

COMPARATIVE EVALUATION OF THE POLYPHENOLIC CONTENT AND THE RESULTING ANTIOXIDANT ACTIVITY OF SEVERAL COMMON *ALLIUM* SPECIES

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ABSTRACT

The Allium genus representatives are recognized as valuable nutrients, widely used in various national cuisines all over the World due to their specific taste. Besides, their evinced antioxidant properties, these species possess some pharmaceutic abilities. That is why these plants are the object of growing research interest. Thus, the present study provides data, regarding the polyphenolic compound contents, especially flavonoids, the resulting antioxidant activity and the functional groups of their molecules. The research activities included garlic, leek, shallot, red, white and yellow onions. These species were submitted to comparative analysis, by UV-Vis spectrophotometry, and Fourier Transform Infrared Spectroscopy (FT-IR). The former method was applied to four procedures on powder extracts for determination of to determine: (i) the total polyphenols and particularly (ii) flavonoids, followed by (iii) ferric reducing antioxidant power (FRAP) and (iv) free radical scavenging activity (also known as “DPPH-method”).

The analysis of the results showed that the yellow onion samples contained the highest amount of polyphenolic compounds, whereas the highest content of flavonoids was registered for the shallot. The results for antioxidant activities of the studied cultivars showed similar trends, completely coinciding with the data for the flavonoid contents. Hence, according to the obtained data, the antioxidant activity and flavonoid content decreased in the following order: shallot > yellow onion > red onion > white onion > leek > garlic.

Keywords: garlic, leek, shallot, onions - red, white, yellow; total polyphenols and flavonoids antioxidant activity.

INTRODUCTION

For centuries, the representatives of the genus *Allium* have been recognised as valuable nutrients and appear among the main ingredients of various national cuisines all over the World, due to their specific taste. Besides their evinced antioxidant properties, these species possess some pharmaceutic abilities [1], especially antimicrobial [2 - 4] and cardiovascular and metabolic protective [5], activities. In this sense, Srivastava et al. remark on some other valuable abilities of these plants, such as preventive benefits for lung problems brought on by exposure to toxic chemicals, as well as for lung cancer, infections, inflammation and allergic disorders [6]. In this aspect, a

recent intensification of the research activities, regarding the activity of the polyphenols against malaria-causing plasmodium appears [7, 8]. Finally, anticancer activity possessed by *Allium* cultivars was recently proposed by Iwar and co-workers [9]. In addition, bacteriostatic effect was established by Marinkova et al. [10]. Further, these authors remark the obvious bactericide activity of the former cultivar against *Pseudomonas fluorescens* and *Arthrobacter oxydans* strains [10]. The anti-infectious activity of the polyphenols, extracted from other plants [11 - 14], especially lavender [15 - 19] is an object of interest, as well.

The use of plant-derived antioxidants for nutrition product preservation is proposed by Deshmukh et al. [20],

Kashtiban et al. [21] and Abeyrathne et al. [22], whereas Sun and co-workers [23] remark on the capability of the flavonoids to suppress Alzheimer's Disease.

Consequently, the aim of the present work is to compare the polyphenolic and especially the flavonoidic contents of several common *Allium* species: garlic, leek, shallot, red, white and yellow onion. The instrumental analytical methods used were UV-vis spectrophotometry and Fourier Transform Infrared Spectroscopy (FT-IR). The former method was applied to four procedures on powder extracts to determine: (i) the total polyphenols and particularly (ii) flavonoids, (iii) ferric reducing antioxidant power (FRAP) and (iv) free radical scavenging activity (also known as "DPPH-method"). The latter method (i.e. FT-IR) was used to determine the functional moieties of the respective polyphenolic compounds in powder samples of the *Allium* species studied.

EXPERIMENTAL

Preliminary sample preparation

For the needs of the present study, six commercially available (Kaufland, Bulgaria) *Allium* species: garlic, leek, shallot and red, white and yellow onions were investigated. After sorting them to remove damaged parts of the vegetables, the respective samples were dried for 48 h at 55°C. The obtained dry biomass was crushed by a coffee grinder and stored in a desiccator. Then the six dry biomass powders were divided into two parts for each cultivar.

The former part of each powder underwent extraction. It was performed in acidified methanol solution for overnight at room temperature. The extraction solution was prepared by addition of 2.3 mL of 37 %_{wt.} M HCl to 1 L analar grade methanol purchased from Merck (Germany). The extraction proportion was 5 g of dry biomass powder to 50 mL of the above-described extraction solution.

Characterization procedure conditions

UV-Vis spectrophotometry

This method was applied for the determination of: (i) the total polyphenols (at 760 nm) and particularly (ii) flavonoids (at 405 nm), followed by (iii) ferric reducing antioxidant power (593 nm) and (iv) free radical scavenging activity (517 nm), after the respective treatment procedures, described below. All

the measurements were performed at least in triplicate using a Cary 100 device. The acquired data were treated by Excell (Microsoft Office 365) software. The data variance was carried out using the ANOVA technique.

The total polyphenols (TP) were determined using the Folin-Ciocalteu method, following Grelliera et al. [24] and Waterhouse [25]. The measurement was performed at 760 nm of analytical reaction mixture, and incubated for 2 h at room temperature. The reaction mixture was prepared by the execution of several consecutive steps. Initially, 20 µL of the methanolic extract was diluted with 1.58 mL water. Then it was mixed with 100 µL Folin-Ciocalteu reagent - Sigma Aldrich (Germany), preliminary diluted with distilled water 1:4 v/v. After incubation, up to 8 min. 300 µL sodium carbonate solution was added. This solution was composed of 200 g pure Na₂CO₃ to 800 mL of distilled water. The measurements were performed against a reference solution without extract, and the results were compared with a gallic acid calibration standard curve.

Analysis of total flavonoids (TF) was performed with a modified colorimetric method using aluminum trichloride [26 - 28]. In brief, 100 µL of each methanolic extract was mixed with 500 µL of distilled water and then with 100 µL of 5 % aqueous solution of NaNO₃ and allowed to stay for 6 min. Then 150 µL of 10 % AlCl₃ solution was added and allowed to stay for 5 min. after which 200 µL of 1 M of NaOH solution was added sequentially. The absorbance of this reaction mixture was recorded at 400 nm.

The antioxidant activity of the methanolic extracts was carried out by both FRAP and DPPH methods, following the prescriptions, respectively [29, 30]. In brief, the FRAP method was performed by spectrophotometric measurements at 593 nm of the preliminary prepared reaction mixture. This mixture was prepared by the addition of 400 µL of the respective methanolic extract to 3 mL of tricomponent solution. This solution contained (i) 25 mL acetate buffer for pH 3.6, (ii) 2.5 mL of 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ) solution, (iii) 2.5 mL of FeCl₃ solution. The acetate buffer consisted of 300 mM of CH₃COONa. The TPTZ solution was prepared by the addition of 31.2 mg of the reagent (Sigma-Aldrich-USA) to 10 mL of 40 mM HCl. The FeCl₃ solution was composed of 54.1 mg of FeCl₃·6H₂O dissolved in 10 mL of distilled water. The mixtures needed 4 min. retaining in the dark

prior to the respective measurements.

The DPPH method was performed by spectrophotometric measurements at 517 nm. For the execution of this method, another reaction solution was prepared. It was made by addition of 400 mL methanol extract to the solution of 2.4 mg of 2, 2-di(4-tert-octylphenyl)-1-picrylhydrazyl radical (DPPH), (Sigma-Aldrich-USA) in 100 mL methanol. This solution needed 15 min exposure to darkness prior to the respective measurement. In both methods, Trolox was used as an antioxidant capacity standard.

Fourier Transform Infrared Spectroscopy

This analytical technique was performed on each of the dry powders from the respective cultivars. 20 mg of each powder was encapsulated in 80 mg KBr pellet by PIKE-USA handle press. The measurements were recorded on the Bruker Tensor 37 FT-IR in the mid-infrared region (MIR), from 4000 to 400 cm^{-1} averaging over 64 scans in transmittance mode with reference KBr pellets at room temperature.

In this way, sample extracts (for the UV-Vis spectrophotometric analyses) and dry powders (for the respective FT-IR analyses) were acquired from the six cultivars as shown in Fig. 1.

RESULTS AND DISCUSSION

As it was mentioned in the previous sections of the present study, six *Allium* cultivars were submitted to five types of analyses by two basic methods. The respective results and comments are represented below.

Total polyphenol and flavonoid contents

Prior to the determination of the antioxidant activities of the investigated species, their total polyphenolic and flavonoid contents were defined spectrophotometrically. The acquired data are summarized in Table 1. There, the respective total polyphenol contents are calculated as milligrams of gallic acid equivalents acquired per 100 g dried powder, whereas the flavonoid contents are expressed as milligrams of quercetin equivalents, again per 100 g dried powder. All the measurements were performed at least in triplicate so that the standard deviations were calculated for at least three individual results.

The studied *Allium* species reveal rather large differences among the total polyphenol contents. The data in Table 1 show that from the highest to the lowest contents, the extracts could be ordered as follows: yellow onion > red onion > shallot > white onion > leek > garlic.

However, this ordering does not correspond completely to the total flavonoid content. In this case, the ordering is: shallot > yellow onion > red onion > white onion > leek > garlic. Hence, the highest total polyphenol content belongs to the yellow onion, whereas the highest flavonoid amount was registered for the shallot. Besides, the comparison among the data in the table reveals that the content of flavonoids for the yellow onion approaches 66.72 % of the total polyphenols. In the case of shallot, this ratio is about 77.24 %, respectively. This fact means that the yellow onion contains a larger quantity of other polyphenolic compounds for 100 g dried powder.

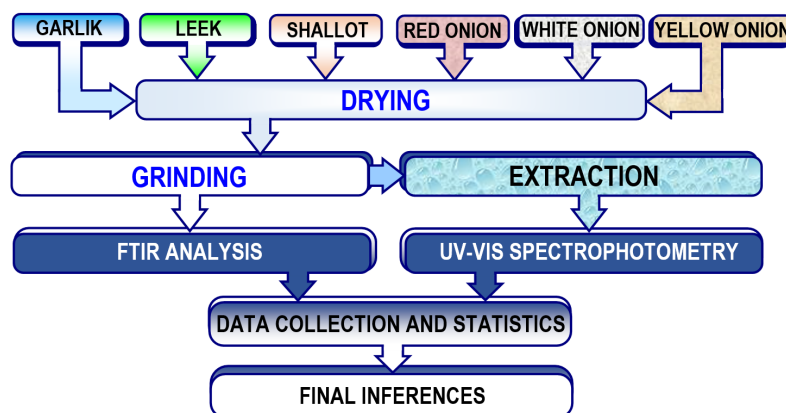


Fig.1. Schematic schedule of the research activities.

Table 1. Total polyphenols and flavonoids of different onion species

Analyzed product	Total Polyphenols	Total flavonoids
Garlic	133.32 ± 3.37	47.09 ± 2.77
Leek	426.07 ± 4.21	303.90 ± 2.80
Shallot	661.03 ± 4.20	510.60 ± 3.59
Red onion	696.30 ± 4.41	428.30 ± 1.98
White onion	479.90 ± 3.95	269.80 ± 3.09
Yellow onion	697.03 ± 2.78	465.03 ± 3.17

Antioxidant activity determination

This activity was defined for all the six *Allium* cultivars by two, independent analytical approaches, assigned as “FRAP” and “DPPH” methods. The respective procedures are described in detail, in the experimental part. In brief, the systematic analyses were performed after a mixture of methanolic extracts with suitable analytical reagents and spectrophotometric determinations at 593 or 517 nm, respectively. The acquired results were summarized and represented in the form of histograms in Fig. 2.

In concordance with the inferences established for the total polyphenols and flavonoids, described in the previous paragraph, the results in Fig. 2 show that garlic possesses the lowest antioxidant activity. Besides, this fact is established by both FRAP and DPPH methods. However, for all the investigated species, the results acquired by the FRAP mode are lower than those obtained by DPPH. Nevertheless, both methods reveal the same trends, positioning the investigated cultivars in the following order: shallot > yellow onion > red onion > white onion > leek > garlic. This order completely coincides with the ordering of the flavonoid contents, represented in the previous paragraph.

Fourier Transform Infrared Spectroscopy

The application of this instrumental analytical technique enables to extend the research activities, related to the present brief systematic study. The FT-IR spectra in the MIR region obtained from the dry powders are shown in Fig. 3.

The acquired spectra look relatively similar, although some differences are noticeable in the peak occurrence, shapes, frequencies and intensities. The

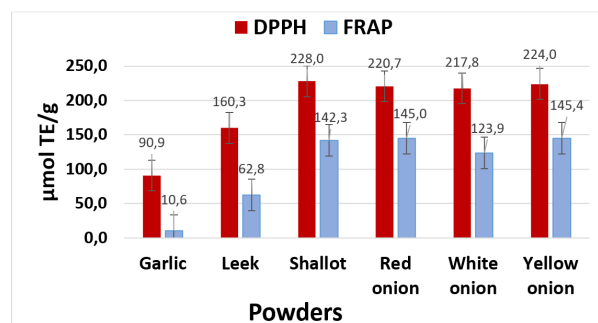


Fig. 2. Histograms showing a comparison of the DPPH test (in blue) and ferric-reducing power (in red) of both as Trolox equivalents per g dried powder.

peak positions in the FT-IR spectra were analyzed, identifying the functional groups of the polyphenolic compounds for each of the studied *Allium* species. The analysis was based on the literature data listed below, regarding the correspondence between the FT-IR peak positions and the respective functional moieties.

According to Abbas et al. [32], Stuart [39] and Nandiyanto et al. [40], the spectral range between 3500 and 3200 cm^{-1} is characteristic of the vibration of the O-H functional group. All the studied cultivars showed a broad peak, with a maximum shifting, inherent for each one of the species. Hence, the lowest wavelength range peak maxima were situated at 3365 cm^{-1} for garlic; 3383 cm^{-1} for leek; 3377 cm^{-1} for shallot; 3362 cm^{-1} for red onion; 3386 cm^{-1} for white onion, and at 3364 cm^{-1} for yellow onion, respectively. This peak is assigned to the stretching vibration of O-H groups from water, phenols, and carboxylic acids by various authors, like Verma et al. [31], Thampi and Shalini [35], Kozłowicz et al. [37], Nandiyanto et al. [40], etc.

The relatively extended peak width is a result of overlapping of peaks, due to symmetric and asymmetric stretching vibrations in various complex molecules. The FT-IR stretching vibrations at 2933 and 2924 cm^{-1} could be ascribed to the C-H group (asymmetrical and symmetrical $-\text{CH}_2-$, $-\text{CH}_3$ groups). These moieties could belong to both alkyl and aromatic radicals, bonded with carbohydrates, according to Kozłowicz et al. [37], or aromatic compounds, regarding the work team of Divya et al. [34]. Small peaks appear in the triple bond region (2000-2500 cm^{-1}) in the spectra acquired from the garlic and red onion powders. The peaks are attributed to $\text{C}\equiv\text{C}$ weak stretching at 2379 and 2353 cm^{-1} [40] or alternatively to the S-H moiety of allicin, as proposed

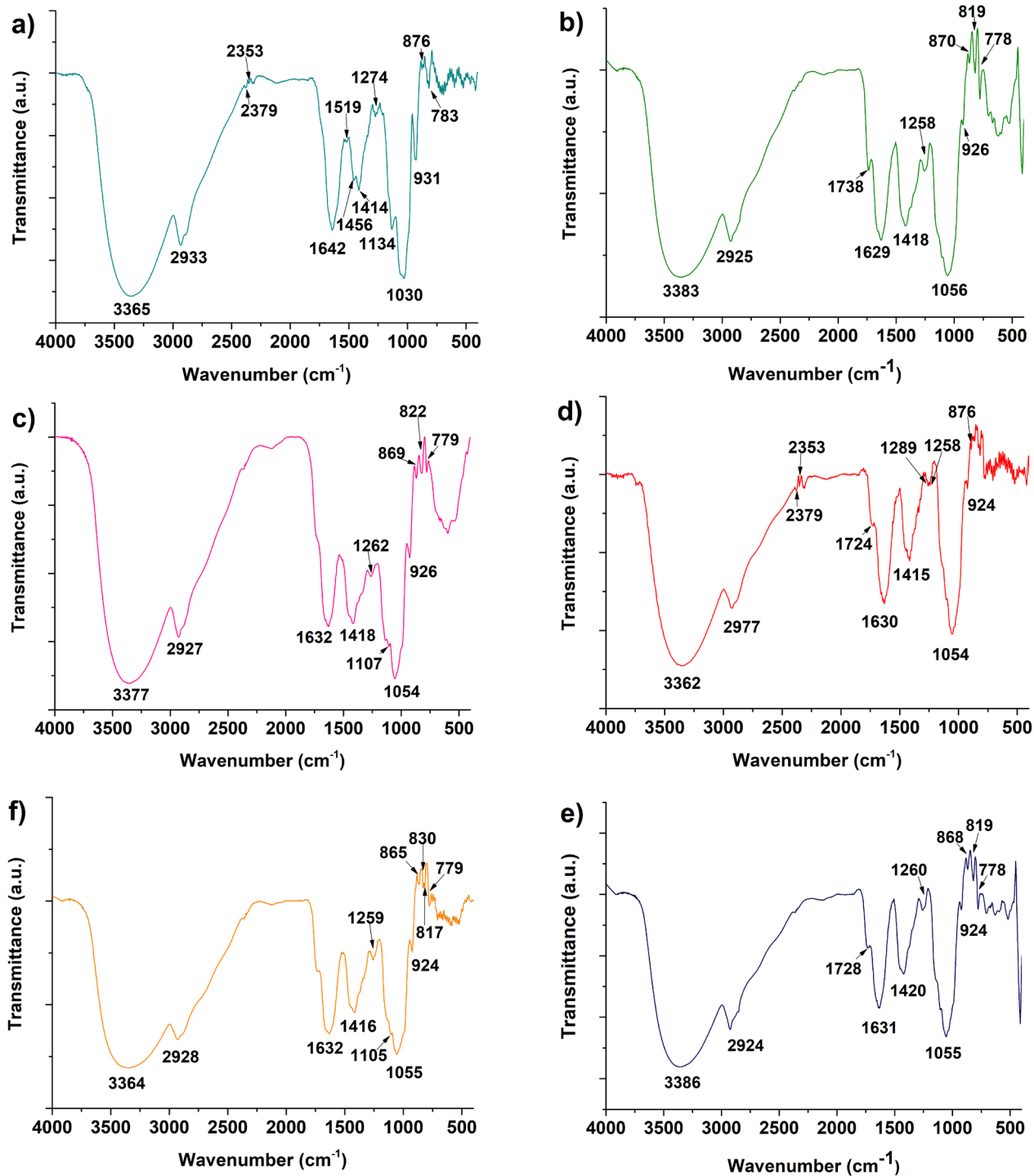


Fig. 3. FT-IR spectra of the studied powders: (a) - garlic, (b) - leek, (c) - shallot, (d) - red onion, (e) - white onion, (f) - yellow onion.

by Songsungkan and Chanthai [41].

A small peak at 1738 cm^{-1} appears in the spectra of leek shifted to 1728 cm^{-1} for white onion and 1724 cm^{-1} for the red onion powders. It reveals the stretching

of the $\text{C}=\text{O}$ group in aldehydes and/or esters [40]. The rather intensive, broad and asymmetric peak was found in the interval between 1629 and 1642 cm^{-1} for almost all powders. It was at 1642 cm^{-1} for garlic and shifted

to 1629 cm^{-1} for leek, to 1630 cm^{-1} for red onion, to 1631 cm^{-1} for white onion and to 1632 cm^{-1} for shallot and yellow onion powders, respectively. According to some authors, like da Silva Barud et al. [42], Oliveira et al. [43], and Danciu et al. [44], it reveals stretching vibration of C=C groups, for instance in allicin molecules for garlic [41], whereas others [45] relay it to aromatic ring bending and C=O stretching vibration. Such bonds can be attributed to the occurrence of moieties of flavonoids and lipids [43], or to occurrence of caffeic acid and its derivatives [46].

An almost indistinguishable peak was observed at 1456 cm^{-1} in the spectra of the garlic samples. It could be related to the bending vibration of C-H of CH_3 , CH_2 , flavonoids and aromatic rings and the stretching vibration of aromatics [43] that only occurred in garlic powder. Its spectra have a peak at 1414 cm^{-1} , which appeared slightly shifted to: 1415 cm^{-1} for red onion, to 1416 cm^{-1} for yellow onion and to 1418 cm^{-1} leek and shallot, respectively. The occurrence of these peaks is probably a response of the benzopyrylium ring and benzo- γ -pyrone structure in-plane bending in region 1650 to 1400 cm^{-1} [32] or Ar-C=O< and >C=C< stretching vibrations [31, 32, 35]. Another peak appears at 1339 cm^{-1} for red onion. It is attributed to O-H bending in C-OH group and/or to C-O stretching vibrations, since these bands belong to the spectral regions 1390-1330 cm^{-1} and 1260-1180 cm^{-1} [31, 32, 37], respectively. There is also a small peak at 1258 cm^{-1} for the yellow, red onions and leek. It is slightly shifted to 1261 cm^{-1} for white onion, and to 1262 cm^{-1} for shallot. Particularly, the red onion reveals an additional peak at 1289 cm^{-1} , as well. According to Oliveira et al. these peaks belong to C-O stretching vibrations of organic acid or ester [43]. An additional peak at 1134 cm^{-1} was observed in the case of the garlic powder. It also indicates the occurrence of stretching of C-O-C group, as is established by Kalak et al. [38]. An additional peak was registered at 1054 cm^{-1} in spectrum of shallot, and the red onion. In the cases of white onion, it is at 1055 cm^{-1} , whereas for the yellow onion, it was registered at 1056 cm^{-1} , respectively. The spectra of the garlic powder, this peak was positioned at 1030 cm^{-1} . Following the works of Kalak et al. [37] and Nandiyanto et al. [40], it can be attributed to bending vibration C-H, C-O, and C-C (carbohydrates) for the peak at 1054 cm^{-1} . Some flavonoids are linked

to carbohydrates. The peak is assigned to the stretching C-O-C of pyranose structure [30] and bending C-O of polysaccharides [35, 36] and for garlic powder indicates the S=O group revealing the presence of organosulfur compounds including alliin, allicin and diallyl disulphide [32, 39].

Finally, at the lowest wavelength region, peaks in 931-924 and 875-865 cm^{-1} ranges with different intensities were also detected. Besides, a weak shift was observed for the former peak. For leek and shallot powders it was positioned at 926 cm^{-1} . It was shifted to 924 cm^{-1} for red, white and yellow onions, whereas for garlic it was at 931 cm^{-1} . Supplemental peak was registered at 876 cm^{-1} for the garlic and red onion powder, and it shifted to 870 cm^{-1} for leek, to 869 cm^{-1} for white onion, to 865 cm^{-1} yellow onion and to 870 cm^{-1} for shallot. Those peaks were stretching vibrations C-C in carbohydrate structure; C-H bending vibration [34].

At last, it is worth noting that a recent extended research work, summarizes that any peak, belonging to 1755-1630; 1629-1400; 1399-1200 or 1199-700 cm^{-1} could be response of the occurrence of anthocyanin, anthocyanidin, flavonol, flavone, isoflavone, flavanol, or flavanone [30]. Consequently, all the peaks of the spectra in Fig. 3 are rather probable response to some representatives of the phenolic class compounds.

In concordance with the statement done, regarding the highest antioxidant activity and total flavonoid content of the shallot and the yellow onion, their FT-IR spectra possess almost identical shapes. The peaks in their spectra are clearer, whereas in the rest spectra there are supplemental peaks corresponding to other groups of compounds.

Although the red onion has shown rather similar results to these of the yellow one, regarding the polyphenols, and antioxidant activity, its FT-IR spectrum possessed more peaks. The difference between their spectra (Fig. 3d and e) are due to the occurrence of compounds with different functional groups, which was registered by the DPPH test results illustrated in Fig. 2.

The possessor of the lowest antioxidant activity and the lowest polyphenols and flavonoids (i.e. the garlic) has shown the most distinguishable spectra, revealing the highest content of other kinds of compounds. For instance, it has peaks at 1456, 1414 and 1134 cm^{-1} , which were not observed for the other studied *Allium*

species. Hence, obviously it has other compounds with beneficial effects, but their determination is not an object of the present study.

CONCLUSIONS

The present study represents the data, acquired from the methanol extracts of six *Allium* species. The respective data were obtained by systematic comparative analyses, performed at similar conditions for all the investigated cultivars. This approach has enabled us to determine the total polyphenol and particularly flavonoid contents and to compare them with the antioxidant activity, defined by two independent methods. Finally, near-range Fourier Transform Infrared Spectroscopy was applied for acquisition of additional data, regarding the chemical contents of the investigated samples.

The results reveal that the investigated *Allium* species reveal rather large difference among the total polyphenol contents. From the highest to the lowest contents, the extracts could be ordered as follows: yellow onion > red onion > shallot > white onion > leek > garlic. Respectively, according to the flavonoid contents the ordering is: shallot > yellow onion > red onion > white onion > leek > garlic. Hence, the highest total polyphenol content belongs to the yellow onion, whereas the highest flavonoid amount is owed by the shallot. Furthermore, it was established that the yellow onion contains a larger quantity of other polyphenolic compounds, for 100 g dried powder.

The antioxidant activities of the characterized cultivars were evaluated by two, independent methods, assigned as "FRAP" and "DPPH", respectively. Although the FRAP results are lower than those, acquired by the DPPH method, both analytical techniques reveal the same trend. Besides, it is completely concordant with this, established for the flavonoid contents of the investigated *Allium* species.

The research activities were carried out by systematic FT-IR analyses. The peak positions in the respective spectra were analysed, identifying the functional groups of the polyphenolic compounds for each of the studied *Allium* species. The correspondence between the FT-IR peak positions and the respective functional moieties was defined on the basis of a variety of recent research and review papers.

Hence, all the studied cultivars showed a broad peak, with a maximum shifting, inherent for each one of the species. This peak was assigned to the stretching vibration of O-H groups from water, phenols, and carboxylic acids.

Small C≡C weak stretching peaks were registered for the garlic and red onion powders. In turn, the spectra of leek and red onion powders revealed stretching of C=O group in aldehydes and/or esters.

Almost indistinguishable peak was observed in the spectra of the garlic samples, related to the bending vibration of C-H of CH₃, CH₂, flavonoids and aromatic rings and the stretching vibration of aromatics.

Other peak, revealing benzopyrylium ring, a benzo-γ-pyrone structure or Ar-C=O< and >C=C< stretching vibrations was registered for garlic, leek, shallot and onions, respectively.

The garlic and red onion powders, have shown occurrence of C=S stretching and O-H bending in C-OH group and/or to C-O stretching vibrations. C-O stretching vibrations, typical for organic acid or ester were observed for red, yellow, white onions, and shallot.

Additional peak was registered in the cases of leek, shallot, garlic as well as for the white, red and yellow onions, due to bending vibration C-H, C-O, and bonds C-C in carbohydrate structures, like carbohydrates. The spectra acquired from garlic showed S=O group, revealing the presence of organosulfur compounds including alliin, allicin and diallyl disulphide. Peaks indicating stretching vibrations C-C in carbohydrate structure; C-H bending vibration were registered for all the studied powders.

The results for shallot and the yellow onion as possessors of the highest antioxidant activity and total flavonoid content correspond to their FT-IR spectra, which were different from the rest ones.

Although the red onion has shown rather similar results to these of the yellow one, regarding the polyphenols, and antioxidant activity, its FT-IR spectrum possessed more peaks, due to the occurrence of compounds with different functional groups. It was registered by the DPPH test results.

The possessor of the lowest antioxidant activity and the lowest polyphenols and flavonoids (i.e. the garlic) has shown the most distinguishable spectra, revealing high content of other kinds of compounds.

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