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VARIABILITY OF BIOACTIVE COMPOUNDS IN LEEK (ALLIUM PORRUM L.)

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ABSTRACT

Leek (*Allium porrum* L.), a commonly grown vegetable widely used in the food industry, has gained increased interest in recent years in connection with its content of bioactive substances. Therefore, this study aimed to determine and evaluate the influence of cultivar and morphological part on the total content of polyphenols, sulfur compounds, and the antioxidant activity in the monitored morphological parts of leek (*Allium porrum* L.). The total polyphenol content (TPC) in the white part of analyzed leek cultivars ranged from 446.30 to 1580.55 mg GAE.kg^{-1} DM. TPC in the green part of analyzed leek cultivars ranged from 748.09 to 2172.78 mg GAE.kg^{-1} DM. TPC in the leaves of analyzed leek cultivars ranged from 2301.24 to 5477.73 mg GAE.kg^{-1} DM. The antioxidant activity (AA) in the white part of analyzed leek cultivars ranged from 3.70 to 14.23 mmol TE.kg^{-1} DM. AA in the green part of analyzed leek cultivars ranged from 6.89 to 14.38 mmol TE.kg^{-1} DM. AA in leaves of analyzed leek cultivars ranged from 30.85 to 106.92 mmol TE.kg^{-1} DM. The total sulfur content (TSC) in the white part of analyzed leek cultivars ranged from 0.25 to 0.52 %. TSC in the green part of analyzed leek cultivars ranged from 0.27 to 0.52 %. TSC in the leaves of analyzed leek cultivars ranged from 0.31 to 0.59 %. Based on the analysis of variance, we can state that statistically higher ($p< 0.05$) TPC, AA, and TSC were determined in the leaves of the leek. Statistically higher ($p< 0.05$) TPC, AA, and TSC in all morphological parts were determined in the cultivar De Carentan. The results confirmed the variability of bioactive substances and antioxidant activity in the individual monitored cultivars, but also in the monitored morphological parts of the leek.

Keywords: Allium porrum L.; leek, total polyphenol content; antioxidant activity; total sulfur content

INTRODUCTION

The *Allium porrum* L., known as the leek, is a tough biennial crop that has been consumed since ancient Egypt and is today an irreplaceable part of gastronomy grown in many countries around the world **(Benedé *et al.*, 2019)**. Leek is well recognized for its distinctively strong aroma and pungent flavor. It is one of the essential culinary, spicy, ornamental, and aromatic plants, along with garlic and onion. The entire plant or its separate parts—the leaves, white, and green parts—are most frequently served by cooking. For example, they can also be eaten fresh as a component of different salads. In addition to their well-known culinary benefits, leeks are said to offer therapeutic qualities that are similar to those of garlic. Leek consumption has been linked to the observation of positive benefits on human health, including anti-inflammatory, anticancer, anti-diabetic, and gastroprotective effects. It has been demonstrated that including leeks in one's diet on a regular basis has positive effects on the body, particularly the circulatory system **(Chen *et al.*, 2010; Ognyanov *et al.*, 2020)**. **Benedé *et al*. (2019)** suggest that due to its antioxidant and anti-inflammatory properties, *A. porrum* extract may have potential anti-allergic effects.
Plants belonging to the *Allium* genus are considered rich sources of secondary metabolites including phenolic acids and their derivatives, flavonoids (flavans, flavanones, flavones, flavonols, dihydroflavonols, flavan-3-ols, flavan-4-ols and flavan-3,4-diols) and flavonoid polymers (proanthocyanidins or condensed tannins) **(Strati *et al.*, 2018)**. Phenolic compounds have many functions in plants. Some act as defense elements against herbivores and pathogens, while others have antioxidant properties and protect cellular structures from chain reactions of free radicals that occur during certain chemical reactions and UV radiation. Many of them absorb light in the ultraviolet region of the electromagnetic spectrum, thereby acting as protection against harmful solar radiation. They also play structural roles, linking cell wall polysaccharides and anchoring lignin to the polysaccharide domain. They may also play an important role in the organoleptic properties of plants **(Marchiosi *et al.*, 2020; Rana *et al.*, 2022; Aloo *et al.*, 2023)**. Polyphenolic chemicals have antioxidant characteristics that can protect cells from oxidative stress and reduce the risk of developing a variety of degenerative disorders **(Sharma, 2014)**. Numerous studies that focus on polyphenolic substances point to the fact that long-term intake of a diet high in polyphenol substances considerably lessens the negative effects of diseases linked to the liver, heart, kidney, brain, pancreas, and genetic disorders, including malignant tumors and cancer **(Hossen *et al.*, 2017)**. According to **Abbas *et al.* (2017)**, polyphenolic compounds are effective in the treatment of cardiovascular diseases, osteoporosis, neurogenerative diseases, cancer, and diabetes. Plants belonging to the genus *Allium* also contain a wide range of sulfur compounds, which are responsible for the specific taste, smell, lachrymatory effects, and antioxidant properties **(Upadhay, 2017)**. According to **Zeng *et al.* (2017)**, sulfur compounds present in plant species belonging to the genus *Allium* have a protective effect against several chronic diseases and exhibit anti-inflammatory, anti-obesity, anti-diabetic, antibacterial, neuroprotective, and immunological properties.
The aim of the study was to determine the total polyphenol content, total sulfur content, and antioxidant activity in the white shaft, green shaft, and leaves of selected cultivars of leek (*Allium porrum* L.), and to evaluate the variations of determined parameters among the individual morphological parts and cultivars.

MATERIAL AND METHODS

Plant material

10 cultivars of leek, namely Bavaria, Golem, Starozagurski kamuš, October, Zwitserse rauzen, Tango, De Carentan 2, Tétényi áttelelo, Titus, and Elefant were collected in the state of full ripeness. Collection of samples was realized at the turn of August and September for summer cultivars (Bavaria, Golem, Starozagurski kamuš, Zwiterse rauzen), at the end of October for autumn cultivars (October, Titus, Elefant) and in December for winter cultivars (Tango, De Carentan 2, Tétényi áttelelo,). All samples were grown under the same agroenvironmental conditions, in the Experimental Garden of the Slovak University of Agriculture in Nitra, Slovakia. which belongs to a warm, dry climate area. The soil from the cultivation area is medium-heavy, sandy-clay with an alkali soil reaction, 3.53 % of humus, and 2.05 % of organic carbon.
For obtaining an average sample, 15 plants of each cultivar were used (cca 4 500 g).

Extract preparation

Extracts were prepared by shaking 25 g of homogenized sample in 50 g of 80% methanol on Heidolph Promax 1020 horizontal shaker (Heidolph Instruments GmbH, Schwabach, Germany) for 16 hours at room temperature. The extracts were filtered through Munktell No. 392 filtrating paper (Munktell & Filtrac GmbH, Bärenstein, Germany) and stored at 4°C before analysis.

Total polyphenol content

Total polyphenol content (TPC) was determined by the spectrophotometric method according to **Lachman *et al.* (2003)**, using a Folin –Ciocalteau phenol reagent, 20% Na_2CO_3, and distilled water. 0.1 ml of the prepared methanolic extract was added to the 50 ml volumetric flasks using an automatic pipette and diluted with 2 ml of distilled water. To the diluted sample, 2.5 ml of Folin-Ciocalteu reagent was added and mixed. After 3 minutes, 7.5 ml of 20% Na_2CO_3 was added and mixed. Using a syringe containing distilled water, the missing volume was added to the mark on the measuring flask (up to a volume of 50 ml) and mixed. The samples were then left for 2 hours to form the blue color complex. On a spectrophotometer (Shimadzu UV/VIS 1800) the absorbance was measured at a wavelength of 765 nm against a blank sample. The results are expressed as mg of gallic acid equivalent per kg of dry matter (GAE.kg^{-1} DM).

Antioxidant activity

Antioxidant activity (AA) was determined by the DPPH method according to **Brand-Wiliams *et al.* (1995)**, using 2,2-diphenyl-1-picryhydrazyl and methanol. DPPH solution (3.9 ml) was pipetted into cuvettes and the absorbance was measured on a Shimadzu UV-1800 spectrophotometer at a wavelength of 515.6 nm. After measuring the initial absorbance, 0.1 ml of the extract was added using an automatic pipette and stirred. After ten minutes, absorbance was measured again against a blank sample. The results are expressed as mmol of Trolox equivalent per kg of dry matter (TE.kg^{-1} DM).

Total sulfur content

Total sulfur content (TSC) was determined by the method of **Šapčanin *et al.* (2013)**. 50 mg of a homogenized freeze-dried sample was put into a tin container in the elemental analyzer Vario Macro Cube V 3.1.4, where combustion takes place at a temperature of 1250 °C, using V_2O_5 as a catalyst. The fumes produced during combustion were escorted past the combustion tube, where the oxidation is already completed and the reduction reaction of sulfur dioxide to sulfur dioxide is taking place. The gases are sent to a chromatographic column where gas separation takes place, and gases are relocated to the thermal conductivity detector. The software captures and evaluates individual signals from the detector, using sulfanilamide as a calibration standard. The results are expressed as % of sulfur present in the leek sample.

Statistical analysis

Statistical analysis was performed using XLSTAT software. The non-parametric ANOVA test (Kruskal-Wallis) was used for the comparison between the tested variables. The Spearman correlation was used to determine the relationships between individual parameters.

RESULTS AND DISCUSSION

Total polyphenol content

Table 1 Total polyphenol content of analyzed samples (mg GAE.kg^{-1} DM)

Cultivar / Part	White shaft	Green shaft	Leaves
Bavaria	485.0±25.21^{a,A}	868.7±24.36^{c,B}	3649±26.47^{c,C}
Golem	847.5±41.15^{c,A}	1011±26.50^{e,B}	3402±40.14^{b,C}
Starozagorski kamuš	446.3±25.66^{a,A}	748.1±27.49^{a,B}	2301±47.86^{a,C}
October	1117±42.87^{e,A}	1191±70.66^{f,B}	4232±34.10^{f,C}
Zwitserse rauzen	774.5±35.27^{b,A}	961.1±29.59^{d,B}	4116±30.75^{e,C}
Tango	1578±22.74^{f,A}	1723±7.88^{h,B}	4613±32.18^{g,C}
De Carentan 2	1580±28.32^{g,A}	2172±14.83^{i,B}	5477±49.39^{i,C}
Tétényi áttelelo	792.1±25.32^{b,A}	814.0±22.01^{b,B}	3904±33.91^{d,C}
Titus	933.2±15.05^{d,A}	1190±22.60^{f,B}	4632±44.59^{g,C}
Elefant	1122±8.84^{e,A}	1550±33.01^{g,B}	4803±35.47^{h,C}

Legend: $^{a-i}$ Values in the column marked with a different lowercase letter mean significant statistical differences ($p < 0.05$) between the studied cultivars; $^{A-C}$ Values in rows marked with a different uppercase letter mean significant statistical differences ($p < 0.05$) between the morphological parts; values shown in the table are expressed as mean ± SD (n = 4)

The total polyphenol content (TPC) in the white shaft of analyzed leek cultivars ranged from 446.30 (Starozagorski kamuš) to 1580.55 mg.kg^{-1} DM (De Carentan 2). TPC in the green shaft of analyzed leek cultivars ranged from 748.09 (Starozagorski kamuš) to 2172.78 mg.kg^{-1} DM (De Carentan 2). TPC in the leaves of analyzed leek cultivars ranged from 2301.24 (Starozagorski kamuš) to 5477.73 mg.kg^{-1} DM (De Carentan 2). We can state that the statistically lowest TPC was determined in the white parts, and the statistically highest TPC was determined in the leaves of the analyzed cultivars. **Bernaert *et al.* (2012)** report that several studies focusing on polyphenol content highlight a positive correlation between solar radiation and plant production of polyphenols. For this reason, the green leaves of leek contain a higher content of polyphenols because they grow above the soil, unlike the white parts that are in the soil and do not receive as much sunlight. The total content of polyphenols in garden leek can affect also stress factors, insects, microorganisms, and low environmental temperatures **(Michalak, 2006). Kovarovič *et al.* (2019)** reported similar values of TPC - from 504.22 to 1117.19 mg GAE.kg^{-1} DM in the white shaft, from 776.58 to 1224.09 mg GAE.kg^{-1} DM in the green shaft, and from 3686.81 to 4767.71 mg GAE.kg^{-1} DM in the leaves of leek cultivars grown in Zohor, Slovakia. Other authors reported higher values of TPC in leek. **Bernaert *et al*. (2012)** reported from 5000 to 13960 mg GAE.kg$^{−1}$ DM (7570 mg GAE.kg$^{−1}$ DM in Elefant) in the white shaft, and from 5470 to 15 140 mg GAE.kg$^{−1}$ DM (8830 mg GAE.kg$^{−1}$ DM in Elefant) in the green leaves of the 30 leek cultivars grown in a greenhouse in Belgium. **Koca *et al*. (2015)** reported from 2130 mg.kg^{-1} DM in the white part to 4780 mg.kg^{-1} DM in the green part of leek grown in Turkey. **Turkmen et al. (2005)** reported 3008 mg GAE.kg^{-1} DM in the edible part of leek purchased in Turkey. These differences could be caused by different agro-environmental factors and cultivation methods, or by different processing of plant material and different extraction methods.

Antioxidant activity

The antioxidant activity (AA) in the white shaft of analyzed leek cultivars ranged from 3.70 (Starozagorski kamuš) to 14.23 mmol TE.kg^{-1} DM (De Carentan 2). AA in the green shaft of analyzed leek cultivars ranged from 6.89 (Starozagorski kamuš) to 14.38 mmol TE.kg^{-1} DM (De Carentan 2). AA in the leaves of analyzed leek cultivars ranged from 30.85 (Starozagorski kamuš) to 106.92 mmol TE.kg^{-1} DM (De Carentan 2). We can state that AA was statistically higher ($p < 0.05$) in the leaves than in white and green shafts. **Arfa *et al.* (2015)** also reported that wild leek leaves had higher antioxidant activity compared to the white parts. **Tiveron *et al.* (2012)** reported slightly lower values of AA - 3.2 mmol TE.kg^{-1} DM in the edible part of leek grown in Brazil. Other authors reported similar values of AA in the white part of the leek, but lower values of AA in the leaves. **Bernaert *et al.* (2012)** reported from 2 to 11 mmol TE.kg-1 DM in the white shaft, and from 5 to 14 mmol TE.kg-1 DM in the green leaves of the 30 leek cultivars grown in the greenhouse in Belgium. **Bernaert *et al.* (2013)** reported that the AA of leek organically cultivated in Belgium ranges from 6 mmol TE.kg^{-1} DM (white part) to 8 mmol TE.kg^{-1} DM (leaves). These differences could be caused by different agro-environmental factors and cultivation methods, or by different processing of plant material and different extraction methods.

Table 2 Antioxidant activity of analyzed samples (mmol TE.kg^{-1} DM)

Cultivar / Part	White shaft	Green shaft	Leaves
Bavaria	8.91±0.31^{b,A}	11.00±1.08^{c,B}	88.30±1.70^{g,C}
Golem	8.80±0.64^{b,A}	12.35±0.77^{d,B}	78.56±0.82^{f,C}
Starozagorski kamuš	3.70±0.57^{a,A}	6.89±0.61^{ab,B}	30.85±0.68^{a,C}
October	13.56±1.24^{ef,A}	16.00±0.64^{f,B}	49.53±0.78^{c,C}
Zwitserse rauzen	12.66±0.26^{d,A}	8.08±1.34^{b,A}	88.37±0.63^{g,B}
Tango	8.73±0.77^{b,A}	7.14±0.87^{b,A}	62.42±0.96^{d,B}
De Carentan 2	14.23±0.59^{f,A}	14.38±1.31^{e,A}	106.92±0.95^{i,B}
Tétényi áttelelo	8.60±0.23^{b,A}	13.79±0.69^{e,B}	43.88±1.06^{b,C}
Titus	11.50±0.45^{c,B}	7.66±0.50^{b,A}	75.12±1.39^{e,C}
Elefant	12.68±0.37^{de,B}	7.96±0.36^{b,A}	95.06±0.68^{h,C}

Legend: $^{a-i}$ Values in the column marked with a different lowercase letter mean significant statistical differences ($p < 0.05$) between the studied cultivars; $^{A-C}$ Values in rows marked with a different uppercase letter mean significant statistical differences ($p < 0.05$) between the morphological parts; values shown in the table are expressed as mean ± SD (n = 4)

Total sulfur content

The total sulfur content (TSC) in the white shaft of analyzed leek cultivars ranged from 0.25 (Titus) to 0.52 % (De Carentan 2). TSC in the green shaft of analyzed leek cultivars ranged from 0.27 (Zwitserse rauzen) to 0.52 % (De Carentan 2).

Table 3 Total sulfur content of analyzed samples (% DM)

Cultivar / Part	White shaft	Green shaft	Leaves
Bavaria	0.32±0.04^{bc,A}	0.37±0.02^{d,A}	0.38±0.01^{b,A}
Golem	0.27±0.01^{ab,A}	0.34±0.01^{cd,A}	0.42±0.04^{c,B}
Starozagorski kamuš	0.38±0.01^{d,A}	0.41±0.01^{e,B}	0.39±0.01^{bc,AB}
October	0.26±0.01^{a,A}	0.34±0.04^{cd,B}	0.31±0.01^{a,B}
Zwitserse rauzen	0.26±0.01^{a,A}	0.27±0.01^{a,A}	0.34±0.01^{a,B}
Tango	0.34±0.06^{cd,A}	0.28±0.01^{ab,A}	0.32±0.01^{a,A}
De Carentan 2	0.52±0.01^{e,A}	0.52±0.01^{g,A}	0.59±0.01^{e,B}
Tétényi áttelelo	0.37±0.01^{cd,A}	0.42±0.01^{e,B}	0.53±0.01^{d,C}
Titus	0.25±0.01^{a,A}	0.31±0.01^{bc,B}	0.32±0.01^{a,B}
Elefant	0.26 ±0.01$^{a.A}$	0.48 ±0.02$^{f.B}$	0.59 ±0.01$^{e.C}$

Legend: $^{a\text{-}j}$ Values in the column marked with a different lowercase letter mean significant statistical differences (p < 0.05) between the studied cultivars; $^{A\text{-}C}$ Values in rows marked with a different uppercase letter mean significant statistical differences (p < 0.05) between the morphological parts; values shown in the table are expressed as arithmetic mean ± SD (n = 4)

TSC in the leaves of analyzed leek cultivars ranged from 0.31 (October) to 0.59 % (De Carentan 2). We can state that TSC was statistically higher (p < 0.05) in the leaves than in the white shaft. Other authors reported similar to higher values of TSC. **Mordoğan *et al.* (2019)** reported TSC in leek from 0.40 to 0.90 %. **Gunduz and Akman (2015)** reported TSC in leek from 0.375 to 0.960 %.

Relationships between monitored parameters

Positive correlations were found between the TPC of individual morphological parts, between TPC of leaves and AA of leaves and white shaft, between AA of white shaft and AA of leaves, and between TSC of white shaft and TSC of leaves. The positive correlation of TPC and AA of leek was also confirmed by **Koca *et al.* (2016)** and by **Kovarovič *et al.* (2019).** Positive correlations between TPC and AA were also confirmed in garlic **(Lenková et al., 2016; Čeryová *et al.*, 2021; Čeryová *et al*., 2023)**, onion **(Kavalcová *et al*., 2015; Lenková *et al*., 2016)**, chives **(Lenková *et al*., 2016)** and bear garlic **(Lenková *et al*., 2016; Tóth *et al*., 2018; Kovarovič *et al*., 2019).**

Table 4 Relationship between monitored parameters of analyzed samples

	TPC white shaft	TPC green shaft	TPC leaves	AA white shaft	AA green shaft	AA leaves	TSC white shaft	TSC green shaft	TSC leaves
TPC white shaft	—								
TPC green shaft	0.927 ***	—							
TPC leaves	0.820 **	0.832 **	—						
AA white shaft	0.568	0.577	0.844 **	—					
AA green shaft	0.180	0.108	0.199	0.388	—				
AA leaves	0.349	0.554	0.651 *	0.657 *	0.018	—			
TSC white shaft	0.323	0.463	0.171	-0.089	0.281	0.072	—		
TSC green shaft	0.153	0.378	0.212	0.118	0.320	0.231	0.630	—	
TSC leaves	0.223	0.389	0.324	0.197	0.261	0.373	0.519	0.902 ***	—

Legend: * p < 0.05, ** p < 0.01, *** p < 0.001

CONCLUSION

Our achieved results confirm the variability of bioactive substances in the individual monitored cultivars, but also in the monitored morphological parts of the leek. The leaves of the leek were richer in the total content of polyphenols, sulfur, and antioxidant activity compared to the white and green parts; therefore, we suggest that the leaves could be also utilized in the food industry. While the cultivar has an impact on the content of bioactive substances, further research on the other factors, such as the location of cultivation and the agrochemical composition of the soil, is important to identify conditions that could affect the amount of the bioactive substances in leek.

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