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CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN SELECTED VARIETIES OF ONION (*ALLIUM CEPA* L.)

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ABSTRACT

Onion (*Allium cepa* L.) is one of the most important vegetable crops widely consumed in the world. The bulb onion is grown as fresh shoots and as bulbs for consuming uncooked, cooked, and pickled or production of seed and sets. They can be eaten raw, boiled, steamed and roasted. Onion has high nutritional value. Onions are a good source of vitamins, minerals and major component like polyphenols, flavonoids, fructooligosaccharides, thiosulfinates and other sulfur compounds. Polyphenols belong to significant antioxidants in human diet. Antioxidant compounds (polyphenols, flavonoids) scavenge free radicals, inhibit the oxidative mechanisms that lead to degenerative diseases. Antioxidant compounds in food play an important role as a health protecting factor. Onions have a wide range of beneficial properties for human health, such as anti-cholesterolaemic, anti-mutagenic and antioxidant capacity. In this work we evaluated content of total polyphenols and antioxidant activity in selected varieties of onion. Samples of plant material we collected at the stage of full maturity in the locality of Pružina. Pružina is locality without negative influences and emission sources. Total polyphenols content and antioxidant activity were measured in six varieties of onion, namely in red variety (Red matté), in yellow varieties (Boston, Bingo, Sherpa) and white varieties of onion (Diamond, White dry). The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). Antioxidant activity was measured by using a compound DPPH˙. In the present experiment it was detected, that total polyphenols content in samples ranges from 142.01 $mg.kg^{-1}$ GAE (white variety of onion- White dry) to 1083.04 $mg.kg^{-1}$GAE (red variety of onion- Red matté). Statistically significant highest value of total polyphenols (1083.04 $mg.kg^{-1}$ GAE) was recorded in variety Red matté. Another indicator that has been evaluated and compared was the antioxidant activity. The values of antioxidant activity were in interval from 7.74% to 41.67%. Based on the measured values of antioxidant activity in onion the samples can beclassified as follows: Red matté (41.67%) > Boston (25.77%) > Sherpa (24.34%) > Bingo (19.54%) > White dry (8.10%) > Diamond (7.74%).

Keywords: onion; variety; color; polyphenols; antioxidant activity

INTRODUCTION

Onions are one of the oldest vegetables in continuous cultivation dating back to at least 4,000 BC. Onion (*Allium cepa* L.) is botanically included in the *Liliaceae* and species are found across a wide range of latitudes and altitudes in Europe, Asia, North America and Africa. World onion production has increased by at least 25% over the past 10 years with current production being around 44 million tonnes making it the second most important horticultural crop after tomatoes **Griffiths et al., (2002)**.

Onions are grown mainly as food materials. They are highly valued for their flavour and for their nutritional value. Onion bulb (red, white or yellow in colour) is consumed in its tender state, raw, ripe, pickled or in form of powder. Onion can grow on most soil types. However, well-drained medium textured soils with pH 6-7 are particularly good for the crop. Flat land enhances good yield. Varieties of onion can differ for pungency, sugar content, disease resistance, seed stem formation, double centers, bulb shape, and bulb size.

Onions have a high content of hydrophilic vitamins (vitamin C, B, A) and a lipophilic vitamin (vitamin D and E). Onion is a source of minerals such as iron, selenium, iodine, potassium, calcium, sulfur, and many others. Onion is characterized by not only rich in vitamins and minerals, but is characterized by a strong content of biologically active substances, especially polyphenolic compounds (flavonoids, quercetin, rutin) and phenolic acids (cinnamic acid derivatives and benzoic acid), fructooligosaccharides (FOS), thiosulfinates and other sulfur compounds.

Allium species are referred to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries **Benkeblia (2005)**.

Polyphenols are compounds possessing one or more aromatic rings with one or more hydroxyl groups. Polyphenols are secondary metabolites of plants, currently known more than 8000 phenolic structures ranging from simple molecules (phenolic acids) to highly polymerized

substances (tannins). Phenolics are generally involved in protection against ultraviolet radiation, by pathogens, parasites and predators **D´Archivio et al., (2007)**. Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basisof structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes and lignans **Spencer et al., (2008)**.

Polyphenols are directly involved in the response of plants to different types of stress: they contribute to healing of damaged areas possess antimicrobial properties, and their concentrations may increase after infection **(Parr and Bolwell, 2000)**.

Another factor that directly affects the polyphenol content of the foods is storage. Studies have proved that polyphenolic content of the foods change on storage, the reason is easy oxidation of these polyphenols **Manach et al., (2004)**.

Epidemiological studies suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases **(Pandey and Rizvi, 2009)**. Polyphenols are characteristic in an antioxidant and anti-inflammatory effect.

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants is found in a number of dietary sources **Qusti et al., (2010)**. The main characteristic of antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and inhibit the oxidative mechanisms that lead to degenerative diseases **Prakash et al., (2007)**. The aim of our study was to evaluated content of total polyphenols and antioxidant activity in selected varieties of onion (*Allium cepa* L.).

MATERIAL AND METHODOLOGY

The experiment was established in the year 2014 in the area of Pružina block methods (seeds of varieties of onion was planted by hand to 4 lines, size of the experimental flat- 1 m^2). Samples of plant material were collected at full maturity stages from area of Pružina. The samples of soil (Table 1) and plant material were analyzed individually by selected methodologies, and we used fresh material for analysis. Pružina is located under the Strážovské hills – Strážov. The attitude of the village is in the middle of 381 m.a.s.l. Pružina belongs to the mild cold climate zone, average annual temperature is 7 °C, annual rainfall is 800-1000 mm. Pružina is area without negative influences, emission sources (carbon), relatively pure from point of view of content permissible forms of risk elements.

We determined the soil samples from Pružina as sandy – loam, loam. The soil samples had a value of active soil reaction pH (H_2O) = 7.95. The soil was strongly alkaline. Cox oxidizable carbon content was 1.46 and the humus content was 2.51. The content of potassium and magnesium was high; the content of phosphorus was very high. The total content of heavy metals (aqua regia) in soil sample was determined according to the current legislation Slovak republic (Slovak decree no. 220/2004 Coll., Annex. 2) Cadmium exceeded the limit value of 1.91-times, Zinc exceeded the limit value 2.03-times.

Sample preparation

Samples of fresh onion were homogenized and we prepared an extract: 25 g cut onion extracted by 50 mL 80% ethanol for sixteen hours. These extracts were use for analyze.

Characteristic varieties of onion:

Red matté is red variety of onion. Red matté can be grown from seed or stecklings. It is suitable for consumption and storage.

Diamond is white variety of onion. It is suitable for consumption, industrial processing and short-term storage.

White dry is white variety of onion, suitable for consumption and short-term storage.

Boston is yellow variety of onion. Boston is suitable for consumption and longer storage.

Sherpa is yellow variety of onion. This variety is suitable for consumption and storage. Sherpa can be grown from seed and stecklings.

Bingo is yellow variety of onion, suitable for consumption and longer storage. Onion is medium, firm and round. The yield of this variety in optimal conditions is large.

Table 1 Agrochemical characteristic of soil substrate in mg.kg^{-1} (Pružina).

Agrochemical characteristic	**pH(H_2O)**	**pH(KCl)**	**C_{ox} (%)**	**Humus**				
	7.95	7.18	1.46	2.51				
Nutrients	**P**	**K**	**Ca**	**Mg**				
	259.78	354.40	8049.30	439.60				
Heavy metals	**Cd**	**Pb**	**Cu**	**Zn**	**Cr**	**Co**	**Mn**	**Fe**
Content in aqua regia (mg.kg^{-1})	1.34	29.40	36.90	305.30	21.00	13.3	636.50	25696.4
Limit value (mg.kg^{-1})	**0.7**	**70**	**60**	**150**	**70**	**15**	**-**	**-**

Note: *Limit value for Aqua raegia – Slovak decree no. 220/2004 Coll.

Determination of total polyphenols

Total polyphenols were determined by the method of **Lachman et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay (Merck). The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 mL of extract. The content was mixed and 5 mL of sodium carbonate solution (20%) (Sigma Aldrich) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wave length against blank (Shimadzu UV/VIS-1240, Japan). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid (Sigma Aldrich).

Determination of antioxidant activity

Antioxidant activity was measured by the **Brand and Williams et al., (1995)** method-using a compound DPPH˙ (2.2-diphenyl-1-pikrylhydrazyl)). 2.2-diphenyl-1-pikrylhydrazyl (DPPH˙) was pipetted to cuvette (3.9 m^3) then the value of absorbance, which corresponded to the initial concentration of DPPH˙ solution in time Ao was written. Then 0.1 cm^3 of the followed solution was added and then the dependence A = f(t) was immediately started to measure. The absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer Shimadzu UV/VIS-1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH˙ radical at the given time.

Inhibition (%) = (Ao - At / Ao) x 100

Antioxidant activity was measured by the **Brand and Williams et al., (1995)** method-using a radical DPPH˙ (2.2-diphenyl-1-pikrylhydrazyl) (Merck). 2.2- diphenyl-1- pikrylhydrazyl (DPPH˙) was pipetted to cuvette (3.9 m^3), then the value of absorbance was written, which corresponded to the initial concentration of DPPH˙ solution in time Ao. Then 0.1 cm^3 of onion extract was added. Solution in the cuvette was mixed and then was immediately started to measure the dependence A = f (t). The absorbance after 1, 5 and 10 minutes was measured at 515.6 nm in the spectrophotometer (Shimadzu UV/VIS -1240). The percentage of inhibition reflects how antioxidant compound are able to remove DPPH˙ radical at the given time. Inhibition (%) = (Ao - At / Ao) x 100

Statistical analysis

Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0% LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA).

Table 2 Average content of total polyphenols ($mg.kg^{-1}$) in selected varieties of onion.

vegetable	variety	TPC ($mg.kg^{-1}$)
onion	Boston	441.32 ±26.29 [b]
	Sherpa	455.22 ±44.86 [b]
	Bingo	451.71 ±38.21 [b]
	Red matté	1083.04 ±56.03 [c]
	Diamond	160.49 ±7.30 [a]
	White dry	142.01 ±15.91 [a]
HD 95%	$HD_{0,05}$	52.9127
HD 99%	$HD_{0,01}$	72.4949

Legend: *Multiple Range Tests, Method: 95,0 percent LSD, Different letters (a, b, c,) between the factors show statistically significant differences (p <0.05) – LSD test, TPC- total polyphenols content.

Table 3 Average values of antioxidant activity (% inhibition) in selected varieties of onion

vegetable	variety	AOA (% inhibition)
onion	Boston	25.77 ±0.29 [d]
	Sherpa	24.34 ±0.39 [c]
	Bingo	19.54 ±0.98 [b]
	Red matté	41.67 ±0.70 [e]
	Diamond	7.74 ±0.69 [a]
	White dry	8.10 ±0.31 [a]
HD 95%	$HD_{0,05}$	0.949236
HD 99%	$HD_{0,01}$	1.30053

Legend: *Multiple Range Tests, Method: 95.0% LSD, Different letters (a, b, c, d, and e) between the factors show statistically significant differences (p <0.05) – LSD test AOA- antioxidant activity.

RESULTS AND DISCUSSION

Onion is recognized as one of the most important onion vegetable in our diet for its content of polyphenols compounds, antioxidants with beneficial effect on the human body.

Onions are very low in calories and fats. 100 grams carry just 40 calories. However, onions are rich in soluble dietary fiber. Onion is rich source of chromium and mineral manganese, vitamin C, B- complex group of vitamins. It is also good source of antioxidant polyphenols, which has anti-carcinogenic, anti-inflammatory, and anti-diabetic functions**.**

In this work the content of total polyphenols in selected varieties of onion was evaluated. Total polyphenols content was measured in six varieties of onion, namely in red variety (Red matté), in yellow varieties (Boston, Bingo, Sherpa) and white varieties of onion (Diamond and White dry). Onions are grown and distributed in three colors- white onion (5%), red varieties of onion (8%) and yellow onions (88%).

In the present experiment it was detected, that total polyphenols content in samples ranged from 142.01 $mg.kg^{-1}$GAE (in white variety of onion- White dry) to 1083.04 $mg.kg^{-1}$ GAE (in red variety of onion- Red matté) (Table 2). Statistically the highest value of total polyphenols (1083.04 $mg.kg^{-1}$ GAE) was recorded in variety of Red matté. The lowest content of total polyphenols was recorded in white variety of White dry (142.01 $mg.kg^{-1}$ GAE) and in white variety of Diamond (160.49 $mg.kg^{-1}$ GAE). In the case of yellow varieties of onion Sherpa (455.22 $mg.kg^{-1}$ GAE), Bingo (451.71 $mg.kg^{-1}$GAE) and Boston (441.32 $mg.kg^{-1}$ GAE) lower levels of polyphenols than in red variety of onion (Red matté) were measured. Our results correspond to the results of **Armand et al., (2012)**, which reported the highest values of total polyphenols in variety of red onion (982.03 $mg.kg^{-1}$) yellow variety ($mg.kg^{-1}$) and white variety (280 $mg.kg^{-1}$). **Andrejiová et al., (2011)** also reported the content of total polyphenols in red onion 1088.51 $mg.kg^{-1}$, yellow onion 652.15 $mg.kg^{-1}$ and in white onion 105.19 $mg.kg^{-1}$. **Karadeniz et al., (2005)** reported that the polyphenols in onion was in the amount 536 $mg.kg^{-1}$. **Amin et al., (2013)** reffered that the content of polyphenols was 132.2 $mg.kg^{-1}$. **Brat et al., (2006)** published that the content of total polyphenols in onion was 761 $mg.kg^{-1}$. In comparision to our determined values of polyphenols their results were in similar interval. **Benkeblia et al., (2005)** reported that the highest content of polyphenols was in red onion (473 $mg.kg^{-1}$), followed by yellow variety (347 $mg.kg^{-1}$). **Apak et al., (2007)** reffered that the content of total polyphenols in yellow onion was 880 $mg.kg^{-1}$. In comparision to our measured values in yellow onion (455.22 $mg.kg^{-1}$ GAE – 441.32 $mg.kg^{-1}$ GAE) their results were higher. **Bystrická et al., (2014)** published that the content of polyphenols in yellow onion was 508.16 – 638.2 $mg.kg^{-1}$. In comparision to our determined values of polyphenols in yellow onion, their results were in similar interval.

Lu et al., (2011) found similar results in the evaluation of onions of different colors. From the results we can conclude that the highest content of total polyphenols we measured in variety of Red matté (1083 $mg.kg^{-1}$ GAE), followed by variety of Sherpa 455.22 $mg.kg^{-1}$ GAE. In variety of Red matté is the average content of 6.74-times higher than that of the variety Diamond. The lowest content of total polyphenols was recorded in variety of White dry (142.01 $mg.kg^{-1}$ GAE). Based on the measured values of total polyphenols in onion the samples can be classified as follows: Red matté (1083 $mg.kg^{-1}$GAE) > Sherpa (455.22 $mg.kg^{-1}$ GAE) > Bingo (451.71 $mg.kg^{-1}$ GAE) > Boston (441.32 $mg.kg^{-1}$ GAE) > Diamond (160.49 $mg.kg^{-1}$ GAE) > White dry (142.01 $mg.kg^{-1}$ GAE). The content of polyphenolic compounds in onion also can be affected by the type of variety and color of bulb of onion.

Another indicator that has been evaluated and compared was the antioxidant activity in selected varieties of onion. Antioxidant activity of onion is often associated with L-ascorbic acid and polyphenolic compound such as anthocyanins, quercetin, and rutin. Antioxidant activity was also determined in six varieties of onion (Red matté, Boston, Bingo, Sherpa, Diamond and White dry).

In the present work we found that, antioxidant activity in samples ranges from 7.74% to 41.67%. The DPPH method is frequently used to determine the antioxidant activity. DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. Statistically the highest value of antioxidant activity (41.67%) was recorded in red variety- Red matté. The lowest value of antioxidant activity (7.74%) was recorded in white variety- Diamond. In the case of yellow varieties of onion Sherpa (24.37%), Bingo (19.54%) and Boston (25.77%) lower levels of antioxidant activity than in red variety of onion (Red matté) but higher levels antioxidant activity than in white varieties of onion (Diamond, White dry) were measured. **Škerget et al., (2009)** published that the value of antioxidant activity in yellow onion was 35%. In comparision to our measured values in yellow varieties their results were higher. **Prakash et al., (2007)** publishedthat the value of antioxidant activity in red onion was 50.6% and in white onion 13.6%. In comparision to our measured values of antioxidant activity their results were in similar interval. **Nuutila et al., (2003)** reported that the value of antioxidant activity was in interval from 32.9% (yellow onion) to 44.5% (red onion). **Cheng et al., (2013)** determined that red onion extracts showed good antioxidant activity varying from 53.36% to 85.53%and better than in the yellow variety ranging from 52.32% to 72.25%. In comparision to our measured values their results were higher. **Kavalcová et al., (2014)** published that the value of antioxidant activity in yellow onion was 25.7%.

Based on the measured values of AOA in onion can be samples classified as follows: Red matte > Boston > Sherpa > Bingo > White dry > Diamond.

Polyphenols are natural substances in plants that are antioxidants with the potential to protect the human body from diseases.

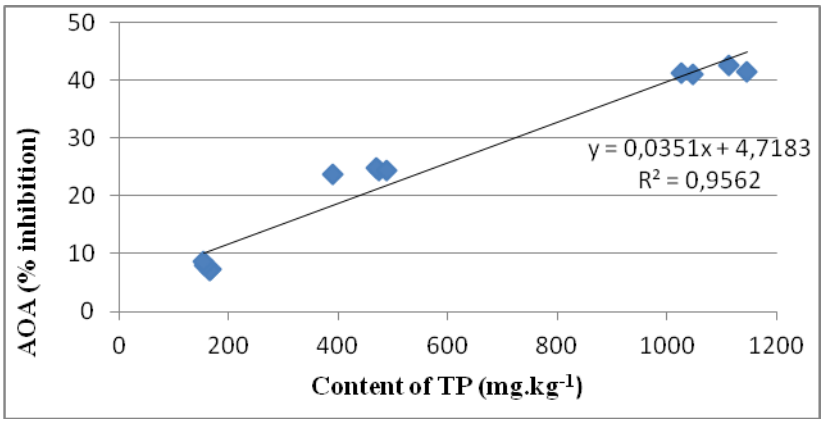

Figure 1 Relatioship between TP (total polyphenols) and AOA (antioxidant activity) (red, yellow and white onion).

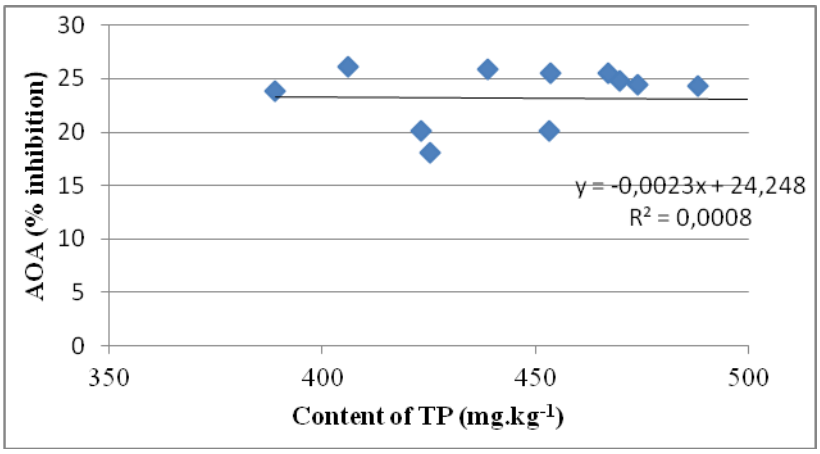

Figure 2 Relationship between TP (total polyphenols) and AOA (antioxidant activity) yellow onions (Sherpa, Bingo, Boston).

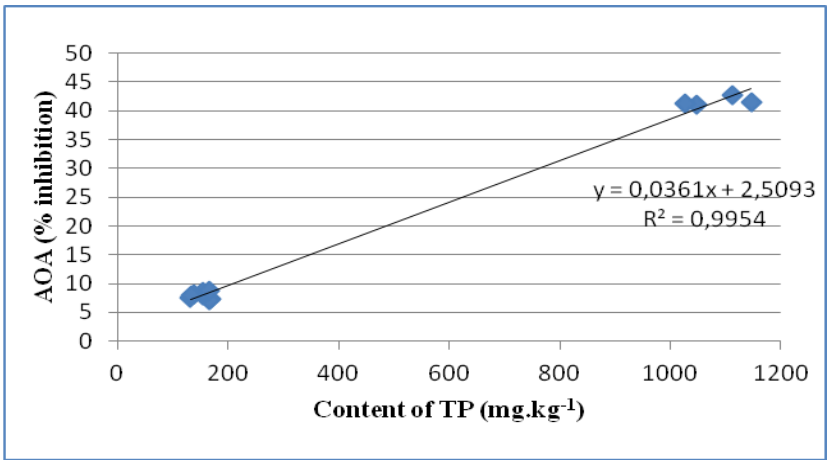

Figure 3 Relationship between TP (total polyphenols) and AOA (antioxidant activity) (Red matte, Diamond and White dry).

Between the content of total polyphenols and antioxidant activity in red, yellow and white onions we have found positive correlation. We can conclude that with increasing the content of total polyphenols also increased antioxidant activity (Figure 1). Several studies have reported a good correlation between the TP content of plant extracts and antioxidant activity. **Lu et al., (2011)** found the positive relationship between the content of total polyphenols and antioxidant activity. Most of the researches have mentioned that high phenolic content will lead to high radical scavenging activity **(Silva et al., 2007; Tawaha et al., 2007)**.

In the case of yellow varieties of onion, we have not found relationship between the content of total

polyphenols and antioxidant activity (Figure 2). **Mamamury (2002)** also not found positive relationship between TPC and AOA. Antioxidant activity depends not only on the total phenolic content but is significantly influenced by the structure of these phenolic compounds, in particular the position and the number of hydroxyl groups. The antioxidant activity of onion is often associated with a L-ascorbic acid, vitamin E and present polyphenolic compounds such as quercetin, rutin.

Red onion is rich in anthocyanins and yellow onion has high concentration of flavonoids (quercetin, kaempherol). We recorded the highest content of polyphenols and antioxidant activity was in red variety of onion (Red matté) (Figure 1 and Figure 3). Anthocyanins are known to have the most potent antioxidant effects. **Kong et al. (2003)** reported that for high antioxidant activity in red varieties of onion are responsible anthocyanins.

CONCLUSION

Onions are characterized by a high content of nutritionally valuable components, which are essential for the management of biochemical processes that positively affect the cardiovascular, cancer and other civilization diseases. The contribution focused on the total of polyphenol content and antioxidant activity in selected varieties of onion. The results suggest that statistically the highest value of total polyphenols and antioxidant activity was in red onion. In the case of white varieties of onion, we determined significantly the lowest values of total polyphenols and antioxidant activity. From the results we can conclude that more colorful varieties of onions have a higher content of polyphenols and a higher antioxidant activity. This statement is not always true, because not all polyphenols have antioxidant effects. Polyphenolic compounds in onion are quite variable, may be affected by type of variety, post-harvest, climatic condition, agrochemical composition of soil.

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