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Proceeding Paper

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

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Abstract: Our study aimed to carry out the nutritional characterization and discrimination of *Allium fistulosum*, *A. nutans*, *A. odorum*, *A. schoenoprasum*, *A. ampeloprasum* var. *ampeloprasum*, and *A. sativum* var. *sagittatum* samples grown in Serbia. Samples were recorded using an XploRA MicroRaman spectrometer at a 532 nm wavelength, spectra were preprocessed using Spectragryph, and PCA was performed by PAST software. According to the vibrational spectra, *Allium* samples are rich in carbohydrates, mostly polysaccharides, plant pigments, and proteins, while the minor constituents are pectic acid and pectin. A 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 the variables with the highest positive contribution along the PC1 axis corresponded to chlorophyll a and b, carotenoids, carbohydrates, and proteins. According to PC2, the most influential parameters indicated a similar carbohydrate composition and the predominance of carotenoid constituents in the 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 benefits for human health. Moreover, *A. cepa* (onion) occupies an important place in human nutrition, and it is the most important bulb crop globally. The 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, and anti-inflammatory properties [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 combined with a microscope could give detailed information on the spatial distribution of the different bioactive components of fresh food samples and could give important insight into the characterization and evaluation of the crops, which are widely known as plants for different agricultural applications. The 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 the results are acquired quickly [5].

In the *Allium* genus, Raman spectroscopy was used to examine the antibacterial [6] and antimicrobial effects [7] and to study volatile organic compounds [8]. Most of the studies on *Allium* species regarding 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 potential sources of bioactive components.

Based on the above facts, our study aimed to carry out the nutritional characterization and discrimination of *Allium fistulosum* (F), *A. nutans* (N), *A. odorum* (O), *A. schoenoprasum* (S), *A. ampeloprasum* var. *ampeloprasum* (A), and *A. sativum* var. *sagittatum* (R) grown in Serbia using an 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), and *A. sativum* var. *sagittatum* (R), were grown in an 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 the life cycle (when the leaves were dried). Whole plants (F), leaves (N, O, and S), and bulbs (A and R) were used, depending on what part of the plant is used as food.

The 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 a plastic cuvette and immediately used for analysis.

2.2. Raman Instrumentation

The 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 that was 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 an exposure time of 10 s and scanning the sample 15 times.

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

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

3. Results and Discussion

The available scientific results indicate that the chemical compositions of *Allium* species are dominated by carbohydrates (5 to >30%, depending on the species) [11]. The contents of proteins vary from 1.1% for *A. cepa* [12] to 17.2% for *A. sativum* [13]. The *Allium* species examined in this paper possessed contents of proteins similar to the content determined for *A. cepa* (unpublished results). In [14], the authors indicated that pigments in *Allium* species are present in moderate amounts and that they are characteristic of spring onions. Vuković et al. obtained similar results, i.e., in *Allium* species in which leaves or whole plants are used in the diet, the presence of pigments (carotenoids and 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, and cellulose as well as proteins, chlorophylls, and 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 of leaves (for S-K, N-K, and O-K), bulbs (for A-K and R-K), and whole plants (for F-K) by Raman spectroscopy Spectra were recorded in the spectral region from 200 to 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.

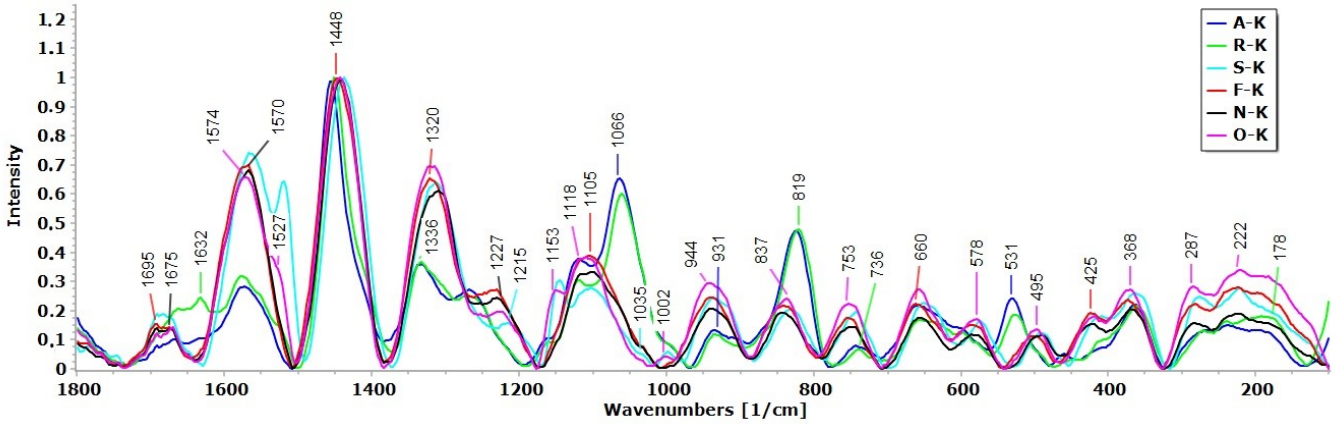

Figure 1. Averages of normalized Raman spectra of six *Allium* species samples, recorded in the spectral range from 200 to 1800 cm^{-1}, with bands specific for carotenoids (1002, 1153, and ~1527 cm^{-1}), chlorophyll (~1570 and 1632 cm^{-1}), and glucosidic structure (1118 and 1448 cm^{-1}).

The Raman spectra of all *Allium* species samples (Figure 1) showed the highest intensity band at ~1448 cm^{-1}, which could be related to the CH_2 vibrational mode, associated with the glucosidic structure, and 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 by the band located at 1632 cm^{-1}, are probably directed to the occurrence of chlorophyll *a* and *b* [18,19], while its shoulder as a lower-intensity band at ~1527 cm^{-1}, specific to the S-K sample and involving the vibration of C=C bonds, most probably originated from the polyenic chain [20,21]. Similarly, the medium-intensity band observed in leaf-containing samples (S-K, O-K, and 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 could also indicate chlorophylls and was assigned to the bending vibration of C-H and CH_2 described for leaf samples [19,22]. The additional presence of polysaccharide polymers that occurred in bulb samples (Figure 1) indicated the most common band for all samples at 1118 and 1105 cm^{-1}, which indicated C-O-C and 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 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], which occurred only in leaf-containing samples. As the samples of *Allium* species were rich in various carbohydrates, the lower-intensity bands observed below 1000 cm^{-1} are probably related to this class of compounds [16,25,29], and we assigned the bands at 944, 495, and 425 and at 1066, 819, and 368 cm^{-1} to polygalacturonic (pectic) acid and pectin, respectively. In the region bellow 1000 cm^{-1}, the occurrence of bands could be useful for the interpretation of amino acids occurring only in *Allium* sp. bulb samples, such as the medium-intensity peaks as well as the lower-intensity bands

at 736 cm^{-1}, which is related to the C–C–H and C–O–C bending vibration of tryptophan; ~660 cm^{-1}, which refers to the C-S vibration of methionine; and 531 cm^{-1}, which = is probably assigned to the S-S vibration of cysteine [24,30], observed only in bulb-containing samples (A-K and R-K). Proteins also indicated a band in the region from 1670 to 1700 cm^{-1}, which is associated with the C=O vibration of amide I [24]. The bands ranging from 180 to 300 cm^{-1} probably refer to the deformation of pyranosyl rings and the C–O–C and C-C-C vibration of glycoside linkage [31], which were common for all analyzed samples. Therefore, the Raman spectra of the samples of six *Allium* species are mainly dominated by bands of polysaccharides, plant pigments, and proteins, while the minor constituents are pectic acid and pectin.

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

Combining the Raman spectra results with a principal component analysis (PCA) enabled a spectral fingerprint that is related to the biochemical changes in different *Allium* species samples to be distinguished. The first PCA model obtained for samples of *Allium* species resulted in two principal components explaining 77.66% (PC1—65.26% and 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, e.g., the A-K and R-K samples clearly differ from all other samples.

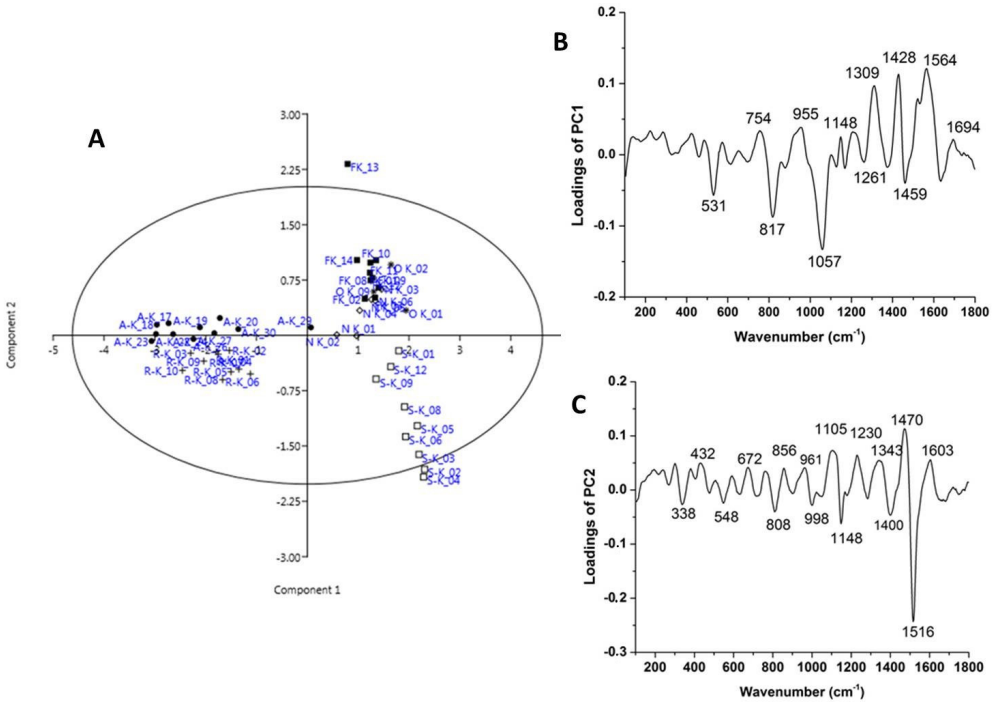

Figure 2. PCA analysis applied to the data obtained from the Raman spectra of *Allium* species samples: (**A**) score plot, (**B**,**C**) loading plots.

The loading plot (Figure 2B) shows that the variables with a positive influence along the PC1 axis corresponded to the signals at 754, 955, 1148, 1309, 1428, 1564, and 1694 cm^{-1}, while the signals at 531, 817, 1057, 1261, and 1459 cm^{-1} had negative contributions. The highest positive-intensity loadings along PC1 at 1564 cm^{-1} and at 1428 cm^{-1} are mostly responsible for the differentiation of S-K, N-K, O-K, and F-K from the other samples, and they are probably attributed to leaf pigments or, more precisely, to chlorophyll *a* and *b* [18,19]. The lower extent of the differences between the mentioned samples (Figure 2A,B) also indicated medium- and lower-intensity loadings at 1309 and 1148 cm^{-1}, which could indicated the occurrence of leaf β-carotene content [18,22]. The lower extend of the differences

also has an impact on 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 loadings of PC1 placed at 1057, 817, and 531 cm^{-1} are mostly responsible for the differentiation of A-K and R-K from all other samples, with the differences mainly depending on pectin and amino acids, respectively [24]. The following bands in the lower extent 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, most of F-K, N-K, O-K, and partially A-K differ from R-K and especially from the S-K samples, and they are probably in the group because of a similar carbohydrate composition, which suggests the higher positive-intensity band at 1470 cm^{-1} as well as 1105, 961, and 856 cm^{-1} and lower-intensity loadings are related to the (CH_2) and glycosidic bonds from polysaccharides [29]. The lower extent of the differences also impacts the loadings at 1230 and 1343 cm^{-1}, indicated by the β-carotene [19,22] occurrence in leaf-containing samples (F-K, N-K, and O-K) and the loading at 1603 cm^{-1} (C-C vibration) of phenylalanine [30]. Conversely, the S-K samples had characteristic signals at 1516 cm^{-1}, suggesting higher carotenoid constituents [24,26,32]. Negative lower-intensity signals at 998 and 1148 cm^{-1} indicated chlorophylls, assigned to the stretching and bending vibration of C-N and C-N-C [17,22], together with the highest-intensity loading, indicated by the predominance of leaf-containing plant parts in the investigated *Allium* samples. The negative lowest-intensity loadings in the region from 808 to 330 cm^{-1} could be assigned to the bending vibration of C–C–C and 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 obtaining more detailed information concerning 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. Chlorophylls *a* and *b* and carotenoids are mostly responsible for the differentiation of the leaf-rich samples (S-K, N-K, O-K, and F-K) from the other samples, while bulb-rich samples (A-K and R-K) mainly differ from all other samples in their pectic and amino acid compositions. Based on PC2, there is a separation of F-K, N-K, and O-K from the R-K and S-K samples, mainly based on the polysaccharide and carotenoid compositions. Further research will be focused on the chemical compositions of the selected *Allium* species, grown both in open field conditions and in a protected area, with the application of different fertilizers, using Raman spectroscopy and spectrophotometric analysis.

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References

1. Li, Q.Q.; Zhou, S.D.; He, X.J.; Yu, Y.; Zhang, Y.C.; Wei, X.Q. Phylogeny and biogeography of *Allium* (*Amaryllidaceae*: *Allieae*) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. *Ann. Bot.* **2010**, *106*, 709–733. [CrossRef] [PubMed]
2. Marrelli, M.; Amodeo, V.; Statti, G.; Conforti, F. Biological properties and bioactive components of *Allium cepa* L.: Focus on potential benefits in the treatment of obesity and related comorbidities. *Molecules* **2019**, *24*, 24010119. [CrossRef] [PubMed]
3. Fredotović, Ž.; Puizina, J. Edible *Allium* species: Chemical composition, biological activity and health effects. *Ital. J. Food Sci.* **2019**, *31*, 19–39.
4. Fritsch, R.M.; Keusgen, M. Occurrence and taxonomic significance of cysteine sulphoxides in the genus *Allium* L. (*Alliaceae*). *Phytochemistry* **2006**, *67*, 1127–1135. [CrossRef] [PubMed]
5. Buzgar, N.; Apopei, A.I.; Buzatu, A. Romanian Database of Raman Spectroscopy. 2009. Available online: http://www.rdrs.ro (accessed on 17 January 2021).
6. Lu, X.; Rasco, B.A.; Jabal, J.M.; Aston, D.E.; Lin, M.; Konkel, M.E. Investigating antibacterial effects of garlic (*Allium sativum*) concentrate and garlic-derived organosulfur compounds on *Campylobacter jejuni* by using Fourier transform infrared spectroscopy, Raman spectroscopy, and electron microscopy. *Appl. Environ. Microbiol.* **2011**, *77*, 5257–5269. [CrossRef]
7. Lu, X.; Rasco, B.A.; Kang, D.H.; Jabal, J.M.; Aston, D.E.; Konkel, M.E. Infrared and Raman spectroscopic studies of the antimicrobial effects of garlic concentrates and diallyl constituents on foodborne pathogens. *Anal. Chem.* **2011**, *83*, 4137–4146. [CrossRef]
8. Si, M.Z.; Zhang, D.Q.; Liu, R.M. Study of volatile organic compounds of fresh *Allium* species using headspace combined with surface-enhanced Raman scattering. *Guang Pu Xue Yu Guang Pu Fen Xi* **2014**, *34*, 2449–2452.
9. Menges, F. Spectragryph Optical Spectroscopy Software, Version 1.2.14. Available online: http://www.effemm2.de/spectragryph/ (accessed on 27 March 2021).
10. Hammer, Ø.; Harper, D.A.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
11. Hanen, N.; Fattouch, S.; Ammar, E.; Neffati, M. *Allium* Species, Ancient Health Food for the Future? In *Scientific, Health and Social Aspects of the Food Industry*; Valdez, B., Ed.; IntechOpen: London, UK, 2012; pp. 343–354. Available online: https://www.intechopen.com/chapters/27393 (accessed on 17 January 2021).
12. Upadhyay, R. Nutraceutical, pharmaceutical and therapeutic uses of *Allium cepa*: A review. *Int. J. Green Pharm.* **2016**, *10*, 46–64.
13. Hacıseferoğulları, H.; Özcan, M.; Demir, F.; Çalışır, S. Some nutritional and technological properties of garlic (*Allium sativum* L.). *J. Food Eng.* **2005**, *68*, 463–469. [CrossRef]
14. Hedges, L.; Lister, C. *The Nutritional Attributes of Allium Species*; Crop & Food Research Confidential Report No. 1814; Crop & Food Research: Christchurch, New Zealand, 2007. [CrossRef]
15. Tsiaganis, M.; Laskari, K.; Melissari, E. Fatty acid composition of *Allium* species lipids. *J. Food Compos. Anal.* **2006**, *19*, 620–627. [CrossRef]
16. Synytsya, A.; Čopíková, J.; Matějka, P.; Machovič, V. Fourier transform Raman and infrared spectroscopy of pectins. *Carbohydr. Polym.* **2003**, *54*, 97–106. [CrossRef]
17. Zeng, J.; Ping, W.; Sanaeifar, A.; Xu, X.; Luo, W.; Sha, J.; Huang, Z.; Huang, Y.; Liu, X.; Zhan, B.; et al. Quantitative visualization of photosynthetic pigments in tea leaves based on Raman spectroscopy and calibration model transfer. *Plant Methods* **2021**, *17*, 4. [CrossRef]
18. Picaud, T.; Le Moigne, C.; Gomez de Gracia, A.; Desbois, A. Soret-Excited Raman Spectroscopy of the Spinach Cytochrome b6f Complex. Structures of the *b*- and *c*-Type Hemes, Chlorophyll *a* and β-Carotene. *Biochemistry* **2001**, *40*, 7309–7317. [CrossRef]
19. Cai, Z.L.; Zeng, H.; Chen, M.; Larkum, A.W. Raman spectroscopy of chlorophyll d from Acaryochloris marina. *Biochim. Biophys. Acta* **2002**, *1556*, 89–91. [CrossRef]
20. Adar, F. Carotenoids—Their Resonance Raman Spectra and How They Can Be Helpful in Characterizing a Number of Biological Systems. *Spectroscopy* **2017**, *32*, 12–20.
21. Vítek, P.; Novotná, K.; Hodaňová, P.; Rapantová, B.; Klem, K. Detection of herbicide effects on pigment composition and PSII photochemistry in *Helianthus annuus* by Raman spectroscopy and chlorophyll *a* fluorescence. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *170*, 234–241. [CrossRef]
22. Vrettos, J.S.; Stewart, D.H.; De Paula, J.C.; Brudvig, G.W. Low-Temperature Optical and Resonance Raman Spectra of a Carotenoid Cation Radical in Photosystem II. *J. Phys. Chem. B* **1999**, *103*, 6403–6406. [CrossRef]
23. Edwards, H.G.; Farwell, D.W.; Webster, D. FT Raman microscopy of untreated natural plant fibres. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **1997**, *53A*, 2383–2392. [CrossRef]
24. Schulz, H.; Schütze, W.; Baranska, M. Fast determination of carotenoids in tomatoes and tomato products by Raman spectroscopy. *Acta Hortic.* **2006**, *712*, 901–905. [CrossRef]
25. Farber, C.; Sanchez, L.; Rizevsky, S.; Ermolenkov, A.; McCutchen, B.; Cason, J.; Simpson, C.; Burow, M.; Kurouski, D. Raman Spectroscopy Enables Non-Invasive Identification of Peanut Genotypes and Value-Added Traits. *Sci. Rep.* **2020**, *10*, 7730. [CrossRef]
26. Schulz, H.; Baranska, M.; Baranski, R. Potential of NIR-FT-Raman spectroscopy in natural carotenoid analysis. *Biopolymers* **2005**, *77*, 212–221. [CrossRef]

27. Collins, A.M.; Jones, H.D.; Han, D.; Hu, Q.; Beechem, T.E.; Timlin, J.A. Carotenoid distribution in living cells of *Haematococcus pluvialis* (Chlorophyceae). *PLoS ONE* **2011**, *6*, e24302. [CrossRef]
28. Vítek, P.; Veselá, B.; Klem, K. Spatial and Temporal Variability of Plant Leaf Responses Cascade after PSII Inhibition: Raman, Chlorophyll Fluorescence and Infrared Thermal Imaging. *Sensor* **2020**, *20*, 1015. [CrossRef]
29. Agarwal, U.P. 1064 nm FT-Raman spectroscopy for investigations of plant cell walls and other biomass materials. *Front. Plant Sci.* **2014**, *5*, 490. [CrossRef]
30. Volić, M.; Pajić-Lijaković, I.; Djordjević, V.; Knežević-Jugović, Z.; Pećinar, I.; Stevanović-Dajić, Z.; Veljović, Đ.; Hadnadjev, M.; Bugarski, B. Alginate/soy protein system for essential oil encapsulation with intestinal delivery. *Carbohydr. Polym.* **2018**, *200*, 15–24. [CrossRef]
31. da Silva, C.E.; Vandenabeele, P.; Edwards, H.G.; de Oliveira, L.F. NIR-FT-Raman spectroscopic analytical characterization of the fruits, seeds, and phytotherapeutic oils from rosehips. *Anal. Bioanal. Chem.* **2008**, *392*, 1489–1496. [CrossRef]
32. De Oliveira, V.E.; Castro, H.V.; Edwards, H.G.M.; de Oliveiraa, L.F.C. Carotenes and carotenoids in natural biological samples: A Raman spectroscopic analysis. *J. Raman Spectrosc.* **2010**, *41*, 642–650. [CrossRef]
33. Wiercigroch, E.; Szafraniec, E.; Czamara, K.; Pacia, M.Z.; Majzner, K.; Kochan, K.; Kaczor, A.; Baranska, M.; Malek, K. Raman and infrared spectroscopy of carbohydrates: A review. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2017**, *185*, 317–335. [CrossRef]