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Determination of phenolic acids in raw garlic (Allium sativum L.*) and onion (Allium cepa* L.*) bulbs*

Oznaczanie kwasów fenolowych w świeżych cebulach czosnku (*Allium sativum* L.) i białej cebuli (*Allium cepa* L.)

INTRODUCTION

Garlic (*Allium sativum* L.*)* and onion (*Allium cepa* L.), belonging to the Alliaceae family, have been commonly used worldwide for culinary (flavouring) and medicinal purposes to treat various diseases, including common cold, fever, headache, toothache, circulatory, nervous and gastric problems, or anaemia [7,10]. Both have been reported to have antispasmodic, antioxidant [11,15], hypotensive, anticholesterolemic, and hypoglycemic [4,9] properties. They were also found active against some bacteria (*Staphylococcus* sp., *Micrococcus* sp., *Streptococcus* sp., *Klebsiella* sp., *Corynebacterium* sp., *Proteus* sp. and *E. coli*) and fungal species (dermatophytes, saprophytes and *Candia* sp.) [3,18]. Moreover, according to the available study findings, garlic and onion compounds are capable of disturbing the life cycle of schistosoma parasites or paralyzing the roundworm's and fluke's muscles (9, 19). Other potential health benefits include their anti-inflammatory effects. Furthermore, their role in prevention and treatment of various cancers (breast, stomach, colorectal, lung, prostate or endometrium) has been of much interest [1,5].

The pharmacological properties of garlic and onion are strictly associated with the presence of such chemical compounds as aromatic sulphur-based compounds, phenolic compounds (phenolic acids, flavonoids), polysaccharides and proteins [1,11].

Phenolic acids are an important adjunctive, physiological constituent of major active substances. The anti-inflammatory effects of ellagic, ferulic, caffeic and p-coumaric acids, antibacterial properties of p-hydroxybenzoic and vanillic acids or antiseptic activity of caffeic and p-coumaric acids are well known. Moreover, some acids, including chlorogenic, ferulic, p-coumaric, caffeic and protocatechuic acid, show immunostimulating effects. Ferulic, protocatechuic and gallic acids are potential protective agents against oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. Recent studies indicate that they can be used to treat or prevent cancer and cardiovascular diseases [6,15,16]. Thus, the aim of this study was the identification and determination of phenolic acids in *Allium sativum* L. and *Allium cepa* L.

MATERIAL AND METHODS

Extraction of plant material. The raw bulbs of garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) were harvested from the crops near Lublin (Poland) in August 2008.

Five hundred grams of fresh bulbs (only fleshy leaves) were used in the study. The plant material was homogenized with 98% ethanol, macerated (24 h) at room temperature using 95% ethanol (2 l) and after decantation extracted twice (1h) by reflux with hot 95% ethanol (1,2 l). The obtained extracts were filtered, mixed and the solvent was evaporated in the vacuum. The dry remains were eluted with hot water (200 ml), cooled and placed in a refrigerator for 18 h. Free phenolic acid fractions (A), those released after acid (B) and alkaline hydrolysis (C) were isolated from the water extract using the method described earlier (12).

Chromatographic analysis. The obtained fractions A, B and C were analyzed using the two dimensional thin-layer chromatography (2D-TLC) and high performance liquid chromatography (HPLC).

The qualitative analysis was performed by 2D-TLC using cellulose plates (DC-Fertigplatten, Merck, Cellulose 100 x 100 x 0.1 mm) against standards of phenolic acids (Sigma, St. Louis. MO, USA). The plates were developed by one- or two-dimensional technique in the horizontal DS-chambers according to the procedure described earlier [21]. After drying, the chromatograms were observed in UV light (λ = 366 nm) before and after treatment with ammonia vapour and in daylight after spraying with the typical reagents [12]. The results of TLC analysis of each fraction are presented in Table 1.

The RP-HPLC analysis was performed in the Knauer chromatographic system (Berlin, Germany) consisting of a Model K-1001 pump equipped with a Rheodyne Model 7125 injection valve (Cotati, CA, USA) with a 20-µl sample loop, UV/VIS detector operated at 254 nm, and the EuroChrom 2000. A Hypersil ODS column (250 x 4.6 mm, i.d. 5 µm, Merck, Darmstadt, Germany) was used. The isocratic mobile phase consisted of: methanol: water (22:78, v/v) with 1% (v/v) trifluoroacetic acid. The flow rate was 1.0 ml/min.

The organic solvents were of HPLC grade (Merck). After preparation, the mobile phase was filtered through the 0.45 µm filter (J.T. Baker, Phillipsburg, NY, USA). Stock solutions and standard solutions (Sigma, St. Louis. MO, USA) of phenolic acids were prepared according to the procedure described earlier [13]. The volumes injected amounted to 10 µl.

Calibration curves were obtained by plotting the peak height (y) against the concentration of standard solutions (x) and all showed linear relationships. All phenolic acids were quantified using the external standard method.

The phenolic acids were identified by comparing their retention times with those of appropriate standard compounds.

The results of HPLC analysis are presented in Table 2 and Fig. 1.

The water content in the raw material studied was determined using the FPVII standard method [14].

Table 1. Phenolic acids in raw garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) bulbs

No.	Phenolic acids	*Allium sativum* L.			*Allium cepa* L.		
		A_S	B_S	C_S	A_C	B_C	C_C
1.	ellagic	+	-	-	+	+	-
2.	chlorogenic	-	-	-	+	+	-
3	homoprotocatechuic	+	+	+	-	-	-
4.	protocatechuic	-	-	-	+	+	+
5.	caffeic cis	+	+	+	+	+	+
	trans	+	+	+	+	+	+
6.	gentisic	+	+	-	-	+	+
7.	p-hydroxyphenylacetic	-	-	+	-	-	-
8.	p-hydroxybenzoic	+	+	+	+	+	+
9.	p-coumaric cis	+	-	+	+	-	+
	trans	+	-	+	+	-	+
10.	vanillic	+	+	+	+	+	+
11.	syringic	-	-	+	-	-	-
12.	sinapic cis	-	-	+	-	-	+
	trans	-	-	+	-	-	+
13.	ferulic cis	+	+	+	+	+	+
	trans	+	+	+	+	+	+

Explanation: + detectable, - not detectable,
A – fractions of free phenolic acids,
B – fractions of phenolic acids released after acidic hydrolysis,
C – fractions of phenolic acids released after alkaline hydrolysis.

Table 2. The content of phenolic acids in *A. sativum* L. and *A. cepa* L. [µg g^{-1}]

No.	Phenolic acids	*Allium sativum* L. [µg g^{-1}]			*Allium cepa* L. [µg g^{-1}]		
		A_S	B_S	C_S	A_C	B_C	C_C
2.	chlorogenic	-	-	-	tr	•2.6±0.1 *34.2±1.7	- -
3	homoprotocatechuic	•8.5±0.3 *25.0±1.2	• 85.8±2.6 *252.1±7.6	•1.7±0.1 *5.1±0.26	- -	- -	- -
4.	protocatechuic	-	-	-	•5.8±0.2 *76.3±3.1	•2.1±0.1 *27.6±0.8	•1.3±0.05 *17.1±0.5
5.	caffeic cis	tr	•18.0±0.95 *52.8±2.6	•0.06±0.01 *0.17±0.02	•1.7±0.1 *22.4±1.2	•0.4±0.05 *5.3±0.4	tr
	trans	•0.25±0.02 *0.73±0.07	•48.0±2.4 *141.1±7.1	•0.05±0.01 *0.15±0.02	•0.17±0.02 *22.4±1.1	•0.7±0.05 *9.2±0.5	•2.4±0.2 *31.6±2.5
8.	p-hydroxybenzoic	•0.2±0.01 *0.6±0.05	•11.4±0.6 *33.5±2.1	•0.05±0.01 *0.1±0.01	•1.8±0.1 *23.7±1.2	•1.4±0.1 *18.4±0.9	•0.8±0.05 *10.5±0.6
9.	p-coumaric cis	•38.5±3.1 *113.2±9.1	tr	•2.1±0.1 *6.2±0.5	•3.3±0.2 *43.4±2.2	-	•0.05±0.01 *0.65±0.05
	trans	•12.8±0.6 *37.5±1.9	-	tr	•2.7±0.1 *35.5±1.8	-	•0.1±0.01 *1.32±0.1
13.	ferulic cis	•14.8±1.1 *43.4±3.5	•0.4±0.05 *1.1±0.1	•4.3±0.2 *12.6±0.6	tr	•2.3±0.1 *30.3±1.2	•0.1±0.01 *1.3±0.1
	trans	•12,9±0.65 *37.9±1.9	•0.63±0.05 *1.75±0.15	•0.3±0.02 *0.88±0.1	-	•2.7±0.14 *35.5±1.8	•0.05±0.01 *0.7±0.05

Explanations:
A – fractions of free phenolic acids,
B – fractions of phenolic acids released after acidic hydrolysis,
C – fractions of phenolic acids released after alkaline hydrolysis.
s – *Allium sativum* L.
c – *Allium cepa* L.
• – content of compound in fraction in µg/g of raw material
* – content of compound in fraction in µg/g of dried material
tr – trace amount

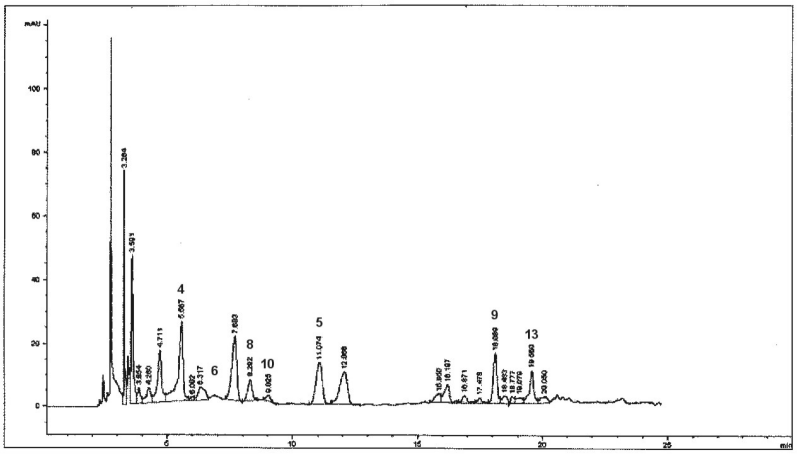

Fig. 1. HPLC chromatogram of the fraction of phenolic acids released after alkaline hydrolysis from *Allium cepa* L.(C_C). The numbers of compounds in accordance with Table 2

RESULTS AND DISCUSSION

The qualitative and quantitative analysis of phenolic acids in raw *Allium sativum* L. and *Allium cepa* L. bulbs was conducted using 2D-TLC and HPLC methods. A diversity and high concentration of phenolics was observed. Free (A) and bound (liberated by acid (B) and alkaline (C) hydrolysis) fractions of phenolic acids were isolated. Thirteen phenolic acids were identified: ellagic, chlorogenic, homoprotocatechuic, protocatechuic, caffeic, gentisic, p-hydroxyphenylacetic, p-hydroxybenzoic, p-coumaric, vanillic, syringic, sinapic and ferulic, which occurrence was confirmed by literature data [2,8,15,17,20]. Additionally, new phenolic acids were detected: ellagic, homoprotocatechuic, gentisic, p-hydroxyphenylacetic, syringic, sinapic acids.

Caffeic, p-hydroxybenzoic, vanillic and ferulic acids were common, both in their free and bound state (in all fractions).

P-hydroxyphenylacetic, syryngic and sinapic acids were found exclusively after alkaline hydrolysis, which is likely to indicate that they occur only in ester conjugates with different compounds.

P-coumaric acid was found in fractions A and C (free and ester-bound state).

Caffeic acid, gentisic acid, p-hydroxybenzoic acid, p-coumaric acid, vanillic acid, sinapic acid and ferulic acid were detected in both plants. Chlorogenic and protocatechuic acids were found only in *Allium cepa* L. bulbs whereas homoprotocatechuic, p-hydroxyphenylacetic and syringic acids – only in *Allium sativum* L. bulbs. Ellagic acid detected in Allium cepa L. was both free and glycoside-bound. Otherwise, ellagic acid in *Allium sativum* L. was present only in the free fraction (A).

Seven major phenolic acids found in garlic and onion were quantified using RP-HPLC. Their amounts were within the range of 0.05–85.8 μg/g of fresh material (0.1–252.1 μg/g of dried material) and 0.05–5.8 μg/g of the fresh material (22.4–76.3 μg/g of dried material), respectively. The water

content determined using the FPVII standard method was 65.94% in raw *Allium sativum* L. bulbs and 92.4% in *Allium cepa* L.

Significant differences in quantitative proportions and forms of phenolic compounds were observed in the material studied, e.g. in *Allium sativum* L., the concentration of caffeic acid in fraction B (glycoside-bound) was found to be high; in *Allium cepa* L., on the other hand, its level in fraction B was lower whereas that in free state (fraction A) and in the ester-bound form (fraction C) higher. The concentrations of some phenolic acids were higher in hydrolyzed samples, suggesting that they are present in the plant material both in free and glycoside- or ester-bound forms.

It was shown that the raw bulbs of garlic (*Allium sativum* L.) had richer qualitative and quantitative composition of phenolic acids in comparison with the raw bulbs of onion (*Allium cepa* L.).

The fraction of unbound phenolics (As) in garlic contained about ten times higher amounts of p-coumaric acid *cis* (38.5 μg/g) and several times higher amounts of p-coumaric acid *trans* (12.8 μg/g), ferulic acid *cis* (14.8 μg/g) and ferulic acid *trans* (12.9 μg/g) compared to onion.

In the fraction B from *Allium sativum* L., high levels of homoprotocatechuic acid (85.8 μg/g) and caffeic acid *trans* (48.8 μg/g) as well as low levels of ferulic acid *cis* (0.4 μg/g) and ferulic acid *trans* (0.63 μg/g) were observed. In *Allium cepa* L., however, the fraction B contained lower amounts of the phenolic acids in question (with homoprotocatechuic acid lacking).

In the fraction C from *Allium sativum* L., ferulic acid *cis* was determined (4.3 μg/g) and a low amount of p-hydroxybenzoic acid (0.05 μg/g). The fraction C from *Allium cepa* L., high amounts of protocatechuic acid (1.3 μg/g) and p-hydroxybenzoic acid (0.8 μg/g) were found.

Recently we observe rapidly increasing world-wide interest in garlic and onion. Our study has supported the idea that the consumption of garlic and onion has beneficial effects on health. Additionally, garlic and onion are cheap and readily available in various parts of Poland. Therefore, we recommend their use for the treatment of upper respiratory tract infections and for prevention of arteriosclerosis, asthma and cancer.

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SUMMARY

The analysis of free and liberated (by acidic and alkaline hydrolysis) phenolic acids in raw garlic (*Allium sativm* L.) and onion (*Allium cepa* L.) bulbs was performed by 2D-TLC and RP-HPLC methods. 13 phenolic acids were identified: ellagic, chlorogenic, homoprotocatechuic, potocatechuic, caffeic, gentisic, p-hydroxyphenylacetic, p-hydroxybenzoic, p-coumaric, vanillic, syringic, sinapic and ferulic. Using the RP-HPLC, the content of 7 major phenolic acids was determined, which ranged from 0.05 to 85.8 μg/g of the fresh material (0.1-252.1 μg/g of dried material) in garlic (*Allium sativm* L.) and from 0.05 to 5.8 μg/g of the fresh material (22.4-76.3 μg/g of dried material) in onion (*Allium cepa* L.).

Keywords: Allium sativm L., *Allium cepa* L., phenolic acids, 2D-TLC and RP-HPLC analysis.

STRESZCZENIE

Przeprowadzono analizę wolnych i związanych (uwolnionych na drodze hydrolizy kwasowej i zasadowej) kwasów fenolowych w świeżych cebulach czosnku (*Allium sativm* L.) i białej cebuli (*Allium cepa* L.) za pomocą metod 2D-TLC i RP-HPLC. Zidentyfikowano 13 fenolokwasów: elagowy, chlorogenowy, homoprotokatechowy, protokatechowy, kawowy, gentyzynowy, p-hydroksyfenylooctowy, p-hydroxybenzoesowy, p-kumarowy, wanilinowy, syryngowy, synapinowy i ferulowy. Metodą HPLC określono zawartość 7 głównych fenolokwasów. Wynosiła ona odpowiednio dla czosnku (*Allium sativm* L.) od 0,05 do 85,8 µg/g świeżego surowca (0,1 – 252,1 µg/g suchej masy surowca), a dla cebuli (*Allium cepa* L.) od 0,05 do 5,8 µg/g świeżego surowca (22,4 – 76,3 µg/g suchej masy surowca).

Słowa kluczowe: Allium sativm L., *Allium cepa* L., kwasy fenolowe, analiza 2D-TLC i RP-HPLC.