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Total phenol content and antioxidant potential of different *Brassica oleracea* varieties

Original Article

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Abstract:

Varieties of *Brassica oleracea* are commonly used in human nutrition. Beside nutritive role it is suggested that diets reach in cabbage play important role in disease prevention. The aim of this study was to determine total phenol content and the antioxidant potential of different *Brassica oleracea* varieties (broccoli, brussel sprouts, cauliflower, green cabbage and red cabbage). The highest content of total phenols was found in red cabbage and the lowest in broccoli. Red cabbage had the highest ability to remove free radicals, while the lowest radical scavenging activity was recorded for broccoli. Strong correlation between phenolic content and antioxidant activity suggests the importance of phenolics in health benefits of cabbages. The study identified differences between different varieties suggesting different nutritional advantages, providing useful information in selection of right cabbage variety as diet supplement in disease prevention.

Key words:
Brussel sprouts, Broccoli, cauliflower, DPPH radical scavenging, green cabbage, phenols, red cabbage

Apstract:

Ukupan sadržaj fenola i antioksidativni potencijal različitih varijeteta *Brassica oleracea*

Različiti varijeteti vrste *Brassica oleracea* često se koriste u ishrani ljudi. Pored hranljive uloge, smatra se da ishrana bogata kupusom ima važnu ulogu u prevenciji bolesti. Cilj ovog istraživanja bio je da se utvrdi ukupan sadržaj fenola i antioksidativni potencijal različitih varijeteta vrste *Brassica oleracea* (brokoli, kelj pupčar, karfiol, zeleni kupus i crveni kupus). Najveći sadržaj ukupnih fenola utvrđen je u crvenom kupusu, a najmanji u brokoliju. Crveni kupus je imao najveću sposobnost uklanjanja slobodnih radikala, dok je najmanja aktivnost uklanjanja radikala zabeležena kod brokolija. Izražena korelacija između sadržaja fenola i antioksidativne aktivnosti ukazuje na značaj fenola u zdravstvenim prednostima kupusa. Studija je identifikovala razlike između različitih varijeteta koje sugerišu različite nutritivne prednosti, pružajući korisne informacije u izboru prave sorte kupusa kao dodatak ishrani u prevenciji bolesti.

Ključne reči:
kelj pupčar, brokoli, karfiol, DPPH uklanjanje radikala, zeleni kupus, fenoli, crveni kupus

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Introduction

Brassicaceae (Cruciferae) or the cabbage family includes many economically important, edible and industrial types of oilseeds, vegetables, spices and fodder. This family is also known to comprise more than 120 wild species, some of which are very important for agriculture e.g. *Sinapsis arvensis* and *Raphanus sativus* (Warwick, 2011). The long history of plant selection has resulted that this crop group is highly variable in use, appearance and phytochemistry. *Brassica oleracea* is a good example with several important varieties: broccoli, cauliflower, Brussel sprouts, cabbage, kale (Bjorkman et al., 2011).

Oxidative stress has been identified as a major cause of the development and progression of several diseases. Supplementation with exogenous antioxidant or enhancement of endogenous antioxidant defence of the body is a promising way of counteracting the reactive oxygen species (ROS) side effects that cause oxidative damage. Plants have long been a source of exogenous antioxidants. Two-thirds of

the worlds' plant species are thought to be medically significant, and almost all of them have excellent antioxidant potential. Plants have effective, complex enzymatic and non-enzymatic antioxidant defence systems to avoid the toxic effects of free radicals and non-enzymatic antioxidant system including secondary metabolites as antioxidants (Kasote et al., 2015).

Phenolic compounds from plants represent the largest group of secondary plant metabolites found in fruits, vegetables and teas. They are characterized by antioxidant, anti-inflammatory, anticancerogenic and other biological properties, and can protect plants from oxidative stress and some diseases (Ahmed et al., 2017).

Over the last few decades, vegetables from the Brassicaceae family have come under scientific attention because of numerous epidemiological studies that provide evidence that diets rich in these vegetables are associated with a reduces risk of several types of cancer and other chronic diseases. Because of that, these vegetables are considered promising sources of metabolites for cancer prevention, and mechanism of anticancerogenic activity is mostly associated with glucosinolate degradation products (Šamec et al., 2019).

The aim of this study was to determine the content of total phenols and antioxidant potential of selected *Brassica oleracea* varieties (broccoli, cauliflower, Brussel sprouts, green cabbage, and red cabbage).

Material and methods

Plant material

Five mostly consumed *Brassica oleracea* varieties were selected for the analysis: *B. oleracea* var. *capitata* (green cabbage) – fresh leaves; *B. oleracea* var. *capitata* f. *rubra* (red cabbage, Bosnia and Herzegovina) – fresh leaves; *B. oleracea var. italica* (broccoli) – broccoli flowers (frozen by rapid freezing technique); *B. oleracea* var. *botrytis* (cauliflower) – fresh flowers of cauliflower; *B. oleracea* var. *gemmifera* (Brussel sprouts) – axillary buds were used (frozen by rapid freezing technique).

Extract preparation

All samples were air-dried and extraction was performed by maceration with 80% ethanol. Prepared macerate was incubated in the dark at 4 °C for 24 hours. After incubation, macerate was centrifuged at 5000 rpm for 30 minutes, and supernatant was collected for the analysis. Extract yield was determined for all samples.

Analysis of total phenol content

Analysis of phenol content in ethanol extracts of different *B. oleracea* varieties was performed using Folin Ciocalteu reagent (FCR), by diluting 20 µL of extract with 1580 µL water and adding 100 µL of FCR, followed by addition of 300 µL 7.5% Na_2CO_3 after 3 min of incubation (Wolfe et al., 2003). Total phenols were quantified based on the direction of the calibration curve of gallic acid. The obtained values are expressed as mg of gallic acid equivalents per gram of dry material (mg GA/gDW).

Analysis of antioxidant capacity

The DPPH (2,2 – diphenyl – 1 – picrylhydrazyl) method was used to determine the antioxidant activity of the tested extracts (Mishra et al., 2012). A stock solution of DPPH was prepared by weighing 50 mg of DPPH and dissolving in 100 mL of 96% ethanol. A working DPPH was prepared by diluting 5 mL of stock solution with 96% ethanol to the 50 mL mark and by adjusting DPPH absorbance reading to 1.123 at 517 nm (Lambda 25; Perkin Elmer). Extract testing was performed by mixing 100 mL of extract with diluted DPPH and final absorbance reading was recorded after 30 minutes against 96% ethanol (blank). Free radical scavenging activity was calculated by the formula:

$$AA\% = \frac{(A_{(t0)} - A_{(60)})}{A_{(t0)}} \times 100$$

AA% - antioxidant capacity; A(t0) – absorbance at 0 seconds; A(60) – absorbance after 60 seconds. Naringenin was used as a standard in concentration of 1 mg/ml.

Statistical analysis

The data were expressed as means of three independent replicates ± standard deviation. All data were statistically evaluated by analysis of variance with the ANOVA test ($p< 0.05$ significance level), and the means were analyzed by regression analysis by Newman Keuls test. Pearson's correlation was used to assess relationships between the variables. All statistical testing was performed using STATISTICA 10 software (Copyright© StatSoft, Inc. 1984-2011).

Results and Discussion

Differences in extract yield for different *B. oleracea* cultivars were recorded. The highest yield was noted for green cabbage (21.5%), followed by red cabbage, cauliflower and sprouts, and the lowest yield was registered for broccoli (2.6%) (**Tab. 1**). The differences in extract yield for the analyzed *B. oleracea* varieties can be attributed to the availability of the components that are extracted from the sample, which in itself depends on the analyzed variety (Hsu et al., 2006). Also, the effectiveness of the solvent

Table 1. Extract yield, total phenol content and antioxidant capacity of different *Brassica oleracea* varieties

SAMPLE	EXTRACT YIELD (%)	Total phenol content (mg GA/g DW)	Antioxidant capacity (%)
Red cabbage *B. oleracea* var. *capitata f. rubra*	$12.00^{b} \pm 0.10$	$27.97^{a} \pm 3.23$	$86.63^{a} \pm 1.97$
Green cabbage *B. oleracea* var. *capitata*	$21.50^{a} \pm 0.12$	$7.89^{bc} \pm 0.88$	$19.88^{c} \pm 0.60$
Broccoli *B. oleracea* var. *italica*	$2.60^{d} \pm 0.09$	$3.41^{d} \pm 0.73$	$7.14^{d} \pm 0.30$
Brussel sprouts *B. oleracea* var. *gemmifera*	$9.30^{c} \pm 0.09$	$6.78^{c} \pm 0.67$	$25.76^{b} \pm 0.26$
Cauliflower *B. oleracea* var. *botrytis*	$11.20^{bc} \pm 0.11$	$8.78^{b} \pm 0.90$	$23.49^{b} \pm 1.28$
Standard (naringenin)	-	-	$90.00^{a} \pm 3.89$

Data expressed as means ± standard deviation. Means of samples not sharing the same letter in one column are significantly different ($p < 0.05$); GA - equivalents of gallic acid; DW - dry weight.

used for extraction to dissolve endogenous components has a significant effect on the yield of the extracts (Siddhuraju & Becker, 2003).

Other factors that can affect yield of extract solvent polarity, extraction time and temperature as well as the chemical nature of the sample itself (Sun & Ho, 2005). The choice of extraction solvent was selected according to targeted components (phenolics).

The analysis of total phenolic content showed high concentrations in red cabbage, while broccoli contained only small amounts of phenolics (**Tab. 1**). Compared to other extraction solvents, 80% ethanol is less effective and methanol extracts are richer in phenolics as previously recorded by Jaiswal et al. (2011). High phenolic content in red cabbage is probably result of high sinapic acid content as previously recorded in other studies (Mattila & Hellström, 2007).

Analysis of DPPH radical scavenging activity by ethanol extracts of different varieties of *B. oleracea* are shown in **Tab. 1**. The antioxidant capacity of the tested extracts ranged from 86.63% to 7.14% for red cabbage and broccoli, respectively. Total phenolic content strongly correlated with DPPH scavenging activity (R2 0,9755; p<0,05). High antioxidant capacity of red cabbage has been previously recorded by several authors (Podsędek et al., 2006; Isabelle et al., 2010; Rokayya et al., 2013; Liang et al., 2019). Miller et al. (2000) studied the antioxidant effects of different vegetables and noticed a striking difference between red and green cabbage, suggesting a strong role of red cabbage pigment in antioxidant activity.

Oxidative stress is one of major causes for pathophysiology of several cardiovascular diseases (Lahera et al., 2007). Dietary phenolic antioxidants play significant role in counteracting cardiovascular diseases (Chiu et al., 2018) and intake of red cabbage is beneficial for human health, as suggested by our study as well.

Conclusion

Presented study evaluated different *Brassica oleracea* varieties for their phenolic content and antioxidative capacity. Among the tested varieties, red cabbage had the highest phenolic content and significant antioxidant capacity, suggesting the importance of red cabbage in human nutrition.

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