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Proceeding Paper

Raman Spectroscopy as a Useful Tool for Tentative Identification of Nutritional Ingredients and Distinction of *Allium* Species †

Sandra Vuković [1,*], Đorđe Moravčević [1], Jelica Gvozdanović-Varga [2], Aleksandar Ž. Kostić [1], Ana Vujošević [1], Sofija Kilibarda [1] and Ilinka Pećinar [1,*]

[1] Faculty of Agriculture, University of Belgrade, Nemanjina 8, 11080 Zemun–Belgrade, Serbia; djordjemor@agrif.bg.ac.rs (Đ.M.); akostic@agrif.bg.ac.rs (A.Ž.K.); ana@agrif.bg.ac.rs (A.V.); sofija.kilibarda@agrif.bg.ac.rs (A.V.)

[2] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia; jelica.varga@ifvcns.ns.ac.rs

* Correspondence: sandra.vukovic@agrif.bg.ac.rs (S.V.); ilinka@agrif.bg.ac.rs (I.P.)

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Abstract: Our study aimed to nutritional characterization and discrimination of *Allium fistulosum*, *A. nutans*, *A. odorum*, *A. schoenoprasum*, *A. ampeloprasum* var. *ampeloprasum*, *A. sativum* var. *sagittatum* samples grown in Serbia. Samples were recorded using XploRA MicroRaman spectrometer at a 532 nm wavelength, spectra were preprocessed using Spectragryph, and PCA was performed by PAST software. According to vibrational spectra, *Allium* samples are rich in carbohydrates, mostly polysaccharides, the plant pigments, proteins, while minor constituents are pectic acid and pectin. Multivariate analysis, based on PCA, was applied in order to differentiate between the chemical compositions of six *Allium* samples. The score plot suggests the existence of two groups of objects along the PC1 axis and variables with the highest positive contribution along the PC1 axis corresponded to chlorophyll a, b, carotenoids, carbohydrates and proteins. According to PC2, the most influential parameters indicated on similar carbohydrates composition and predominance of carotenoid constituents in other group of samples.

Keywords: *Allium* species; PCA; fingerprint region of carbohydrates; plant pigments; proteins

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1. Introduction

The genus *Allium* includes over 800 herbaceous biennial or perennial species [1]. Many of these have multiple uses: as vegetable, medicinal, ornamental or spice plants.

Allium species, especially *Allium sativum* or garlic, have been known for centuries, due their health benefits on human health. Also, *A. cepa* (onion) occupies an important place in human nutrition and it is the most important bulb crop, globally. Phytochemicals present in these species exhibit numerous health effects that are well described in the scientific literature [2]. A great number of studies indicate their antioxidant, antifungal, antibacterial, anti-inflammatory properties, etc. [3].

In addition to garlic and onion, according to [4] about 20 species of the genus *Allium* are grown, usually locally. Considering the richness of the genus *Allium* in species and the fact that a small number of species are cultivated, the study of other species of this genus can provide new important scientific information.

Raman spectroscopy combines with a microscope could give detailed information on the spatial distribution of different bioactive components of fresh food samples, and could give important insight in characterization and evaluation of the crops, widely known as

plants for different agricultural applications. Further, advantages of this method are numerous: sample preparation is simple, a small amount of sample is used, it does not require the use of chemicals and dyes and results are acquired quickly [5].

In *Allium* genus, the Raman spectroscopy was used to examine the antibacterial [6], antimicrobial effects [7] and to study volatile organic compounds [8]. Most of the studies on *Allium* species regarding to Raman spectroscopy have focused on garlic and onion, while data on other *Allium* species are either lacking or very scarce. The application of modern spectroscopy methods gives us the opportunity to enrich the diet by examining new, poorly tested, *Allium* species that are a potential source of bioactive components.

Keeping in view of the above facts, our study aimed to nutritional characterization and discrimination of *Allium fistulosum (F)*, *A. nutans (N)*, *A. odorum (O)*, *A. schoenoprasum (S)*, *A. ampeloprasum* var. *ampeloprasum (A)*, *A. sativum* var. *sagittatum (R)* grown in Serbia by using XploRA MicroRaman spectrometer.

2. Material and Methods

2.1. Plant Material

For this study, six *Allium* species: *A. fistulosum* (F), *A. nutans* (N), *A. odorum* (O), *A. schoenoprasum* (S), *A. ampeloprasum* var. *ampeloprasum* (A), *A. sativum* var. *sagittatum* (R) were grown in open field condition, in Serbia. The samples of selected species were collected in the phase of intensive growth for F, N, O and S, while in the case of A and R the samples were collected at the end of life cycle (when the leaves were dried). As samples were used: whole plants (F), leaves (N, O and S) and bulbs (A and R) depending on what plant part is used as food.

Preparation of samples involved cleaning from the ground, washing with water and grinding in an electric mill (Bosch MKM6000, 180 W). The prepared samples were placed in plastic cuvette and immediately used for analysis.

2.2. Raman Instrumentation

Raman microspectroscopy of six *Allium* species samples was focused on the direct measurement of bulb and leaf cells. Spectra were recorded using an XploRA Raman spectrometer from Horiba Jobin Yvon. Raman scattering was excited by a laser at a wavelength of 532 nm equipped with 600 lines/mm grating. The spectral resolution was ~3 cm^{-1} and the calibration was checked by a 520.47 cm^{-1} line of silicon. Spectra were acquired by applying exposure time 10 s and scanning the sample 15 times.

2.3. Chemometric Sample Classification Based on PCA of the Raman Spectra

Principal component analysis (PCA) was carried out on data normalized by the highest intensity band and using spectral region from 200 to 1800 cm^{-1}. The spectra preprocessing was realized using the Spectragryph software, version 1.2.13 [9]. Spectra were baseline corrected and Savitzky–Golay filters with 5 points and a second-order polynomial function was used for spectra smoothing. PCA analysis was performed using the PAST software [10].

3. Results and Discussion

Available scientific results indicate that in the chemical composition of *Allium* species dominated carbohydrates (5 to >30% depending on the species) [11]. The content of proteins varies from 1,1% for *A. cepa* [12] to 17,2% for *A. sativum* [13]. The *Allium* species examined in this paper possessed content of proteins similar to content determined to *A. cepa* (unpublished results). In research of [14] indicate that pigments in *Allium* species are present in moderate amounts and that they are characteristics of spring onions. Vuković et al. have obtained similar results, i.e., in *Allium* species in which leaves or the whole plant are used in the diet, the presence of pigments (carotenoids, chlorophylls *a* and *b*) was determined, while in bulb crops pigments were not detected (unpublished results).

According to [11,15] the least abundant class of nutrients in *Allium* species are fibers and lipids (usually <0.5%).

3.1. Raman Signature of Allium Samples

Allium species are comprised of a diverse group of metabolites, including polysaccharides, pectins, cellulose as well as proteins, chlorophylls, carotenoids, with each molecular class having a particular molecular conformation and interacting with neighboring molecules in a specific way. Figure 1 shows the characterization of *Allium sp*. samples: leaves (for S-K, N-K and O-K), bulbs (for A-K, R-K) and whole plants (for F-K) by Raman spectroscopy, spectra were recorded in the spectral region from 200 and 1800 cm^{-1}. In the fingerprint region the intense and specific Raman skeletal features contain several polymers of different types, such as carotenoids and carbohydrates.

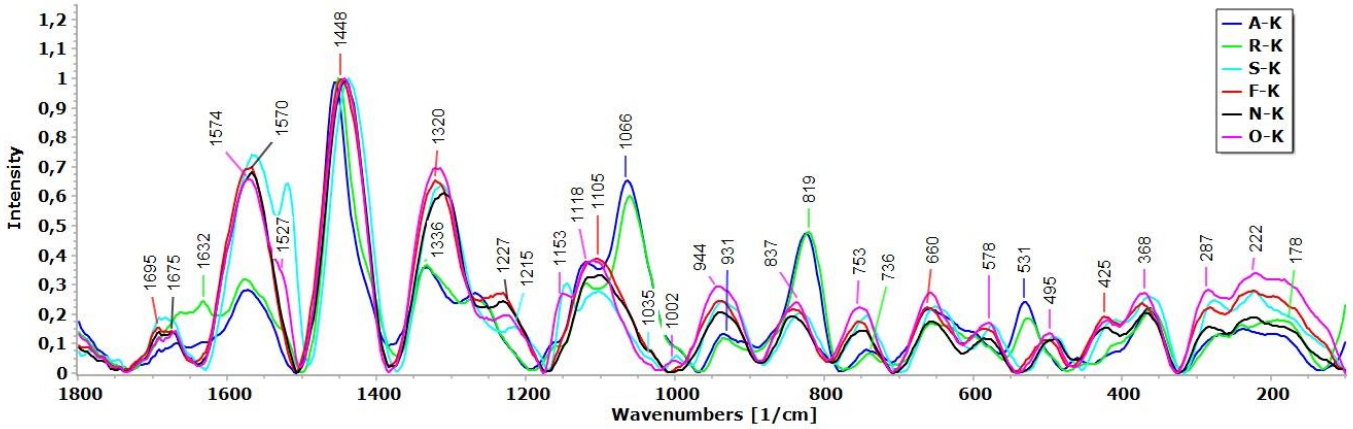

Figure 1. Averages of normalized Raman spectra of six *Allium* species samples recorded in the spectral range from 200–1800 cm^{-1}, with bands specific for carotenoids (1002, 1153, and ~1527 cm^{-1}), chlorophyll (~1570, 1632 cm^{-1}), glucosidic structure (1118, 1448 cm^{-1}).

The Raman spectra of all *Allium* species samples (Figure 1) showed the highest intensity band at ~1448 cm^{-1} which could be related to CH_2 vibrational mode, associated to glucosidic structure, probably originated from cell wall compounds [16,17]. In addition, the medium intensity band common for the leaf base samples of *Allium* species at ~1570 cm^{-1}, following with the band located at 1632 cm^{-1} are probably directed to the occurrence of chlorophyll *a* and *b* [18,19], while its shoulder as lower intensity band at ~1527 cm^{-1}, specific only for S-K sample, involving vibration of C=C bonds, most probably originated from polyenic chain [20,21]. Similarly, the medium intensity band observed in leaf contained samples (S-K, O-K, F-K) at ~1320 cm^{-1} is probably directed to the occurrence of β-carotene together with its shoulder located at 1227 cm^{-1}, this band also could indicated on chlorophylls, was assigned to bending vibration of C-H, and CH_2 and described for leaf samples [19,22]. On the additional presence of polysaccharide polymers occurred in bulb samples (Figure 1.) indicated most common band for all samples at 1118 and 1105 cm^{-1} indicated on C-O-C, C-O-H glycoside bonds to the fructose moiety in sucrose [23–25]. In this region lower intensity bands at 1153 (medium) and 1002 cm^{-1} (week) indicated on the carotenoids and can be assigned as the stretching of the C–C (ν2) bonds coupled to C–CH_3 in-plane bending of the central polyene chain (ϱ(C–CH_3)), respectively [18,26–28] occurred only in leaf containing samples. As samples of *Allium* species are rich in various carbohydrates, the lower intensity bands observed below 1000 cm^{-1} may probably be related to this class of compounds [16,25,29] and assigned the bands at 944, 495, 425 and at 1066 and 819, 368 cm^{-1} to polygalacturonic (pectic) acid and pectin, respectively. In the region bellow 1000 cm^{-1} could be occurred bands useful for interpretation of amino acids occurring only in *Allium* sp. bulb samples, such as medium intensity peaks positioned cm^{-1}, as well as lower intensity band at 736 cm^{-1} related to C–C–H and C–O–C bending vibration of

tryptophan,~ 660 cm^{-1} refers to C-S vibration of methionine, 531 cm^{-1} is probably assigned to S-S vibration of cysteine [24,30], observed only in bulb containing samples (A-K and R-K). On the proteins also indicated band in the region from 1670–1700 cm^{-1} associated with C=O vibration of Amide I [24]. The bands ranging from 180 to 300 cm^{-1} probably refer to the deformation of pyranosyl rings deformation and C–O–C, C-C-C vibration of glycoside linkage [31] common for all analysed samples. Therefore, the Raman spectra of the samples of six *Allium* species are dominated mainly by bands of polysaccharides, the plant pigments, proteins, while minor constituents are pectic acid and pectin.

3.2. PCA of the Data Obtained from Raman Spectra of Allium Species Samples

Combining Raman spectra results with principal component analysis (PCA) can enable to distinguish spectral fingerprint that can be related to the biochemical changes in different *Allium* species samples. The first PCA model obtained for samples of *Allium* species resulted in two principal components explaining 77.66% (PC1-65.26%, PC2-12.40%) of the original Raman spectra and their loadings for the first two PCs are shown in Figure 2. The score plot of PC1 and PC2 (Figure 2A) suggests the clear existence of two groups of objects along the PC1 axis, eg. A-K, R-K samples are clearly differing from all other samples.

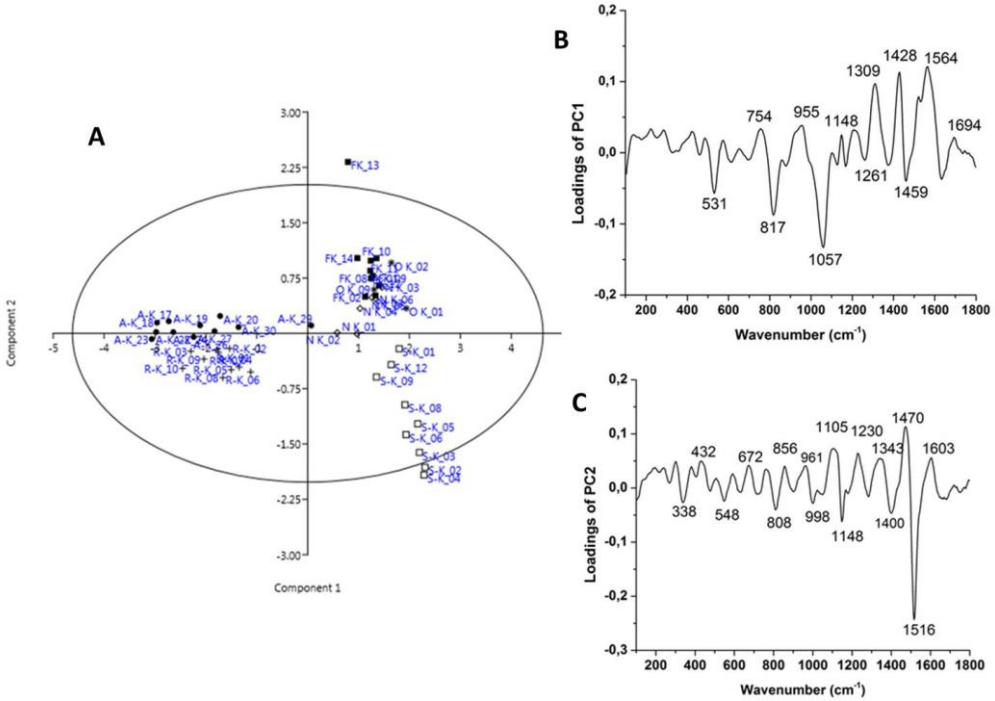

Figure 2. PCA analysis applied to the data obtained from Raman spectra of *Allium* species samples: (**A**) score plot, (**B**,**C**) loading plots.

The loading plot (Figure 2B) shows that the variables with the positive influence along the PC1 axis corresponded to the signals at 754, 955, 1148, 1309, 1428, 1564 and 1694 cm^{-1} while signals at 531, 817, 1057, 1261 and 1459 cm^{-1} have the negative contribution. The highest positive intensity loadings along PC1 at 1564 cm^{-1} and following at 1428 cm^{-1} are mostly responsible for the differentiation among the S-K, N-K, O-K and F-K from other samples, and they are probably attributed to leaf pigments, or more precisely to chlorophyll a and b [18,19]. In the lower extend on the differences between mentioned samples (Figure 2A,B) also indicated medium and lower intensity loadings at 1309 and 1148 cm^{-1}, they could be indicated on occurrence of leafs β-carotene content [18,22]. In the lower extend on the differences also have impact the lowest intensity bands at 754 and 955 cm^{-1} (from γ(C–O-H) of COOH and C-C-H and C-O-H bending vibration, respectively) related

to pectin and polygalacturonic (pectic) acid [16,25,29]. The higher negative intensity loading of PC1 placed at 1057, 817 and 531 cm^{-1} are mostly responsible for the differentiation of A-K, R-K from all other samples, the differences mainly depending on pectin and amino acids, respectively [24]. The following bands in the lower extend contribute to separation between samples. The bands at 1261 and 1459 cm^{-1}, are related to (CH_2) of polygalacturonic (pectic) acid and methyl and acetyl ester groups in pectins [16]. The second PCs did not give a good separation of the investigated *Allium* sp. samples. According to PC2, the most of F-K, N-K, O-K, partially A-K differ from R-K and especially from S-K samples, and there are in the group probably because of similar carbohydrates composition, which suggest the higher positive intensity band at 1470 cm^{-1} as well as 1105, 961, 856 cm^{-1} and lower intensity loadings are related to the (CH_2) and glycosidic bonds from polysaccharides [29]. In the lower extend on the differences have also impact the loadings at 1230 and 1343 cm^{-1} indicated on β-carotene [19,22] occurrence in leaf contained samples (F-K, N-K, O-K) and loading at 1603 cm^{-1} (C-C vibration) of phenylalanine [30]. Differently, samples S-K had characteristic signals at 1516 cm^{-1}, suggesting a higher carotenoid constituents [24,26,32]. Negative lower-intensity signals at 998 and 1148 cm^{-1} indicated on the chlorophylls, assigned to stretching and bending vibration of C-N, C-N-C [17,22] together with highest intensity loading indicated on the predominance of leaf contained plant parts in the investigated *Allium* samples. Negative lowest-intensity loadings in the region from 808–330 cm^{-1} could be assigned to bending vibration of C–C–C, C–C–O from carbohydrates [33].

4. Conclusions

The results of the study have brought to light the potential of Raman spectroscopy as a fast method for getting more detailed information concerning to the nutritional composition and differences among investigated *Allium* sp. samples. The analysis of the Raman spectra combined with PCA provided clear differences in the chemical profiles of the different *Allium* species samples. The chlorophylls *a*, *b*, and carotenoids are mostly responsible for the differentiation among the leaf rich samples (S-K, N-K, O-K and F-K) from other samples, while bulb rich samples (A-K, R-K) from all other samples differ mainly in pectic and amino acids composition. Based on PC2, there is a separation of F-K, N-K, O-K, from R-K and S-K samples, mainly based on polysaccharides and carotenoid composition. Further research will be focused on chemical composition of the selected *Allium* species, grown both in the open field conditions and in a protected area, with the application of different fertilizers, using Raman spectroscopy and spectrophotometric analysis.

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Conflicts of Interest:

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