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ANTIOXIDANT ACTIVITY IN SELECTED *BRASSICACEAE* VEGETABLES

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Abstract

Vegetables from Brassicaceae family are an excellent source of bioactive phytochemicals and may help to reduce the risk of chronic diseases. These vegetables contain various biologically active substances such as glucosinolates, polyphenols, flavonoids and vitamins. White cabbage (Brassica oleracea var. capita), red cabbage (Brassica oleracea var. capitata f. rubra), broccoli (Brassica oleracea var. italica), cauliflower (Brassica oleraceae var. botrytis) and kohlrabi (Brassica oleracea Gongylodes Group) were investigated for their contents in antioxidants: polyphenols, vitamin C, carotenoids, and chlorophyll. The antioxidant efficacy was assessed using DPPH free radical scavenging. Ascorbic acid and phenolic content varied considerably between different Brassica varieties. The results of vitamin C content ranged between 38.99-74.25 mg/100 g fresh weight (FW) and 21.69-71.64 mg/100 g fresh weight for polyphenols. Regarding pigments, the examined vegetables were found to contain between 0.095 and 0.85 mg/100 g FW carotenoids and between 1.37 and 3.41 mg/100 g FW chlorophyll. Regarding DPPH• assay, red cabbage had an exceptionally high antioxidant activity followed by cauliflower, broccoli, kohlrabi and white cabbage.

Key words: *Brassicaceae, DPPH method, pigments, polyphenols, vitamin C.*

INTRODUCTION

Regular consumption of fruits and vegetables confers significant benefits to human health. Epidemiological data as well as *in vitro* studies, strongly suggest that foods containing phytochemicals with antioxidation potential have strong protective effects against major disease risks (Kaur and Kapoor, 2002). Many epidemiological studies have correlated the intake of a diet rich in vegetables and fruits with a reduced risk of incidence of chronic diseases. In particular, current studies reported an inverse correlation between consumption of *Brassicaceae* and risk of cancer (Yagar et al., 2016).

Brassicaceae family or *Cruciferae* family contains 350 genera and about 3,500 species and includes several genera such as *Camelina, Crambe, Sinapis, Thlaspi* and *Brassica.* The genus *Brassica* is the most important within the *Brassicaceae*, which includes some crops and species of a great worldwide economic importance such as *Brassica oleracea* L., *Brassica napus* L. and *Brassica rapa* L.

The main species of *B. oleracea* includes vegetable such as kale, cabbage, broccoli, Brussels sprouts, cauliflower and others (Cartea et al., 2011).

According to Food and Agriculture Organization (FAO) data, the production of *Brassica* vegetables in the world covers a total of approximately 139 million tons: 33.5 million tons of cauliflower and broccoli, 105.7 million tons of cabbage and other *Brassica* species. China is the biggest *Brassica* vegetables producer in the world and constitutes over half of all *Brassica* vegetables production in the world [FAOSTAT]. *Brassicaceae* vegetables are an abundant source of health-promoting substances. Apart from anticarcinogenic glucosinolates, they possess antioxidants of both hydrophilic (vitamin C, polyphenols) and hydrophobic properties (carotenoids, vitamin

E), which can neutralize active oxygen species and quench free radicals. These vegetables are also rich in potassium, magnesium, calcium, phosphorus and vitamins C, E, K (Heimler et al., 2006; Hagen et al., 2009; Jahangir et al., 2009). The antioxidants mentioned above, scavenge radicals and inhibit chain initiation or break the chain propagation (the second defence line). Thus, the contribution of the *Brassicaceae* vegetables (e.g. broccoli, cauliflower, cabbage, kale) to health may be related to the antioxidant capacity of these vegetables due to the presence of phenolic compounds, carotenoids, vitamins and minerals (Mulțescu et al., 2020).

The previous studies indicate the importance of these vegetables as the richest potential source of these substances. The studies emphasize the need to increase the proportion of these products in the human diet.

The current study investigated the content of the main antioxidants between of the selected *Brassicaceae* vegetables (white cabbage, red cabbage, broccoli, cauliflower and kohlrabi) cultivated in Romania.

MATERIALS AND METHODS

Materials

Samples of white cabbage (*Brassica oleracea* var. *capita*), red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), broccoli *(Brassica oleracea* var. *italica)*, cauliflower *(Brassica oleracea* var. *botrytis)* and kohlrabi (*Brassica oleracea zhanggylodes* Group) were provided by the Development and Research Station for Vegetables growing from Buzau, Romania. The different *Brassica* varieties were cultivated in the same pedoclimatic conditions with respect to their own cultivating requirements. After harvesting, the vegetables were analyzed within 3 days.

Chemicals like Trolox, DPPH (2,2-difenil-1-picrililhidrazil) and 2,6 diclorphenol-indophenol were purchased from Sigma-Aldrich. Meta-phosphoric acid, ethylenediaminetetraacetic acid, sodium hydrogen carbonate and sodium carbonate were purchased from Roth, while Folin-Ciocâlteu reagent, ascorbic acid and analytical grade organic solvents (methanol and acetone) were purchased from Merck.

Methods

Sample preparation

The inflorescences of selected vegetables were separated from inedible parts and were visually evaluated for damages or infections. Only vegetables with intact integrity were selected. The samples were washed in tap water and dried at room temperature, cut into small pieces and homogenized. For obtaining the vegetables extracts, a 1: 10 ratio between fresh plant material and selected extraction solvent was used. Finally, the extracts were vortexed and centrifuged, keeping the supernatants.

Analytical methods

Determination of total phenolic content

Total phenolic content of fresh vegetables was determined using Folin-Ciocâlteu reagent (Singleton and Rossi, 1965) with modifications and adaptations to the working conditions of the laboratory. Briefly, 1 ml of 50% (v/v) methanolic extract was treated with 5 ml Folin-Ciocâlteu reagent (10% v/v) and 4 ml sodium carbonate (7.5% w/v). After 20 min keeping in the dark, the blue-colored compound was measured spectrophotometrically at 752 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). Phenolic content was calculated using a calibration curve obtained with gallic acid standard, over an interval of 10-50 μg acid gallic/ml. The results were expressed as mg GAE (gallic acid equivalents)/100 g fresh weight (FW).

Determination of ascorbic acid

The level of ascorbic acid was determined using dye-titration method, according to AOAC procedure, 2000. Metaphosphoric acid extracts of fresh vegetables were subjected to titration with 2,6-dichlorophenolindophenol. In this oxidation-reduction reaction, the ascorbic acid from the extract was oxidized to dehydroascorbic acid and the indophenol dye was reduced to a colourless compound. The end point of the titration was detected when an excess of the unreduced dye gave a rose pink in acid solution. Dehydroascorbic acid was not analysed in this study. The results were expressed in mg ascorbic acid/100 g FW.

Determination of pigments

Carotenoids and chlorophyll were determined according to Lichtenthaler (1987) by extracting pigments using a solvent mixture of acetone/water (80:20, v/v). For a more efficient extraction, the vegetable sample mixed with the solvent was vortexed at 2000 rpm, at 20°C, for 15 min and then separated by centrifugation at 3500 rpm, at 20°C, for 15 min. The clear supernatant was further used for absorbance determination at 470, 646, 663 nm with a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). The content of total chlorophyll, chlorophyll a and chlorophyll b and carotenoid pigments were calculated on the basis of the absorbance values and the equations according to Lichtenthaler (1987) were as follows:

$$Chll\ a = (12.25\text{xA663}) - (2.79\text{xA646})$$

$$Chll\ b = (21.50\text{xA646}) - (5.10\text{xA663})$$

$$Car = \frac{(1000xA470) - (1.82xChll\ a) - (85.02xChll\ b)}{198}$$

The results were expressed in mg chlorophyll/100 g FW and mg carotenoids/ 100 g FW, respectively.

DPPH "scavenging" radical capacity

The antioxidant activity was determined based on the reduction of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical. A modified protocol (Culețu et al., 2016) was used consisting in the extraction of the samples in methanol: water (1:1, v/v). After vortexing (2000 rpm, 20°C, 15 min) and centrifugation (3500 rpm, 20°C, 15 min), 1 ml of supernatant was treated with 6 ml DPPH solution. Prepared solutions were left in the dark for 30 min and then the absorbance was measured at 517 nm using Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). The antioxidant activity was calculated using a calibration curve obtained with Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standards, over an interval of 0-0.40 mg Trolox/ml. The results were expressed in µmol Trolox/100 g FW.

RESULTS AND DISCUSSIONS

Five *Brassica* vegetables were assayed for variability between cultivars regarding antioxidant phyto-chemicals.

Total phenolic content

Phenolic compounds are major antioxidants of *Brassicaceae* vegetables (Podsędek, 2007). Vegetables containing phenolics are an integral part of human diet. Some of the health protective effects of phenolic compounds have been ascribed to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial properties (Xu et al., 2008).

In plants, phenols occur in soluble forms and along with cell wall components-bound phenols. Bound phenols were quantified in the extracts after base hydrolysis of residues following the solvent soluble extraction (Podsędek, 2007). Figure 1 presents the total phenolic content of analysed raw vegetables. The content of phenolic content was highest in red cabbage. According to Charon et al. (2007), red cabbage is a good source of phenolic compounds, and the most abundant class is the anthocyanins.

In this study, the greatest quantity was found in red cabbage (71.64 mg/100 g) followed by broccoli (56.62 mg/100 g) and cauliflower (51.09 mg/100 g). Total polyphenol content of red cabbage was extremely high, four times higher than in white cabbage.

Leja et al. (2010) found that the level of phenolic substances (total, as well as those of individual groups) was much higher in red cabbage compared with the two white cabbage cultivars tested.

Previous studies indicated different levels of total phenolic content for white cabbage ranging between 134.73-393.1 mg GAE/100 g FW (Podsędek et al., 2006; Leja et al., 2010; Chun et al., 2004). Considering that *Brassica* species are the most efficient vegetables regarding polyphenolic content, they are acting as an efficient antiradical agent (Podsędek, 2007).

In present study, kohlrabi and white cabbage presented the lowest quantity of phenolics, 21.69 mg GAE/100 g FW and 22.18 mg GAE/100 g FW, respectively. Yi et al. (2017) reported that kohlrabi is rich in antioxidants from the group of phenolic compounds with a total phenolic content of 27.33 mg GAE/100 g and contains antioxidant vitamins. However, the content of polyphenols in fresh white cabbage was reported to range from 15.3 mg GAE/100 g to 203 mg GAE/100 g (Bahorun et al., 2004; Wu et al., 2004).

The differences in the previous reported data may be due to the variation of the phenolic compounds according to the cultivars, agronomic conditions, harvesting stage, extraction solvent and the details of the analysis method. In the case of phenolic compounds, which are highly reactive species, sample preparation method is also essential.

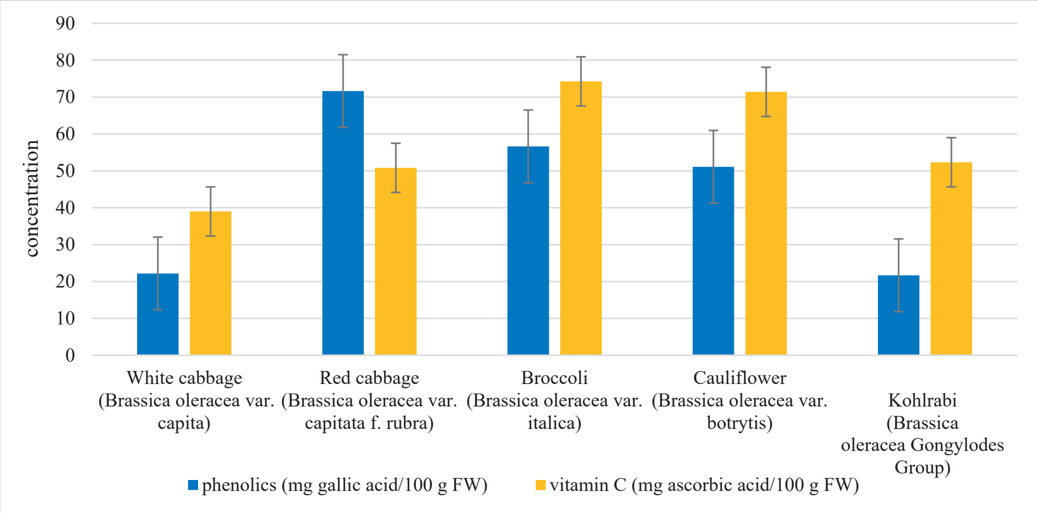

Figure 1. Phenolics and vitamin C composition of *Brassicaceae* vegetables

Ascorbic acid

Vitamin C plays an important role in the human body as an antioxidant, protecting the immune cells against intracellular ROS production, during inflammatory responses. Ascorbic acid is an electron donor and acts as a cofactor for some biosynthetic and gene regulatory enzymes. It maintains tissue integrity and plays a crucial role in skin formation, epithelial and endothelial barriers (Holmannová et al., 2013). Therefore, plant sources become important because of their high content of vitamin C, up to 5,000 mg/100 g (Chambial et al., 2013).

Cruciferous vegetables are considered as a high source of vitamin C in the human diet. The content of vitamin C among *Brassica* vegetables varies significantly between and within their subspecies (Figure 1). The levels of ascorbic acid (AA) determined in the fresh vegetables analyzed in this study, ranged between 38.99-74.25 mg AA/100 g FW. The highest content of AA was recorded for broccoli, followed by cauliflower. The determined AA level in broccoli (74.25 mg/ 100 g) compliece with other reported data ranging between 34.00 to 131.35 mg AA/100 g FW (Favell, 1998; Shams El-Din et al., 2013; Sikora et al., 2008; Koh et al., 2009).

Referring to cauliflower, the average content of vitamin C was 71.41 mg AA/100 g. The resultsof previous studies revealed that the content of vitamin C in cauliflower varies from 40.60 to 396.70 mg AA/100 g (Sikora et al., 2008; Shams El Din et al., 2013).

The results showed that the lowest AA level was found in white cabbage (38.99 mg AA/ 100 g FW). Specialized studies (Chun et al., 2004; Singh et al., 2006; Sham El Din et al., 2013) examined the amount of vitamin C in white cabbage immediately after harvest and found it to be between 5.66 and 41.00 mg/100 g FW. Lower values may be the result of different cabbage varieties used or of growing conditions. It is also possible that the content of this sensitive compound decreased by storage or preliminary processing of vegetables. The ascorbic acid levels from freshly harvested red cabbage heads and kohlrabi were greatly close, 50.82 mg/100 g and 52.33 mg/100 g, respectively. Reported data revealed that the ascorbic acid content in red cabbage ranged from 22.6 mg/100 g to 72.56 mg/100 g (Kurilich et al., 1999; Podsedek et al., 2006).

Our results are also similar to other researchers (Patras et al., 2020) that reported the vitamin C content in kohlrabi is 60-65 mg AA/100 g FW.

According to our study and previous research, the content of AA showed considerable variation depending on vegetables cultivars, sample extraction and analytical method.

Chlorophyll

Chlorophylls (Chl) are photosynthetic pigments that are widely distributed in nature. They can be found in a large number of green vegetables, such as broccoli and cabbage plants. It is classified as blue chlorophyll (chlorophyll a), green chlorophyll (chlorophyll b) and chlorofucin (chlorophyll c1, chlorophyll c2). The absorbance properties of pigments facilitate the qualitative and quantitative analysis of them (Fernandez-Leon et al., 2010). All green vegetables contain chlorophyll a and chlorophyll b. In higher plants, chlorophyll a is the major pigment and chlorophyll b is an accessory pigment. The ratio a/b is usually 3 to 1. The ratio depends and varies with growth conditions and environmental factors. The content of chlorophylls can be determined spectrophotometrically following extraction of the pigments using an organic solvent, such as acetone or dimethyl formamide. The spectrophotometric determination for chlorophylls permits direct calculation of chlorophyll a and chlorophyll b in the plant extract, without separation into individual pigments.

Our results (Table 1) showed a variation in the chlorophyll content of the analysed vegetables, with levels of pigments ranging between 1.37 mg/100 g and 3.41 mg/100 g FW. The highest concentration of chlorophyll was found in broccoli (3.41 mg/100 g) while kohlrabi presented the smallest level of total chlorophyll (1.37 mg/100 g). In cauliflower, no pigments level was detected.

Ramos dos Reis et al. (2015) observed that the content of chlorophyll in fresh broccoli inflorescences was 30.8 mg/100 g, 10 times higher than our value. The authors, also reported the absence of these pigments in cauliflower.

In this study, white cabbage and red cabbage contain 1.96 mg/100 g and 2.77 mg/100 g, respectively.

Lower values may be the result of varieties used or growing conditions. It is also possible that the content of this sensitive compound decreased by storage or preliminary processing of the vegetables.

Overall, chlorophyll levels differed depending on the cultivar and plant part in most cases.

Carotenoids

Carotenoids are natural pigments, that are - synthesized by plants, algae and photosynthetic bacteria. They are responsible for the yellow, orange and red colours in various fruits and vegetables (Namitha and Negi, 2010). Carotenoids in plants can be found in free form or esterified with fatty acids. Though, esterification does not alter the chromophore properties of the carotenoid, it does modify the chemical and biological properties by changing its immediate environment (Pérez-Gálvez & Mínguez-Mosquera, 2005). These pigments can be found in green leaves and fruits along with chlorophylls. The natural functions and properties of carotenoids are determined by their molecular structure. Thus, chromophore is the part of a carotenoid molecule responsible for its colour and photoprotective actions. The colour develops when a chromophore absorbs particular wavelengths of visible light and transmits or reflects others (Britton, 2008). Carotenoids are important for antioxidant activity, intercellular communication and immune system influence (Skibsted, 2012; Stephensen, 2013).

According to the results of this study presented in Table 1, *Brassica oleracea* varieties that were analyzed contain low concentrations of carotenoids. The obtained data confirm that *Cruciferous* vegetables are not a major dietary source of carotenoids.

Kaulmann et al. (2014), reported similar levels of carotenoids in these vegetables as follows: white cabbage (0.51 mg/100 g), red cabbage (0.16 mg/100 g), cauliflower (0.07 mg/100 g) except broccoli, which presented a higher concentration than our result, 4.69 mg/100 g. Ramos dos Reis et al. (2015) analysed the total carotenoid content in fresh broccoli and cauliflower and they obtained lower results 0.76 mg/100 g for broccoli and 0.0035 mg/ 100 g for cauliflower. Zhang and Hamauzu (2004) investigated total carotenoids of broccoli florets and stem and they reported that the stem contained 0.10 mg/100 g FW of carotenoids, less than 3% of total carotenoids present in the floret, 3.75%. Sikora et al. (2008) examined the amount of total carotenoids in broccoli and cauliflower immediately after harvest and found it to be 0.26 mg/100 g for broccoli. In cauliflower, these compounds were absent. Among the 22 species of vegetables investigated by Muller (1997), he observes that broccoli (1.6 mg/100 g), red cabbage (0.43 mg/100 g) white cabbage (0.25 mg/100 g) and kohlrabi (0.07 mg/100 g) contain a small quantity of total carotenoid content.

Content and types of carotenoids in plants depend on several pre- and post-harvesting factors, genotype, ripening time, cultivation method and climatic conditions, processing.

Table 1. Chlorophyll and carotenoid content of selected *Brassicaceae* vegetables

	White cabbage (*Brassica oleracea* var. *capita*)	Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*)	Broccoli (*Brassica oleracea* var. *italica*)	Cauliflower (*Brassica oleracea* var. *botrytis*)	Kohlrabi (*Brassica oleracea* Gongylodes Group)
Total chlorophyll (mg/100 g FW)	1.96±0.31	2.77±0.61	3.41±0.43	Nd	1.37±0.59
Chlorophyll a (mg/100 g FW)	1.38±0.42	2.47±0.49	2.75±0.54	Nd	0.99±0.42
Chlorophyll b (mg/100 g FW)	0.58±0.29	1.30±0.32	0.66±0.15	Nd	0.38±0.06
Total carotenoids (mg/100 g FW)	0.29±0.10	0.11±0.009	0.85±0.06	0.095±0.008	0.28±1.22

Antioxidant activity

The model of scavenging the stable DPPH· radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann, 1979). The DPPH· radical is one of the few stable organic nitrogen radicals, which has a deep purple. *Brassicaceae* vegetables extracts have been screened for antioxidant activity using different oxidation systems and methods to measure antioxidant capacity (Honer & Cervellati, 2002).

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. These methods differ in terms of their assay principles and experimental conditions. The antioxidant capacity was expressed as μmol Trolox/100 g of vegetables extract. The values are displayed in Figure 2. Broccoli, cauliflower and red cabbage show high antioxidant potential, whereas white cabbage has a rather low antioxidant activity Samec et al. (2011) found that the antioxidant activity of white cabbage leaves reached its maximum in the juvenile stages. The maximum of antioxidant activity was recorded in red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), 431.82 μM Trolox/100 g, followed by cauliflower (*Brassica oleracea* var. *botryitis*), 394.88 μM Trolox/100 g). The minimum of DPPH was recorded in white cabbage (*Brassica oleracea* var. *capita*), 252.85 μM Trolox/100 g, followed by kohlrabi (*Brassica oleracea* Gongylodes Group), 329.56 μmol Trolox/100 g and broccoli (*Brassica oleracea* var. *italica*), 380.05 μM Trolox/100 g.

Ramos dos Reis et al. (2015) analyzed the antioxidant activity of fresh broccoli and cauliflower. They found that broccoli and cauliflower have an antioxidant scavenging of 719.34 μM Trolox/100 g and 391.13 μM Trolox/100 g and 391.13 μM Trolox/100 g, respectively. Compare with our study, the value of broccoli was approximately two-fold higher. Some authors (Soare et al., 2016) estimated that the level of 5 cultivars of white cabbage was 91.53-161.12 μM Trolox/100 g, lower than our reported value. The variation of antioxidant activity is caused by many factors such as geographical region, climate, variety, harvest maturity, growth conditions, soil condition and post-harvest conservation and processing methods (Gonçalves et al., 2004). Because the contribution of the antioxidant to the overall antioxidant capacity is different, a correlation analysis was performed. The results showed a strong positive correlation of total phenolic content with antioxidant activity on DPPH

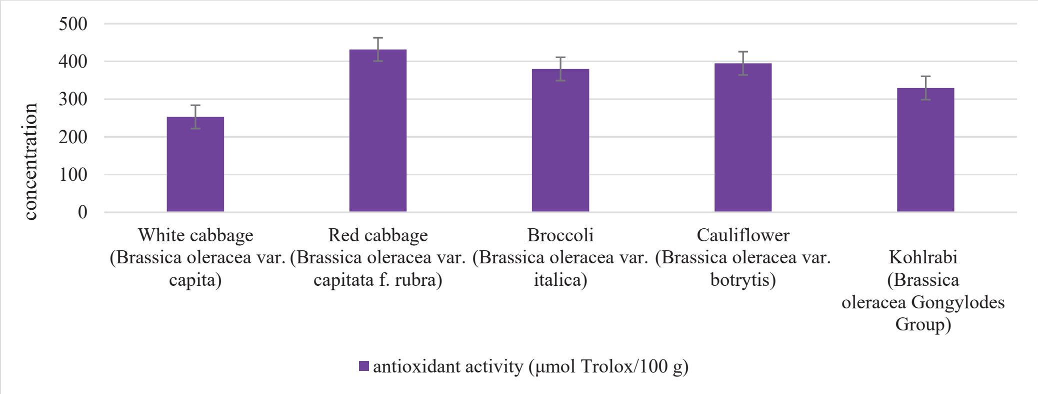

Figure 2. Total antioxidant activity of fresh vegetables determined by DPPH assay

radical, with a correlation coefficient of 0.814. Moderate correlation was recorded for ascorbic acid (0.362), while for chlorophyll and carotenoids the relationship was insignificant. The results complies with other studies that reported strong correlation of antioxidant activity with total phenolic content, correlation coefficient ranging between 0.61-0.86. according to analyses of *Brassica* varieties.

CONCLUSIONS

In this study, 5 subspecies of *Brassica oleracea* were analysed of the content of the most important antioxidants and also of antioxidant activity. All the varieties were cultivated in the same area, the different results were not giving by soil quality, agricultural practices or postharvest conditions. That means that the values obtained for the determination were influence only by different subspecies and the environmental conditions (because they have different cultivation period).

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