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Antiradical Properties of Selected Alium Cultivars

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Abstract

Antioxidant capacity of edible parts and tunic of nine selected onion cultivars of yellow, red and white color was estimated in freshly harvested bulbs and after long-term winter storage. Phenolic substances such as total, phenylpropanoids, flavonols and anthocyanins were detected by spectrophotometric and HPLC methods. Radical scavenging activity was measured in tissue extracts and expressed as the percentage of DPPH neutralization. Great variability of phenolics was observed in the onion flesh as well as in the tunic, the level of them determined in the skin was considerably higher in comparison with the edible parts. Antiradical activity of the flesh was poor, however, in the tunic exceeded 60% and was correlated with content of total phenols. Five month storage did not affect either phenolic substances or RSA in onion tunic while in flesh the significant decrease of antiradical activity was noted.

Keywords: Onion; Phenolics; Antiradical activity

Introduction

Biological and medical functions of Alium species are, first of all, due to their sulphur compounds, such as S-alk(en)yl-Lcysteine sulphoxides, however, presence of dietary phenolics (mainly flavonoids) is also beneficial for human health [1]. According to great number of recent data quercetin and its glucosides predominate in onion bulbs [1-6]. In onion cultivars of red color anthocyanins, mainly derivatives of cvanidin and peonidin were identified [6] as well as those of delfinidin and petunidin [7]. These phenolic substances posses ideal chemical structure for antiradical activity associated with number of hydroxyl groups bounded to the aromatic ring and seem to be more effective antioxidants than vitamin C and E [8]. The aim of the present study was to compare antioxidative capacity of edible parts and skin of nine selected onion cultivars of yellow, red, and white color. The contents of phenolic compounds (total, phenylpropanoids, flavonols and anthocyanins) were determined as well as antiradical activity of onion extracts. Onion bulbs were analyzed after harvesting and after long-term winter storage to simulate the commercial conditions.

Material and Methods

The experiment was carried out in 2007-2008. Seven onion cultivars and two breeding lines were grown in Krakow area, Poland, in field conditions. All cultivars, used in the experiment were qualified as the long-day onion genotypes of different growing periods and different color of tunic (Table 1). Plants were produced in greenhouse from seeds obtained by the "Spojnia" Vegetable Plant Breeding and Seed Production Company and planted on 19th April 2007. During growth the common cultivation and protection procedure recommended for onion was applied.

After harvesting (the end of July) onion bulbs were kept for 2-3 weeks at low humidity and then taken for chemical analyses or stored for five months in cold chamber at 5-8°C at 70% humidity. Thirty bulbs were used as the laboratory mean sample. All analyses were carried out both in the edible parts of bulbs and in tunic. Phenolic substances (total, phenylpropanoids, flavonols and anthocyanins) were determined by the spectrophotometric method given by Fukumoto and Mazza [9] based on UV/VIS absorption measurements. Chlorogenic acid, caffeic acid and quercetin were used as the standars for total phenols, phenylpropanoids and flavonols, respectively.

Anthocyanin content was expressed as the cyanidin, according to its molar extinction. Additionally, phenolics were estimated by the HPLC chromatography. Chromatographic determination of concentration of phenolic compounds was performed using gradient HPLC analysis method on chromatograph Shimadzu LC-10AS with detector SPD-10AV, on a Li Chrospher 125 mm RP18 (5 µm) column, in the watermethanol system, with the addition of H_3PO_4 . The measurements were executed at two wavelengths: 265 nm and 325 nm for all samples [10]. Radical scavenging activity (RSA) was detected by method with DPPH stable radical and expressed as the percentage of its neutralization [11]. For phenolic compounds and RSA measurements tissue extracts either 25% (flesh) or 2% (skin) in 80% methanol were prepared. According to dry matter values [12] 25% (Figure 1) extracts of fresh bulb tissue corresponded to 2% those of the tunic, hence, comparison of their antioxidative properties was possible. All analyses were made in three replications and the results were statistically verified using Duncan's test for significance α =0.05.

Results

Phenolic substances

The level of total phenols determined in the onion flesh after harvesting, ranged in most cases between 70-80 mg•100g⁻¹FW, however, in red bulbs of Wenta F_1 and Scarlet F_1 as well as in those of yellow Efekt exceeded 100 mg•100g⁻¹FW (Table 1). Contents of individual groups of phenolics were distinctly differentiated: the lowest and the highest contents of cinamic acid derivatives (phenylpropanoids) were observed in white Alibaba and yellow Efekt (8.3 and 22.8 mg•100g⁻¹FW, respectively), flavonols ranged between 9.8 (Alibaba) and 38.6 (red Scarlet F_1) while anthocyanin level was poor and higher than 10

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Figure 1: Chromatogram of flesh extract of purple onion (Wenta cultivar), A – caffeic and chlorogenic acids; B – flavonoid glycosides; C – free flavonoids. Concentration of extract – 25%.



mg•100g⁻¹FW was determined in red bulbs (Noe 8, Wenta F_1 , Scarlet F_1) and in yellow Efekt.

Phenolic constituents, detected by HPLC method in edible part of harvested onion were divided into caffeic and chlorogenic acids (A), flavonoid glycosides (B), free flavonoids (C) and expressed in caffeic acid, rutine and quercetine equivalents for A, B and C, respectively (Table 2). Chlorogenic and caffeic acids differed from 0.27 to 1.48 mg•100g⁻¹FW for Zorza and Wenta, respectively. Great variability was observed also in the case of flavonoid glycosides (9.32-21.24 mg•100g⁻¹FW for LR/2 and Wenta) while free flavonoid level did not differ considerably regarding cultivars.

Very high content of phenolic substances was found in tunic of

examined cultivars, since 1010 (white Alibaba) to 6697 mg•100g⁻¹FW (red Scarlet F₁). Similarly, the great variability in the individual groups of phenolics was observed. The level of phenylpropanoids ranged between 276 to 1735 mg•100g⁻¹FW (Alibaba and Efekt, respectively), that of flavonols was lowest in Alibaba (399 mg•100g⁻¹FW) and highest in Scarlet F₁ and in Efekt (2529 and 2505 mg•100g⁻¹FW). Content of anthocyanins was much lower as compared to the other phenolics and only in the tunic of red bulbs exceeded 300 mg 100g⁻¹FW (Noe 8, Wenta, Scarlet F₁) (Table 3).

According to HPLC analysis of onion tunic phenolic compounds were calculated more precisely into caffeic and chlorogenic acid derivatives (A), flavonoid glycosides (B) and free flavonoids (C) (Table 4). In harvested onion the content of the first group compounds (A) was ranged between 115.5 and 339.5 $mg \cdot 100g^{-1}FW$ (red skin Wenta and yellow Efekt). The great variability was observed in flavonoid glycosides (483.0-1142.5 $mg \cdot 100g^{-1}FW$, Zorza and Noe 8) as well as in free flavonoids (531.7-1579.6 mg $100g^{-1}FW$ – Wenta and Efekt (Table 4).

The changes of phenolic constituents determined in the edible parts of onions affected by the long-term storage were, in most cases nonsignificant (Table 1). The slight increase of total phenols was observed in flesh of LR/2 and Scarlet F_1 while the level of these compounds declined in the bulbs of Zorza cultivar. Similarly, the contents of individual phenolic groups rose slightly in LR/2, Scarlet F_1 and Kristine cultivars, the opposite effect was noticed in the case of Zorza (Table 1). Contents of individual groups of phenolics determined by HPLC in onion flesh did not change significantly after long-term storage (Table 2).

Reaction of onion tunic to five month winter storage seems also to be negligible, however, the slight decrease of phenolic compounds (total and those of individual groups) was observed in skin of Zorza and Alibaba. The level of all phenolics increased after storage in the tunic of Wenta (Figure 2). Increase of anthocyanins was also observed in Noe 8 breeding line (Table 3). HPLC analysis showed, in most cases, decrease both in A and B groups of phenolics. The slight decrease was also noted in flavonoid glycosides (B), excepting the profound increase for red Noe 8 and yellow Efekt cultivars. The level of free flavonoid slightly rose during the storage period, in red skin of Wenta the considerable increase was observed, however, in the yellow skin of Kristine content of these constituents dropped down (Table 4).

Radical scavenging activity

Radical scavenging activity measured in the edible parts of harvested onion bulbs was relatively low, however, varied since 5.4% (white Alibaba) to 18.5% (red Wenta). In flesh of all the red bulbs (Noe 8, Wenta, Scarlet F_1) and in the yellow Efekt RSA was higher than 10% (Table 1). Antiradical activity in onion skin determined after harvesting was much higher in comparison with that of flesh, except for Alibaba cultivar (2.6%). The highest values (over 60%) of this parameter were observed in tunic of red bulbs of Wenta and Scarlet F_1 as well as in the yellow Efekt (Table 3). Winter storage caused the significant decrease in RSA of flesh in all the cultivars. Concerning the tunic its high antiradical activity was maintained during storage (Table 1 and 3).

Discussion

Contents of phenolics found in the edible parts of onion, both total and those of the individual groups, were considerably differentiated. The level of total phenols (88 mg•100g⁻¹FW on average) determined

Cultivar / Skin colour		Phenolic Compounds	Antiradical Activity [%]		
	Total Phenols	Phenyl-propanoids	Flavonols	Anthocyanins	DPPH [•] neutralization
Alibaba / white					
ha	vest 78.5 cd*	8.3 a	9.8 a	6.6 abcd	5.4 a
sto	rage 82.3 d	8.8 a	10.7 a	8.3 def	4.5 a
Efekt / yellow					
ha	vest 105.0 efg	22.8 ef	37.6 f	10.7 gh	10.7 ef
sto	rage 99.5 e	21.1 e	36.7 ef	10.5 gh	5.4 a
Kristine / yellow					
ha	vest 99.7 e	17.5 d	30.3 d	7.4 bcde	8.1 bcd
sto	age 106.5 efg	23.2 ef	40.2 fg	10.0 fgh	6.3 ab
Tęcza / yellow					
ha	vest 65.9 b	13.3 b	23.1 c	5.0 a	8.3 cd
sto	rage 73.5 bc	14.4 bc	22.3 c	7.9 cde	5.3 a
Zorza / yellow					
ha	vest 77.9 cd	15.0 cd	24.9 d	8.6 efg	8.3 bc
sto	rage 53.6 a	10.1 a	15.8 b	6.0 ab	4.6 a
LR/2 (breeding line) / yellow					
ha	vest 78.4 cd	16.2 bcd	29.3 d	6.3 abc	10.0 de
sto	age 103.8 efg	21.8 e	38.4 fg	10.0 fgh	6.3 ab
Scarlet F ₁ / purple					
ha	vest 112.0 g	21.8 e	38.6 fg	11.1 hi	15.2 h
sto	rage 122.4 h	25.0 f	42.9 g	13.9 j	11.9 fg
Wenta / purple					
ha	vest 101.6 ef	15.8 bcd	32.0 de	10.6 gh	18.5 i
sto	age 108.5 fg	22.3 ef	37.0 f	12.6 ij	10.7 ef
NOE 8 (breeding line) / purple					
ha	vest 80.9 cd	16.4 cd	30.9 d	10.7 gh	13.6 gh
sto	age 78.0 cd	16.9 cd	29.4 d	10.7 ghi	10.2 ef

alues marked with the same letter within columns do not differ significantly at α =0.05

Table 1: Level of phenolics compounds and antiradical activity in flesh of examined onion bulbs.

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	Phenolic Compounds [mg•100g-1 FW]			
Cultivar / Skin colour	Caffeic and chlorogenic acid (A)	Flavonoid glycosides [as rutine equivalent] (B)	Free flavonoids [as quercetin equivalent] (C)	
Alibaba / white				
harvest	-	-	-	
storage	-	-	-	
Efekt / yellow				
harvest	1.43 h*	15.37 bcd	3.90 ab	
storage	0.67 d	14.74 bcd	2.79 a	
Kristine / yellow				
harvest	1.06 f	16.23 bcd	4.18 abc	
storage	1.06 f	14.76 bcd	2.92 ab	
Tęcza / yellow				
harvest	0.40 c	13.35 abc	4.65 abc	
storage	0.37 bc	10.20 abc	4.77 abc	
Zorza / yellow				
harvest	0.27 b	11.52 abc	6.33 c	
storage	0.46 c	6.89 a	2.98 ab	
LR/2 (breeding line) / yellow				
harvest	0.76 de	9.32 ab	3.74 ab	
storage	0.81 e	12.23 abc	3.84 ab	
Scarlet F, / purple				
harvest	-	-	-	
storage	1.27 g	29.87 e	3.34 ab	
Wenta / purple				
harvest	1.48 h	21.24 d	4.02 abc	
storage	1.15 f	17.08 cd	5.19 bc	
NOE 8 (breeding line) / purple				
harvest	0.37 bc	10.59 abc	4.43 abc	
storage	0.05 a	13.03 abc	3.43 ab	

*values marked with the same letter within columns do not differ significantly at α =0.05

Table 2: Level of phenolics compounds measured by HPLC in flesh of examined onion bulbs.

in the present study was, in general, higher in comparison with recent reports. Roldan-Marin et al. [2] determined 439 mg•100g⁻¹DW of total phenols, expressed as the chlorogenic acid, and Santas et al. [13] in the flesh of white onion found 2.57-6.53 mg•g⁻¹DW. The level of total phenols in freeze dried onion bulbs, reported by Nuutila et al. [14] was 800-3200 mg•kg⁻¹expressed as gallic acid equivalent. Very high level of total phenols of the onion tunic, corresponded with the results obtained by Nuutila et al. [14] which determined 2600 and 8000 mg•100g⁻¹ in the yellow and red skin, respectively, expressed as the gallic acid. In the present experiment the lowest content of phenolics was found in white onion skin (1000 mg•100g⁻¹), in the yellow one 4000-5000 mg•100g⁻¹, while in the red skins as well as in that of dark-yellow exceeded 6000 mg•100g⁻¹.

Among individual phenolics flavonoids and phenolic acids are the most dominant components of onion, responsible for the protection against free radical damage [15]. Bonaccorsi et al. [3] identified 7 flavonols, among them quercetin-3,4'-diglucoside and quercetin-4'glucoside represented 90% of flavonols. Similarly, Slimestad [5] and Rodrigues [6] considered these two compounds as the main paleyellow flavonols of onion. According to Nuutila et al. [14] quercetin and kaempferol were most abundant flavonols in the hydrolysed samples of onion.

The content of flavonols depended strongly on cultivar and varied from the very low level (7 mg kg⁻¹ of white onion) to 600-700 mg kg⁻¹ with the red and gold varieties [3]. Slimestad [5] found 35-159 mg•100g⁻¹ FW of total flavonols while in the present experiment flavonols ranged between 9.8 (white onion) to 38.6 mg 100g⁻¹ FW (red onion).

The very high level of flavonols in the onion tunic was reported by Nuutila et al. [14]: the dry skin of the red and yellow varieties contained 27-30 g•kg⁻¹FW and 10-17 g•kg⁻¹FW of total quercetin. In the present study the flavonol content determined in the dry skin was much lower, however, distinctly differentiated between 399 mg•100g⁻¹ FW (white onion) to over 2500 mg•100g⁻¹ (red and yellow cultivars). Among flavonoids free flavonoids dominated, followed by flavonoid glycosides.

According to Slimestad [5] 25 anthocyanins were detected in red onion, mainly derivatives of cyanidin, as well as those of peonidin, delphinidin and peonidin. Among them the 3-glucoside and 3-(3"-glucosylglucoside) of cyanidin and their malonylated derivatives

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	Phenolic Compounds [mg•100g-1 FW]				Antiradical Activity [%]
Cultivar / Skin Colour	Total Phenols	Phenyl-propanoids	Flavonols	Anthocyanins	DPPH' neutralization
Alibaba / white					
harvest	1010 a*	276 a	399 a	170 b	2.6 a
storage	771 a	111 a	256 a	83 a	2.5 a
Efekt / yellow					
harvest	6273 gh	1735 gh	2505 f	157 b	67.9 j
storage	6341 h	1736 gh	2322 f	163 b	62.5 hi
Kristine / yellow					
harvest	5106 de	1395 de	1896 e	157 b	58.9 gh
storage	5546 ef	1505 ef	1818 de	144 b	52.1 de
Tęcza / yellow					
harvest	5022 de	1371 cde	1596 cd	154 b	46.0 c
storage	4428 bc	1192 bc	1367 bc	128 ab	41.7 b
Zorza / yellow					
harvest	4674 cd	1262 cd	1370 bc	153 b	41.8 b
storage	3934 b	1065 b	1248 b	137 b	41.2 b
LR/2 (breeding line) / yellow					
harvest	5462 ef	1513 ef	1964 e	160 b	52.6 de
storage	5413 ef	1464 ef	1844 e	154 b	49.7 cd
Scarlet F ₁ / purple					
harvest	6697 hi	1760 gh	2529 f	355 d	62.4 hi
storage	7076 i	1809 h	2517 f	378 de	63.9 i
Wenta / purple					
harvest	6212 gh	1582 fg	1963 e	367 de	63.0 i
storage	7936 j	2079 i	2540 f	493 f	66.1 ij
NOE 8 (breeding line) / purple					
harvest	5428 ef	1370 cde	1952 e	304 c	57.1 fg
storage	5742 fg	1517 ef	2330 f	409 e	53.7 ef
	1		1	1	

*values marked with the same letter within columns do not differ significantly at $\alpha\text{=}0.05$

Table 3: Level of phenolics compounds and antiradical activity in tunic of examined onion bulbs.

dominated. Rodrigues [6] identified six anthocyanins in red onion variety being derivatives of cyanidin. In bulbs of Tropea red onion cyanidin derivatives constituted over 50% of the total anthocyanins, accompanied by delphinidin and petunidin [7]. In the edible part of red onion Gennaro et al. [7] found 90.5 mg kg⁻¹FW of total anthocyanins (delphinidin3-glucosylglucoside, cyanidin3-6"malonylglucoside, and cyanidin3-6"malonyl-3"glucosylglucose), in the present study the similar level of anthocyanins was observed in the flesh of red cultivars (slightly over 10 mg•100g⁻¹FW). In dry skin of red onion Gennaro et al. [7] identified 8881 mg•kg⁻¹FW of total anthocyanins, while in the present study their content was poorer and hardly exceeded 300 mg•100g⁻¹FW.

Radical scavenging activity, measured in the onion flesh was poor, slightly higher values (over 10% of DPPH neutralization) were observed only in red bulbs. Similarly, Nuutila et al. [14] found red onion edible parts more active than yellow ones. Shon et al. [15] who compared free radical neutralization of flesh extracts of onions noted the highest RSA in red bulbs followed by yellow and white ones. Santas et al. [13] found, however, high antioxidant activity in the edible parts of white onion extracts, exceeding even in some cases 80 µmol of TROLOX•g⁻¹DW.

Antiradical activity of the skin was considerably higher and in the case of two red and one yellow varieties exceeded 60%. Nuutila et al. [14] also reported the highest RSA in skin extracts in comparison with those of flesh particularly in red onions. According to Corzo-Martinez et al. [1] the outer layers of onion, usually wasted before food processing are rich in flavonoid substances effective as antioxidants. The antioxidant effect of onion extract against DPPH is mostly due to their phenolic hydroxyl groups [15]. The dry skin of red onion, being only 2% of the total weight, contains about 63% of the total anthocyanins, the outer fleshy layer usually discarded after peeling is particularly rich in cyanidin derivatives [7]. Nuutila et al. [14] found correlation between high radical scavenging activity and high amounts of total phenolics and flavonoids in the onion extracts. In their study the DPPH activity of yellow onion extracts ranged between 20-90% and gave a linear response $R^2 = 0.99$, similarly to those of quercetin and myricetin. Roldan-Marin et al. [2] found the significant inverse correlation (R²=0.71) between total phenols of onion and EC₅₀ antioxidant parameter. Positive correlation between flavonoids and antioxidant capacity in yellow onion variety was reported also by Santas et al. [16]. In the present study higher RSA was in most cases accompanied by the higher contents of both total phenolics as well as those of their individual groups. Regression coefficient calculated for total phenolics and RSA for the whole onion bulbs was 0.98. This value, calculated separately for tunic and flesh was 0.96 and 0.42, respectively.

	Phenolic Compounds [ma•100a-1 FW]				
Cultivar / Skin colour	Caffeic and chlorogenic acid (A)	Flavonoid glycosides [as rutine equivalent] (B)	Free flavonoids [as quercetin equivalent] (C)		
Alibaba / white					
harvest	-	-	-		
storage	-	-	-		
Efekt / yellow					
harvest	339.5 i*	579 ab	1579.0 g		
storage	257.5 h	1024.5 cd	1626.5 g		
Kristine / yellow					
harvest	329.5 i	627.5 b	1012.5 e		
storage	197.0 fg	531.0 ab	739.5 bc		
Tęcza / yellow					
harvest	200.5 fg	639.0 b	879.5 cde		
storage	122.5 ab	638.5 b	909.0 de		
Zorza / yellow					
harvest	169.5 de	483.0 ab	608.5 ab		
storage	211.0 g	403.5 a	799.5 cd		
LR/2 (breeding line) / yellow					
harvest	129.0 abc	653.5 b	657.0 ab		
storage	179.0 ef	473.5 ab	821.0 cd		
Scarlet F ₁ / purple					
harvest	-	-	-		
storage	250.0 h	1048.5 cd	1595.5 g		
Wenta / purple					
harvest	115.5 a	891.5 c	531.0 a		
storage	141.0 bc	454.5 ab	1260.5 f		
NOE 8 (breeding line) / purple					
harvest	181.0 ef	1142.5 d	838.0 cd		
storage	150.9 cd	2190.0 e	998.5 e		

*values marked with the same letter within columns do not differ significantly at α =0.05

Table 4: Level of phenolics compounds measured by HPLC in tunic of examined onion bulbs.

Onion bulbs are the storage organs of reduced metabolic activity and the changes of their chemical composition during long term winter storage in commonly applied conditions were not very distinct. Total phenolics as well as those of individual groups detected either in edible part or in the tunic did not change significantly after 5 month cold storage (5-8°C, 70% RH), their slight increase observed in some cases might have been due to the possible dehydration of tissue. These results contradict those reported by Gennaro et al. [7] who observed drastic losses of whole anthocyanin content after 6 week storage of red onions in different temperature and humidity conditions (5°C/RH30%, 25°C/66%RH, 30°C/50% RH) reaching 64-73%.

In the present experiment the significant decrease of RSA affected by storage was observed in the onion flesh of all examined cultivars, however, not in the dry skin. Similarly, in the study of Gennaro et al. [7] during the storage of onion bulbs antioxidant activity declined parallely to anthocyanin decrease and was reduced by 29% and 36% at 5°C and warm ambient conditions, respectively. To sum up, the presented results show that great variability of antioxidant capacity as well as antioxidant content among onion cultivars was mostly due to the color of bulbs. Cultivars of red and dark-yellow skin seemed to be a better source of health-promoting substances (phenolics) responsible for neutralization of the active oxygen species. The consumption of these onions can provide functional compounds necessary in human diet.

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