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Carotenoids determination of carrot cultivars

Sofija Kilibarda [1*], Ilinka Pećinar [1], Aleksandar Ž. Kostić [1], Steva Lević [1], Zora Dajić Stevanović [1], Slavica Jelačić[1] and Đorđe Moravčević [1]

[1]University of Belgrade-Faculty of Agriculture, Nemanjina 6, 11080 Zemun-Belgrade, Serbia; sofija@ymail.com (S.K.); ilinka@agrif.bg.ac.rs (I.P.); akostic@agrif.bg.ac.rs (A.Ž.K.); slevic@agrif.bg.ac.rs (S.L.); zoradajic@agrif.bg.ac.rs (Z.D.S.); jelacic@agrif.bg.ac.rs (S.J.); djordjemor@agrif.bg.ac.rs (Dj.M.)

* Correspondence: sofija@ymail.com; +381114413191 (S.K.)

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Abstract: Carotenoids play an essential role in human health and they affect the perception of taste and flavour which influence consumer preference. Carrot is one of the most important and consumed vegetables in the world, and a critical source of α- and β-carotene. Commonly grown carrot cultivars (Maestro, Bolero, Natuna and Naval) were obtained from Begeč location in Serbia. Raman spectroscopy was applied as a fast chemical evaluation tool which provided information about the carrot cultivars differences. Raman spectra of the root of four carrot cultivars exhibited three dominant carotenoid signals, mainly related to α- and β-carotene, in the three distinct regions: from 1510-1515 cm^{-1}, 1149-1154 cm^{-1} and 1001-1007 cm^{-1}. According to the PCA of the Raman spectra it is indicated that the PC1 and PC2 are responsible for 94.06% of data variance and it suggested the existence of two groups along PC1 axis. Variables with the positive and negative contribution along PC1 indicate the differences between Maestro and Bolero from Natuna and Naval, which are mainly based on carotenoids, phenolic compounds and in the lower extend in carbohydrates.

Keywords: carrot root; commercial cultivars; Raman spectroscopy; total carotenoid content; PCA

1. Introduction

As a center of diversity for carrot (*Daucus carota* L.) it is considered Afghanistan, where this plant has been cultivated for more than 10 centuries. Local folk enjoyed pleasant taste, but also recognized benefits of the consumption of yellow or purple colored carrot root. Over the years, from this region carrot was introduced worldwide, growing in popularity among other vegetables, but as well as in importance, nutritional quality, number of cultivars and varieties. Today, cultivars with orange root flesh have great commercial value in addition to being crucial for human health. Mentioned color originates from carotenoids, a large group of isoprenoid pigments [1]. Variety of carotenes accumulates in roots during growing season. They may be synthesized and deposited in the different amount in periderm, secondary phloem to primary and secondary xylem, predominantly depending on carrot genotype, cultivar and growing sites [2]. Main carotenoids present are α- and β- carotene. As plant matures, the content of mentioned pigments increases, but also their ratio is changing. Therefore, in ripe orange carrot 60% of total carotenoids is comprised of β- carotene, 20% is reserved for α- carotene, and the rest is represented with lycopene, lutein, γ-carotene, ζ-carotene, 3-zeacarotene and xanthophylls [3]. Moreover, edible carrot is source of vitamin A, in form of provitamin A carotenes [4]. From perspective of human wellbeing, this vitamin is essential for optimal organism function. The higher intake of carrots serves the purpose of lowering the risk of few types of cancer, such as prostate, breast, lung and colorectal [5], as well as the chances of developing cardiovascular diseases.

It is often difficult to find safe and noninvasive method for determination and

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quantification of compounds of interest. Methods such as high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) and UV/visible spectrophotometry have been previously used for quantification of carotenoids [6], but usually include long process for sample preparation, expensive equipment usage for extraction and quantification of pigments, as well as subsequent destruction of pigments [2]. However, these techniques do not provide fast and multiple analyses compared to more modern analytical techniques, such as spectroscopic methods. Even if carotenoids are minor components at low concentration in plant samples, due to the specific excitation in the visible wavelength, detection of carotenoids can be achieved by Raman spectroscopy. In this context, Raman spectroscopy is starting to be applied for carotenoids quantification in carotenoid-rich plant material, such as carrot [7,8].

The objectives of this study were to determine: 1) the carotenoid profiles of the carrot cultivars by using the Raman spectroscopy 2) are there any differences among selected cultivars regarding carotenoid content.

2. Material and methods

2.1. Source of carrot material

Experiment was conducted in Begeč location (northwest Serbia). Four cultivars were the subject of examination (Maestro, Bolero, Natuna and Naval). Seeding of previously mentioned carrot cultivars took place on 16th of July, while harvest was done on November 7th. The most advanced technology had been utilized in the growing of carrots, which resulted in 60 do 90 t/ha of yield (mini-beds, drip irrigation), depending on the cultivar. Average commercial samples of carrot roots, which have undergone the process of examination, were harvested for each cultivar from mini beds at 10 meters mark.

2.2. *Raman Instrumentation and multivariant analysis of Raman spectra*

Raman microspectroscopy of carrot roots of four cultivars was focused on direct measurement of its methanol extracts. Carrot extracts were recorded using XploRA Raman spectrometer from Horiba Jobin Yvon. Raman scattering was excited by a laser at a wavelength of 532 nm equipped with a 1200 lines/mm grating. Spectral resolution was ~3 cm^{-1} and the calibration was checked by 520.47 cm^{-1} line of silicon. The Raman spectra were smoothed using Savitzky-Golay filters with 5 points and a second-order polynomial function. The spectra preprocessing was realized using Spectragryph software, version 1.2.13 [9] PCA analysis was performed using PAST software [10].

3. Results and discussion

3.1. Raman spectroscopy analysis

The peaks in Raman spectra correspond to specific chemical bands and/or functional groups of the molecule, and the band intensity is linked to the quantity of the analyzed compounds. The specific bands together make the so called "fingerprint" of the molecule. The analysis of the carrot root was obtained by Raman microspectroscopy, and the averaged spectra related to four commonly grown carrot cultivars obtained from Begeč location (Vojvodina, Serbia) are presented in Figure 1. In the Raman spectra obtained from cultivated carrot samples (Figure 1) significant bands are associated with carotenoid constituents and were observed at 1510-1515 cm^{-1} (very strong intensity), 1149-1154 cm^{-1} (very strong) and 1001-1007 cm^{-1} (medium) (Figure 1). They represent the characteristic bands of carotenoids and can be assigned as the stretching of the C=C (ν1), C−C (ν2) bonds and the C−CH_3 in-plane group rocking vibrations (ϱ(C−CH_3)), respectively. Literature data regarding the same or different plant samples also associate these three bands with the presence of various carotenoids in the spectral region from 1000-1540 cm^{-1} [2,11,12,13].

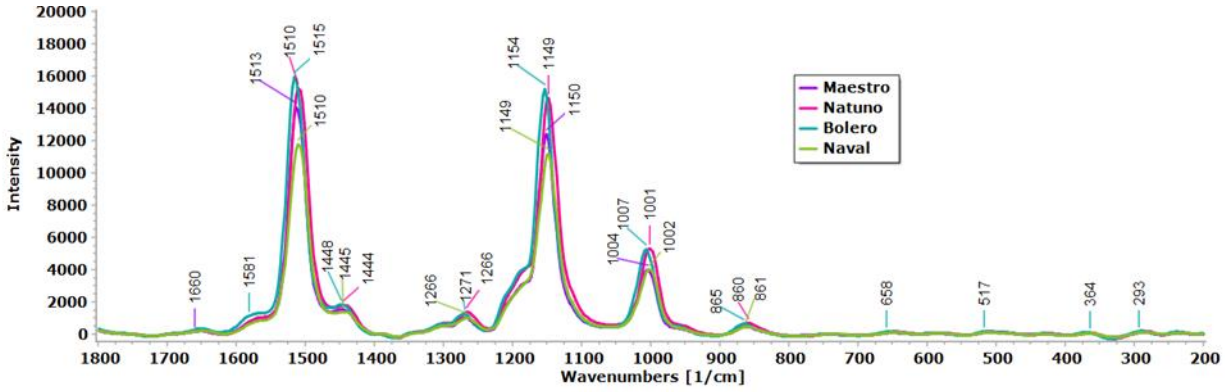

Figure 1. Averages of normalized Raman spectra of four carrot cultivar recorded in the spectral range from 200 to 1800 cm^{-1}. The marked peaks represent mainly carotenoids bands.

However, assigning the spectral data of carotenoids to a specific carotenoid compound is not easy because of similar bands position and due to carotenoids interaction with other cell compounds [13,14]. Very weak bands were identified at 1660 cm^{-1}, in the region from 870 to 290 cm^{-1}, and very low intensity bands at 1581, ~1445 and ~1266 cm^{-1} (Figure 1). According to da Silva et al. [15] the band at ~1445 cm^{-1} is related to δ(CH2) vibrational mode, and is associated to glycosidic structure. As carrot roots are rich in various carbohydrates, the bands observed below 1000 cm^{-1} are probably related to that class of compounds. The bands ranging from 280 to 869 cm^{-1} probably refer to glycosidic link stretches [15,16], such as those at 293, 364, 517 and 658 cm^{-1} (Figure 1). In literature, phenolic compound, were observed at 1550-1700 cm^{-1} [17].

3.2. PCA of the data obtained from Raman spectra of four carrot cultivars

Multivariate analysis, based on PCA, was applied in order to differentiate between chemical compositions of roots od four different carrot cultivars. The PCA analysis was performed using approximately 40 Raman spectra recorded from roots. The score plot of PC1 versus PC2 shows a reasonably good separation between the samples, where the first and second principal components described 94.06% of data variance. Mutual projections of factor scores and their loadings for the first two PCs are shown in Figure 2. The score plot (Figure 2a) suggests the existence of two groups of objects along PC1 axis. The first one includes the Natuna and Naval cultivars, while the Bolero and Maestro form the second cluster. The corresponding loading plot represents the relation between the variables and can be used to identify those with the highest contribution to the object positioning in the score plot. The loading plot (Figure 2b) shows that the variables with the highest positive contribution along PC1 axis corresponded to the signals at 989, 1139 and 1499 cm^{-1}, while signals at 1015, 1162 and 1521 cm^{-1} have the highest negative effects. These variables are responsible for the differences between Natuna and Naval from Bolero and Maestro. The signal at 1499 cm^{-1} is mostly responsible for the differentiation among the cultivars mainly depending on phenolic compounds [17], together with the higher-intensity loading at 1139 cm^{-1}, assigned as C-C stretching mode of conjugated polyenes [15], in the lower extend on the differences also indicated lower intensity loading at 989 cm^{-1}, probably directed to the occurrence of plant cell wall compounds such as cellulose or hemicellulose [18]. The highest negative intensity loading of PC1 placed at the position 1521 cm^{-1}, together with negative intensity loading at 1162 cm^{-1} (Fig. 2b), involving vibration of C=C vibration from polyenic chain [14,19] and C-O, C-CH vibration in pectin molecules [20], respectively. It was found that the negative medium-intensity signal at 1015 cm^{-1} (Figure 2b) originates from in-plane CH_3 rocking of polyene [11].

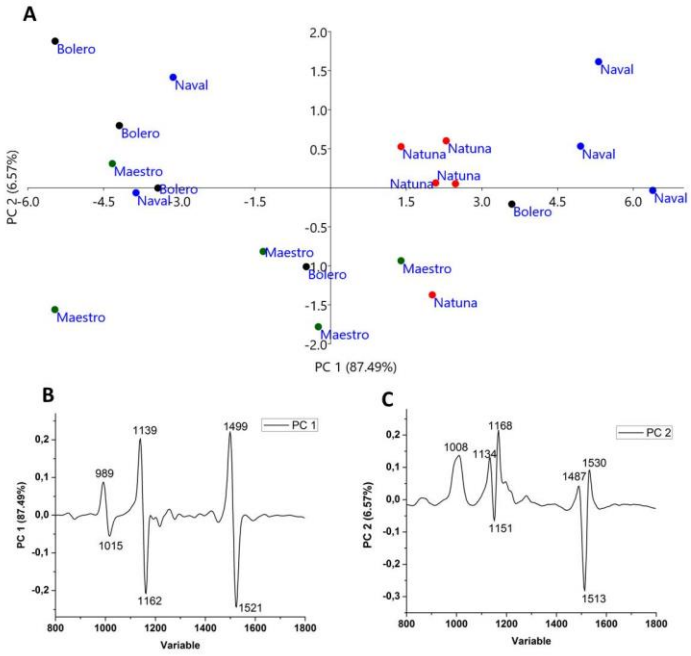

Figure 2. PCA analysis applied to the data obtained from Raman spectra of carrot root samples: a) score plot, b, c) loading plots.

The most influential parameters along PC2 axis corresponded to the signals at 1008, 1134, 1168, 1487 and 1530 cm^{-1} (positive impact) and signals at 1151 and 1513 cm^{-1} with the highest negative impact (Figure 2c). According to PC2, the root samples of Natuna, Naval and Bolero showed differences comparing to Maestro. The signals at 1168 and 1008 cm^{-1} are mostly responsible for the differentiation among the samples involving vibration of C=C, C-C vibrations and CH_3 rocking from polyenic chain, together with the lower-intensity loading at 1530 cm^{-1} attributed to carotenoids, respectively [11,14,21,22]. Lower intensity variable at ~1130 cm^{-1} indicating the presence of coniferyl aldehyde [17,23], 1487 cm^{-1} is probably directed to the terpenoids compunds [24]. The highest negative intensity loading placed at the position 1513 cm^{-1} and lower intensity loading at 1151 cm^{-1} might be associated with stretching vibration in carotenoids [15,25].

4. Conclusion

The results of the present study have brought to light the potential of Raman spectroscopy as a fast and sophisticated method for getting more detailed information concerning the plant metabolites distribution in examined root samples. The analysis of the Raman spectra related to root samples, combined with PCA provided some differences in the chemical profiles of the four samples commonly grown carrot cultivars (Maestro, Bolero, Natuna and Naval). Carotenoids, phenolic compounds and in the lower extend polysaccharides were the classes of compounds that contributed to the variation between the above-mentioned cultivars. Based on PC2, there is a separation of Maestro from Natuna, Naval and Bolero, mainly based on carotenoids composition. The further research will be focused on measuring the changes in carotenoids in selected carrot cultivars obtained from different growing sites and sun expositions, using Raman spectroscopy and spectrophotometric analysis.

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