Total Phenolic Content and Antioxidant Capacity of Radish as Influenced by the Variety and Vegetative Stage

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ABSTRACT

This study investigates the influence of the variety and vegetative stage on the total phenolic content and antioxidant capacity of radish. Samples of seeds, sprouts (day-3, day-5 and day-7) and roots of three varieties (red, white and black) of radish (*Raphanus sativus*) were collected and tested for the above-mentioned parameters. Determination of total phenolic content was performed by Folin-Ciocalteau assay and antioxidant activity by DPPH assay. The total phenolic content ranged between 4.75 and 19.44 mg GAE/g DW and the antioxidant capacity between 12 and 75%. The highest total phenolic content and antioxidant capacity was found in radish sprouts and the lowest in radish roots, and among samples in the black radish variety.

Keywords: *antioxidant capacity, total phenolic content, radish, variety, vegetative stage***.**

INTRODUCTION

Several clinical and epidemiological studies have reported an inverse correlation between the consumption of fruits and vegetables and the occurrence of various diseases such as: as inflammation, cardiovascular disease, cancer and aging-related disorders (Soengas *et al.,* 2011). In the last decades, *Brassica* vegetables have been intensively investigated considering their potential health benefits. Different researches have shown that representatives of this genre improve the immune system, protect against allergies, and reduce the risk of cardiovascular diseases and different types of cancer (Kim *et al.*,2009; Van Horn *et al.*, 2008; Virgili*et et al.*, 2008).

Most of the studies on *Brassica* vegetables are related to their sulfur compounds (Traka and Mithen, 2009; Verkerk*et al.*, 2009), but they were also studied for their phenolic compounds, vitamins (A, C, E, and K), and minerals (Jahangir*et al.*, 2009).

The contribution of *Brassica* vegetables to health improvement has been partly associated with their antioxidant capacity, the phenolic compounds being the major antioxidants of these plants (Soengas*et al.,* 2011). It has been shown that the antioxidant capacity of *Brassica* vegetables is higher compared to other vegetable crops (Zhou *et al.*, 2006).

Radish *(Raphanus sativus)*, a common cruciferous vegetable, is one of the most popular vegetable in Romania. Radishes themselves are available in varieties that differ in terms and

contain different classes of biologically active phytochemicals (Hanlon and Barns, 2011).

The aim of this study was to investigate the influence of the variety and vegetative stage on the total phenolic content and antioxidant capacity of radishes that are commercially available in Romania, in order to highlight which variety and vegetative stage have the maximum antioxidant potential.

MATERIALS AND METHODS

Seeds. Samples of white and black radish seeds *(Raphanus Sativus* L. var. *white* and *niger)* were purchase from a Romanian trade company and samples of red radish seeds (*Raphanus Sativus-Red of Iernut)* were kindly provided by Minerva Heitz, the Manager of the Iernut Vegetable Research and Development Station (Mureş county). Seed samples were divided into three groups. Seeds from the first group were lyophilized, grinded in a fine powder and stored at refrigeration temperature (4°C) until further analysis. Seeds from the second group were germinated up to 7 days while seeds from the third group were cultivated in a greenhouse.

Sprouts. Seeds from the second group were germinated using Perez-Balibrea's *et al*. (2010) protocol with slight modification. Seeds (5 g) were rinsed in MillyQ water, immersed in 5 g/L sodium hypochlorite for 2 h, drained, then washed three times and soaked overnight in MillyQ water. After 16 h, the water was removed and the seeds were lined on the germination bed (trays with vermiculite, water and filter paper). The trays were placed in a growth chamber (SANYO MLR 350) under alternating 16 h lights (25 ºC) and 8 h (20ºC) darkness to simulate daylight conditions for 7 days. During the light cycle, photo synthetically active radiation of 400 µmol $*m^{-2}*s^{-1}$ was provided by 15 fluorescent lamps (FL40SS W/37).

Sprout samples were rapidly and gently collected at 3, 5 and 7 days of germination, then lyophilized, grinded in a fine powder and stored at refrigeration temperature (4°C) until further analysis.

Roots. Seeds from the third group were cultivated in a greenhouse near Alba Iulia following the label instructions provided by the producer for each variety. After harvesting, the radish roots were transported to the laboratory, then washed, lyophilized, grinded in a fine powder and stored at refrigerator temperature (4°C) until further analysis.

Dry matter and moisture. Moisture and dry matter content (%) were determined by lyophilization method using an Edwards freeze-dryer (Modulyo,West Sussex, UK) in all type of samples.

Germination test. The germination percentage was determined using the method described by the Romanian standard SR 1634:1999.

Extraction of bioactive compounds. All radish samples (seeds, sprouts and roots freeze-dried powders) were extracted with acidified methanol (0.826 ml of concentrated HCl in 1000 ml methanol). An amount of 500 mg freeze-dried powder was mixed with 10 ml acidified methanol, then sonicated for 20 min using an Bandelin Sonorex ultrasonic bath (RK100H, Berlin, Germany), and left to macerate overnight (~ 16 h). Further, the macerate was centrifuged using a Hettich centrifuge (EBA20, Tuttlingen Germany) for 20 min at room temperature and the supernatant was collected. The pellet was re-extracted twice with 10 ml of acidified methanol. All supernatants were combined and then evaporated using a Heidolph rotary evaporator (Laborota 4010, Schwabach, Germany). The dry extract was recovered in 3 ml methanol, transferred into Eppendorf tubes and then stored at -20ºC until further analysis.

Total phenolic content. The total phenolic content was determined using the modified Folin-Ciocalteau assay (Socaci*et al*., 2013). An aliquot of 0.1 mL of extract was mixed with 6 mL distilled water and 0.5 mL Folin-Ciocalteu reagent. After 4 min, 1.5 mL Na_2CO_3 solution (7.5%) was added and the sample was brought to a final volume of 10 mL with distilled water. The incubation was carried out at room temperature, in the dark, for 2 h and the absorbance was measured at 750 nm using a Shimadzu UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Kyoto, Japan). The blank was prepared by replacing the extract with distilled water. The results were calculated based on the gallic acid calibration curve ($r^2 = 0.9989$) prepared in various concentrations (0.25-1.25 mg/ml), and expressed in terms of mg of gallic acid equivalents (GAE)/g dry weight (DW).

Antioxidant capacity. The antioxidant capacity was assessed using the DPPH free radical scavenging assay according to Oms-Oliu *et al.*

(2009). An aliquot (10µL) of methanolic extract was mixed with 90 µL of distilled water and 3.9 ml methanolic DPPH solution (0.025 g/L), then incubated for 30 min in darkness. The absorbance was measured at 515nm against methanol as blank, using a Shimadzu UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu Scientific Instruments, Kyoto, Japan). Negative control was prepared with 10 µL methanol, 90 µL distilled water and 3.9 mL DPPH solution. Positive control was prepared with 10 µL gallic acid aqueous solution (0.5 mg/mL), 90 µL distilled water and 3.9 mL DPPH solution in methanol and was used as standard antioxidant. Positive and negative controls were treated the same way as the sample.

The DPPH free radical scavenging activity (RSA) was calculated using the following equation (1):

$$RSA\ [\%] = \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}} * 100 \qquad (1)$$

where:

Abs_{DPPH} is the absorbance of DPPH free radical solution in methanol;

Abs_{sample} is the absorbance of DPPH free radical solution mixed with sample/standard.

Statistical analysis.The effects of variety (V), vegetative stage (SV), and their first- degree interaction (V x VS) on total phenolic content and antioxidant capacity were performed by Analysis of Variance (ANOVA) test using the general linear model. Tukey's honest significance test was carried out at a 95% confidence level ($p< 0.05$). The percentage contribution of each factor and interaction was calculated using eta-square. The Pearson's correlation ($\alpha=0.05$) with two-tailed probability values was used to estimate the strength of association between total phenolic content and antioxidant capacity. Statistical analysis of the data was performed by Minitab Statistical software version 16.1.0 (LEAD Technologies, Inc.).

RESULTS AND DISCUSSION

Both dry matter and germination rate are important, especially from the economic point of view because they help in establishing the yield. Also by knowing these parameters, daily intake values can be calculated.

Regarding the germination rates, as presented in table 1, white radish registered germination rates above 95 % at all-timepoint sample collection, reaching a 98% at day 7 sprouts. Black radish had a low germination rate, reaching only 64.5% at day 7 sprouts. This low germination rate may be explained by the quality of the seeds and by the the fact that black radish is a winter variety and has a longer vegetation stage.

The statistical analysis showed that the two factors taken into study and their first- degree interaction (Table 2) had a significant effect on total phenolic content, as well as on antioxidant capacity; the vegetative stage was the most influential factor (93.3% for total phenolic content and 89.4% forantioxidant capacity).

Tab. 1. Germination rate (%), moisture (%) and dry matter (%) in radish samples

Variety	Vegetative stage	Germination rate	Moisture	Dry matter
Red	Seeds	-	4.54±0.48	95.46±0.48
	Sprouts-day 3	87.25±6.01	74.01±1.40	25.99±1.40
	Sprouts-day 5	88.50±6.36	82.96±1.71	17.04±1.71
	Sprouts-day 7	90.75±3.89	88.04±1.88	11.96±1.88
	Roots	-	95.20±0.14	4.8±0.14
White	Seeds	-	4.27±0.53	95.73±0.53
	Sprouts-day 3	95±2.12	78.51±1.55	21.49±1.55
	Sprouts-day 5	97.25±0.35	88.46±0.11	11.54±0.11
	Sprouts-day 7	97.50±0.71	90.08±1.31	9.92±1.31
	Roots	-	94.12±0.11	5.88±0.11
Black	Seeds	-	4.61±0.15	95.39±0.15
	Sprouts-day 3	60.50±0.71	77.08±0.21	22.92±0.21
	Sprouts-day 5	62.50±1.41	87.47±2.25	12.53±2.25
	Sprouts-day 7	64.50±2.12	91.56±0.89	8.44±0.89
	Roots	-	90.66±1.13	9.34±1.13

Note: Values are expressed as mean± standard deviation of two replicates.

Tab. 2. Effects of variety, vegetative stage and their first- degree interaction on total phenolic content (mg GAE/g DW) and radical scavenging activity (%) in radish

Factor	Total phenolic content	Radical scavenging activity
Variety (V)		
Red	12.9^{b}	51.7^{a}
White	11.6^{c}	45.7^{b}
Black	13.7^{a}	52.0^{a}
p/contribution (%)	*< 0.001***/3.4*	*< 0.01**/2.6*
Vegetative Stage (VS)		
Seeds	10.1^{c}	41.1^{b}
Sprouts-day 3	16.6^{a}	60.9^{a}
Sprouts-day 5	15.8^{b}	66.9^{a}
Sprouts-day 7	16.3^{ab}	59.7^{a}
Roots	4.9^{d}	20.6^{c}
p/contribution (%)	*< 0.001***/93.3*	*< 0.001***/89.4*
V x VS		
RedxSeeds	10.90±0.48^{f}	44.79±1.33^{cde}
RedxSprouts-day 3	15.85±0.64^{cd}	60.71±2.35^{abc}
RedxSprouts-day 5	15.45±0.29^{de}	65.27±0.20^{ab}
RedxSprouts-day 7	16.97±0.02^{bc}	60.72±5.73^{abc}
RedxRoots	5.48±0.11^{h}	27.12±0.10^{fgh}
WhitexSeeds	8.87±0.30^{g}	34.95±2.97^{efg}
WhitexSprouts-day 3	14.41±0.27^{e}	58.10±1.13^{bcd}
WhitexSprouts-day 5	15.64±0.25^{cde}	60.27±6.45^{abcd}
WhitexSprouts-day 7	14.44±0.10^{e}	53.06±8.65^{bcd}
WhitexRoots	4.42±0.07^{h}	22.33±1.04^{gh}
BlackxSeeds	10.52±0.90^{f}	43.70±1.02^{def}
BlackxSprouts-day 3	19.44±0.08^{a}	63.82±2.66^{ab}
BlackxSprouts-day 5	16.18±0.15^{bcd}	75.11±1.02^{a}
BlackxSprouts-day 7	17.43±0.07^{b}	65.36±9.17^{ab}
BlackxRoots	4.75±0.02^{h}	12.23±0.10^{h}
p/contribution (%)	*< 0.001***/3.1*	*< 0.05*/5.3*

Note: Values are expressed as mean± standard deviation of two replicates for each variety x vegetative stage. Different letters in the same column indicate statistically significant differences (Tukey's test $p< 0.05$). Significant differences are denoted by asterisks: *$p< 0.05$; **$p < 0.01$; ***$p< 0.001$; $p \geq 0.05$, non-significant.

The total phenolic content (Table 2; V x VS) found in seeds ranged between 8 and 11 mg GAE/g DW, in sprouts between 14 and 20 mg GAE/g DW, and in radish roots between 4 and 5 mg GAE/g DW. Lower levels of total phenolic content were obtained by Pajak*et al*. (2014) in radish seeds (~ 6mg GAE/g DW) and spouts (~ 12.5mg GAE/g DW) and by Hanlon and Barnes (2011) in radish roots (between 2 and 4 µg GAE/g DW).

Significant differences were found in total phenolic content between all radish varieties; the highest level was noticed in black radish, followed by red and white radish. Regarding the vegetative stage, the highest total phenolic content was found in sprouts-day 3, followed by sprouts-day 7, sprouts-day 5, seeds and roots.

A positive and highly significant correlation was determined between total phenolic content and antioxidant capacity (r^2=0.939, p<0.001).

The antioxidant capacity (Table 2; V x VS) found in seeds ranged between 34.95 and 44.79%, in sprouts between 53.06 and 75.11%, and in radish roots between 12.23 and 27.12%. The negative control registered a 2.84±0.10% RSA value and the positive control 70.38±0.47%. In our previous research (Borş *et al*., 2014) similar radical scavenging activity values were found in radish seeds.

The black and red radish varieties had similar and significantly higher antioxidant capacity than white radish. Significant differences were found in antioxidant capacity between radish seeds, sprouts and roots, but no significant variations were revealed between sprouts day 3, day 5 and day 7.

CONCLUSION

Vegetables are a good source of nutrients, fibers, minerals and phenolic compounds. Because vegetables are seasonal crops, most of the out-of-season vegetables are cultivated under artificial conditions, prematurely harvested and exported to different parts of the world, thereby expensive. Also, all of these factors can result in a decline in nutrient value of vegetables. Therefore, an excellent alternative for plant foods are sprouts.

This study showed that radish sprouts have higher amount of phenolic compounds and higher antioxidant capacity than roots, which are usually consumed. In addition, radish sprouts can be consumed fresh at all times. Therefore, radish sprouts can be considered for future utilization as dietary supplements.

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