharides in these compartments, their structure and distribution during the fruit ripening and storage. Harvested fruits were transported to the laboratory, where samples were properly stored before analyses.

2.2. Raman spectroscopy instrumentation and chemometric sample classification

Sweet cherry (*Prunus avium* L.) cultivars (ground in a blender) were analyzed via Raman spectroscopy to directly assess storage parenchyma cells, using a Raman spectrometer system (Horiba Jobin Yvon, France) with an Olympus BX 41 microscope. A 785 nm laser, directed by a 50 LWD objective (Olympus, Tokyo, Japan), was employed for sample illumination. The spectrometer, featuring a 600 lines/mm grating, operated in the range of 200 to 1800 cm^{-1} in extended mode. Each measurement consisted of a 5 s integration time with 5 spectral accumulations, yielding a spectral resolution of approximately 3 cm^{-1} and 20–25 mW maximum output laser power. Calibration was verified using a 520.47 cm^{-1} silicon line. For each cultivar, ten spectra were recorded, making 50 spectra in total, and fused quartz was used as an alternative transparent substrate. Band assignments were conducted referencing existing literature. The PCA was applied to the data obtained from Raman spectra at the range 200–1800 cm^{-1} for all collected spectra, respectively. Before multivariate analysis, the raw data were pre-processed using the SPECTRAGRYPH software (version 1.2.14, Menges, 2021) baseline correction (weighted least squares) to reduce the baseline drift influence, followed by normalization and mean centering procedures. The standard normal variate, as a commonly applied normalization technique, normalizes the variables from each spectrum to zero and scales them by the standard deviation of the spectral data. On the other side, mean centering is the preferred option when the sample classification is based on parameters measured by the same unit.

We took different randomly selected spots for single-point Raman recording including cell wall and internal cell structure. With these 10 randomly selected points of fresh finely ground fruit samples, we provided insight into the information of vibrations from highly complex plant tissues.

3. Results and discussion

3.1. Raman spectral signature of fresh sweet cherry samples and multivariate analysis

Fruit analysis was performed by Raman microspectroscopy and the averages are related to *Prunus avium* L. cultivars: G-2, Burat, Canetova, Ohriska Crna, and Dolga Šiška cherries (Figure 2, 1–5, respectively).

Significant bands of higher intensity were observed in the Raman spectra (Fig. 2) at 307, 337, 414, and 1336 cm^{-1}, which are associated with the essential components of the fruit and assigned to the glucosidic ring vibrations, fructose, and cellulose (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 CH2 bending vibrations. This indicates the presence of carbohydrates as major components of the storage parenchyma cells of cherries as the main tissue of the pericarp. The intensity of the main carbohydrate bands could indicate some differences between cherry fruits, such as the prominence of a band associated with cellulose at 1336 cm^{-1} in cultivars 1 and 4. Medium intensity bands at 529 and 617 cm^{-1} are

associated with the C-O-C vibration of cellulose and phenylalanine, respectively (Boyaci et al., 2015; Edwards et al., 1997; Da Silva et al., 2008; Camerlingo et al., 2017). While this band at 617 cm^{-1} indicates some phenyl ring vibrations, literature data also suggest the presence of cyanidin (Zaffino et al., 2015). The color of the fruit depends on anthocyanin content; from Fig. 1 and also literature (Gjamovski et al., 2016), it is evident that anthocyanins are highly present in cultivar 4 (Selamovska et al., 2022) compared to other cultivars, where anthocyanin levels are lower.

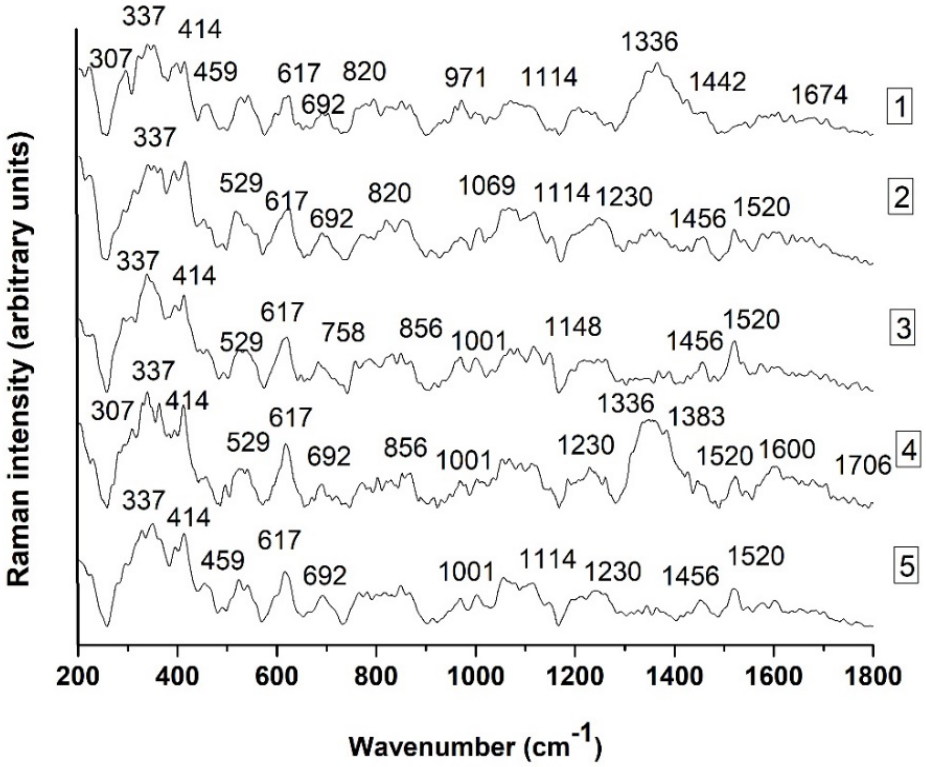

Figure 2. Mean values of normalized Raman spectra of five Prunus avium cultivars from fresh fruit samples, recorded in the spectral range from 200 to 1800 cm^{-1}, with bands specific for carbohydrates (337, 414, 529, 856, and 1336 cm^{-1}), anthocyanidin (617 cm^{-1}), carotenes (1001, 1114 and 1520 cm^{-1}) and phenylpropanoids and flavonoids (1600 cm-1).

According to these bands, the only differences between samples were observed for the band at 617 cm^{-1} associated with anthocyanidin content (Zaffino et al., 2015); for cultivar 1 (lower intensity band) and cultivar 4 (higher intensity band) (Fig. 2). High anthocyanin content for Ohridska Crna cultivar was previously reported by Selamovska et al. (2022). Very weak bands were 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 peak observed at 1600 cm^{-1} might suggest the presence of C=O ring vibrations found in compounds like phenylpropanoids and flavonoids (Zaffino et al., 2015; Krysa et al., 2022). This peak is the most intense in sample 4 (Fig. 2), indicating the differences in phenolic compounds.

PCA was applied to the data obtained from the Raman spectra in the 200–1800 cm^{-1} range to obtain the criteria for the discrimination of the cherry samples. The PCA model obtained for the fresh fruit samples resulted in two prominent principal components that explained 60.39% of the total data variance. The first principal component (PC1) explained 40.16% of the total data variance, while the second (PC2) accounted for 20.23%. The mutual projections of the factor scores and their loadings for two PCs are shown in Figure 3.

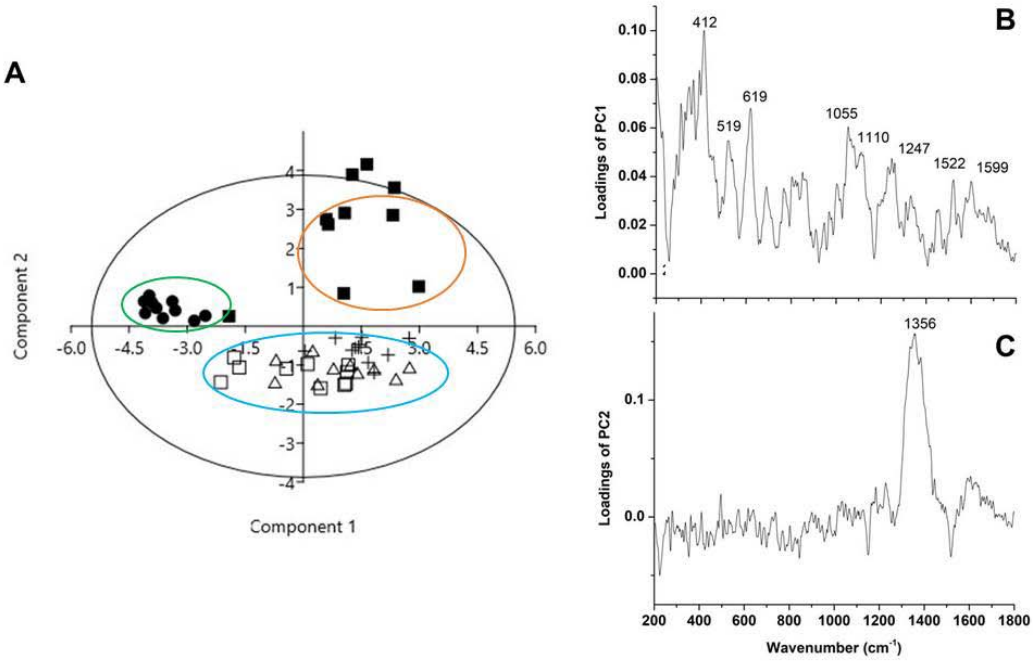

Figure 3. PCA applied to the data obtained from Raman spectra of fresh *Prunus avium* cultivars fruit samples: (A) score plot, (B, C) loading plots (symbols indicated on the fruit samples, 1 – cycle, 2 – cross, 3 – open square, 4 – closed square, 5 – triangle).

The score plot of PC1 and PC2 (Fig. 3A) shows the existence of three groups of objects. Considering the small number of samples of each cultivar, along PC1, cultivar 4 is close to cultivars 5 and 2, but completely differs from cultivar 1, while cultivar 3 is somewhere between. The highest positive intensity loadings along PC1 (Fig. 3B), mostly indicating separation of the samples, at 412 and 619 cm^{-1}, could indicate glucose (Boyaci et al., 2015) and anthocyanidin. Medium intensity loadings at 519, 1055, 1110, and 1247 cm^{-1} denote cellulose and xylan (Yu et al., 2007, Baranski et al., 2005, da Silva et al., 2008, Farber et al., 2020). The lowest intensity loadings at 1522 and 1599 cm^{-1} were probably assigned to C=C and C-C ring vibration and indicated that carotenoids and phenylpropanoids (Agarwal, 2006; da Silva et al., 2008, Farber et al., 2020) in lower amounts have an impact on the differences between cultivar 4 from cultivar 1. The second PC (Fig. 3C) showed good separation between 1 and 4 fresh fruit samples from fresh fruit samples of other cultivars, but the spectra of all other cultivars (2, 3 and 5) show grouping, probably because of their similar chemical composition. The signal at 1356 cm^{-1} with the highest positive influence indicates that carbohydrates have a strong impact on this separation (cell wall carbohydrates, such as arabinose, Wiercigroch et al., 2017). We suggest that the carbohydrate content is reflected in the most prominent band detected in both the raw and mean spectra of cultivars 1 and 4. This wide band is assumed to include the peak mentioned in Fig. 2 (1336 cm^{-1}), as carbohydrates are the main ingredients of plant cell walls (Zhbankov et al., 1997; Wiercigroch et al., 2017).

4. Conclusions

Raman spectroscopy was used to distinguish between different varieties of fresh sweet cherries. The analysis revealed that the fresh fruit samples predominantly contain carbohydrates, fructose, and cellulose, primarily stored in the parenchyma tissue of the pericarp. The presence of anthocyanidins, carotenoids, and phenols is also detected. Among the cultivars studied, Ohridska Crna (cultivar 4) showed the highest potential in terms of nutritive and bioactive compounds present in the fruit. Principal component analysis (PCA) indicated that the fresh fruits of varieties 4 and 1 completely differed, primarily in carbohydrate content, with comparatively lesser distinctions observed in carotenoids and phenylpropanoids.

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Declaration of competing interests

The authors declare that they have no conflict of interest.

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