ANTIOXIDANT ACTIVITY OF EXTERNAL AND INTERNAL LEAVES OF WHITE AND RED CABBAGE CULTIVARS DURING STORAGE

ACTIVITATEA ANTIOXIDANTĂ A FRUNZELOR EXTERNE ŞI INTERNE A SOIURILOR DE VARZĂ ALBĂ ŞI ROŞIE ÎN TIMPUL PĂSTRĂRII

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Abstract. *This study was carried out to determine the changes in the antioxidant activity and phenolic content of the external and internal leaves of different cabbage cultivars during storage. It is also evaluated the anthocyanins content of the red cabbage. The methods used for the determination of antioxidant capacity were : DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging activity and ORAC (oxygen radical absorbance capacity). Trolox, a vitamin E analog, was used as standard antioxidant. Among the cabbage cultivars, red cabbage showed the highest antioxidant potential and the highest phenolic content, both cases, fresh samples and samples kept at 4°C during 10 days.*
There were not found significant differences between antioxidant activity of the external and internal leaves of the cabbage cultivars studied.
Keywords: antioxidant activity, ORAC, cabbage.

Rezumat. *Acest studiu a fost realizat pentru a determina schimbările care intervin în timpul păstrării asupra activităţii antioxidante şi a conţinutului de compuşi fenolici la frunzele externe şi interne ale diferite soiuri de varză. A fost determinat, de asemenea, şi conţinutul de antociani la varza roşie. Capacitatea antioxidantă a soiurilor de varză a fost determinată cu metodele: DPPH (radical liber 1,1 difenil - 2- picrilhidrazil) şi ORAC (capacitatea de absorbţie a radicalilor de oxigen). Trolox, un analog al vitaminei E, a fost folosit ca antioxidant standard. Varza roşie a arătat cel mai mare potenţial antioxidant şi conţinut de compuşi fenolici, dintre toate soiurile studiate, atât la probele prospete cât şi la cele păstrate la 4°C timp de 10 zile.Nu s-au găsit diferenţe semnificative între activitatea antioxidantă a frunzelor externe şi interne a soiurilor de varză analizate.*
Cuvinte cheie: activitatea antioxidantă, ORAC, varză.

INTRODUCTION

Cruciferous vegetables are among the most important dietary vegetables consumed in Europe, owing to their availability in local markets, affordability and consumer preference. Due to its anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and

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duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds.

Numerous studies have highlighted the potential importance of *Brassica* vegetables as a source of antibacterial (Kyung and Fleming, 1994; Hu et al., 2004; Ayaz et al., 2008) and antioxidant substance (Zhou and Yu, 2006; Andarwulan et al., 2010; Isabelle et al., 2010). Cruciferous vegetables, including cabbage (*Brassica oleracea* convar. *capitata* var. *capitata*), have a high nutritional value and contain organo-sulphur phytochemicals that increase their antioxidant capacity, which may have anticarcinogenic effects (Kim et al., 2004; Kurilich at al., 1999).

The aim of this research was to establish the content of compounds with antioxidant properties, i.e. polyphenols and anthocyanins, in selected cabbage cultivars, and the degree to which these substances are active as antioxidants. Because these vegetables are often stored before eaten, the influence of storage on these parameters was investigated.

MATERIAL AND METHOD

One white cabbage and one red cabbage cultivars, fresh and stored at 4°C during 10 days, were analyzed for the antixodant potential. The white cabbage and the red cabbage were purchased from a local market.

For the extract, 1 g of the cabbage was grounded with 10 mL of extraction solvent: acetone (70%), water (28%), acetic acid (2%) (Counet, 2003). The mixture was shaken for 1 h at 4 °C and centrifuged at 17000*g* for 15 min. The supernatant was removed, and the pellet was extracted again with 10 mL of the same solvent, incubated for 15 min, and centrifuged using the same procedure. The extract obtained was kept at -30°C until analyses. Each sample was independently extracted in triplicate.

Total phenolic contents were determined according to the Folin–Ciocalteu method (Caboni, 1997). Appropriately diluted extracts (3.6 mL) were mixed with 0.2 mL of Folin–Ciocalteu reagent, and 3 min later, 0.8 mL of sodium carbonate (20% w/v) was added. The mixture was heated at 30 °C for 1 hour. After cooling, the absorbance at 750 nm was measured. Gallic acid (Sigma) was used as standard, and results were expressed as milligrams of Gallic acid equivalents (GAE) per 100 g of sample. Analyses were performed in duplicate on each sample.

Antioxidant capacity was determined by scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Tadolini et al. (2000). Trolox was used as a standard and methanol as a blank. The absorbance at 517 nm using an Uvikon 931 spectrophotometer (BIOTEK Instruments) of samples, standards, and blanks was determined after 5 min. The results were expressed as micromolar Trolox equivalents (TE) per 100 g of sample. Analyses were performed in duplicate.

ORAC assay was carried out on a fluoroskan Ascent FL Thermolabsystems (Finland) plate reader. The temperature of the incubator was set to 37 °C. Procedures were based on the method of Wu et al. (2004). Briefly, AAPH was used as peroxyl radical generator, Trolox as standard, and fluorescein as fluorescent probe. Fluorescence filters were used for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. 25 of diluted sample, blank, or Trolox calibration solutions were mixed with 150 μL of 4 μM fluorescein and incubated for 15 min at 37 °C before injection of 25 μL of AAPH solution. All samples were

analyzed in duplicate at three different dilutions. The final ORAC values were calculated using the net area under the decay curves and were expressed as micromolar Trolox equivalents (TE) per 100 g of sample.

Anthocyanin quantification was performed by the pH-differential method (Guisti et al., 2001). The extract was diluted in a pH 1.0 solution (0.1 M HCl, 25 mM KCl) and in a pH 4.5 solution (0.4 M CH_3COONa). The absorbance of the mixtures was then measured at 534 and 700 nm against distilled water. The value (Abs535 – Abs700) pH1.0 – (Abs535 – Abs700) pH4.5 corresponds to the absorbance due to the anthocyanins. Results were expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of sample.

Descriptive statistical analysis was performed using Microsoft Excel. Results were expressed as mean values ± standard error.

RESULTS AND DISCUSSIONS

Table 1 lists the total phenolic content (expressed as Gallic acid equivalents) and the antiradical activity measured by ORAC and DPPH (expressed as µmole TE). Oxygen radical absorbance capacity (ORAC) assay is selected for antioxidant capacity measurement as it is the current method widely used by researchers as well as food and supplement industry (Huang et al., 2005).

The amounts of antioxidant activity are very similar to those found with the same method (ORAC) by Isabelle et al., 2010 in the case of cabbage round and red cabbage. Antioxidant activity, measured by ORAC method, of external leaves-white cabbage present an decreasing during the storage while the internal leaves increase their activity. In the case of red cabbage, the internal leaves showed the highest antioxidant activity 5264 µmol TE/100 g fresh sample.

This antioxidant activity decrease during storage until the value of 3514.28 µmol TE/100 g sample. The antioxidant activity of the red cabbage external leaves increase from 2949.42 5264 µmol TE/100 g fresh sample to 3833.12 µmol TE/100 g sample during 10 days at 4°C. In the case of fresh samples, antioxidant activity of the white cabbage external leaves is bigger than internal leaves, at red cabbage the internal leaves present higher antioxidant activity than external leaves. We did not found the same changes in the case of the samples kept at 4°C-10 days. The antioxidant activity of the external leaves of white cabbage presented a lower value than internal one and the red cabbage external leaves presented a bigger value than the internal one.

Regarding the antiradical activity of the fresh samples, determined with DPPH method, the value ranged from 48 µmol TE/100 g sample (white cabbage - external leaves) to 1429 µmol TE/100 g sample (red cabbage - external leaves). Both external and internal leaves of the white cabbage increase their antioxidant activity during storage.

Table 1

Antioxidant activity and total phenolics content of the sample

Samples	Morphological aspects	Fresh samples			Samples kept at 4°C – 10 days		
		ORAC (μM TE/100 g sample)	DPPH (μM TE/100 g sample)	Total phenolics (mg of GAE/100 g sample)	ORAC (μM TE/100 g sample)	DPPH (μM TE/100 g sample)	Total phenolics (mg of GAE/100 g sample)
De Buzău	Ext. leaves	862.84±14	48±3.21	29 ± 6.2	597.66±102	73.79±3.9	33.64±29
	Int. leaves	378±19	68.09±6.8	37 ± 19	806.73±165	71.21±2.9	38.95±6.5
Red cabbage	Ext. leaves	2949.42±439	1429±74.4	263 ± 13	3833.12±543	865.55±36	293.02±16
	Int. leaves	5264±846	735±90.4	256 ± 59	3514.28±45	1672±1.1	211.04±12.8

Red cabbage presented a decreasing of the external leaves antioxidant capacity and an increasing of the internal leaves antioxidant capacity during storage.

Red cabbage exhibits the highest content of total phenolics. The content of total phenolics registered an increase during the storage, except the case of the red cabbage internal leaves which decrease from 256 mg of GAE/100 g sample to 211 mg of GAE/100 g sample.

White cabbage cultivar showed a bigger amount of total phenolics in the internal leaves than the external leaves during the storage. In the case of red cabbage the external leaves seems to present more total phenolic content than the internal leaves. Variation in the antioxidant contents of *Brassica* vegetables is caused by many factors: variety, maturity at harvest, growing condition, soil state, and condition of post-harvest storage (Jeffery et al., 2003; Kurilich et al.,1999).

Table 2

Anthocyanins content of the red cabbage (mg CE/100 g sample)

Samples	Morphological aspects	Fresh samples	Samples kept at 4°C, 10 days
Red cabbage	Ext. leaves	345.44± 25.6	295.73 ± 8.26
	Int. leaves	202.25 ± 13.24	224.9 ± 16.04

The anthocyanins content (table 2) of the fresh external leaves decrease during storage from 345.44 mg CE/100 g samples to 295.73 mg CE/100 g samples, instead the content of the internal leaves is increasing.

Table 3

Correlations between antioxidant activity and total phenolics

Corellations	Fresh samples	Samples kept at 4°C, 10 days
ORAC - TP	$R^2 = 0.791$	$R^2 = 0.964$
DPPH - TP	$R^2 = 0.829$	$R^2 = 0.577$

Table 3 present the correlations between antioxidant activity, determined by ORAC and DPPH methods, and total phenolics. At fresh samples it was found a good correlation between DPPH and TP (R^2 = 0.829) and at stored samples corellatioms between ORAC and TP registered a high value (R^2 = 0.964). These correlations proves that TP are the major contributors to the antiradical activity of the cabbage samples analyzed.

CONCLUSIONS

1. Red cabbage had bigger antioxidant activity than white cabbage cultivar, in both methods tested.

2. Antioxidant activity of the external and internal leaves varied greatly among the cabbage cultivars used in this study and during the storage

3. Red cabbage is an important source of phenolic compounds and anthocyanins also.

4. Very good correlations were obtained between antioxidant activity, measured by ORAC assay, and total phenolics, in the case of samples stored. We can conclude that the 10 days storage at 4 °C improved antioxidant potential of the cabbage.

REFERENCES

1. **Andarwulan N., Batari R., Sandrasari D.A., Bolling B., Wijaya H., 2010 -** *Flavonoid content and antioxidant activity of vegetables from Indonesia*. Food Chemistry, 121, p. 1231–1235.
2. **Ayaz F.A., Hayırlıoglu-Ayaz S., Alpay-Karaoglu S. et al., 2008 -** *Phenolic acid contents of kale (Brassica oleraceae L. var. acephala DC.) extracts and their antioxidant and antibacterial activities*. Food Chemistry, 107, p. 19–25.
3. **Caboni E., Tonelli M. G., Lauri P., Iacovacci P., Kevers C., Damiano C., Gaspar T., 1997 -** *Biochemical aspects of almond microcuttings related to in vitro rooting ability*. Biol. Plant, 39, p. 91–97.
4. **Counet C., Collin S., 2003** - *Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate*. J. Agric. Food Chem., 51, p. 6816–6822.
5. **Hu S.H., Wang J.C., Kung H.F., Wang J.T., Lee W.L., Yang Y.H., 2004** -*Antimicrobial effect of extracts of Cruciferous vegetables.* The Kaohsiung Journal of Medical Sciences, 20, p. 591–599.
6. **Huang D., Ou B., Prior R. L., 2005** - *The chemistry behind antioxidant capacity assays*. Journal of Agricultural and Food Chemistry, 53, p. 1841–1856.
7. **Guisti M. M., Wrolstad R. E., 2001**- *Characterization and measurement of anthocyanins by UV–visible spectroscopy*. Unit F1.2. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Schwartz, S. J., Eds.; Wiley: New York, F1.2.1–F1.2.13.
8. **Isabelle M., Lee B.L., Lim M.T., Koh W.P., Huang D., Ong C.N., 2010 -** *Antioxidant activity and profiles of common vegetables in Singapore*. Food Chemistry, 120, p. 993–1003.
9. **Jeffery E.H., Brown A.F., Kurilich A.C., Keek A.S., Matusheski N., Klein B. P. et al., 2003** - *Variation in content of bioactive components in broccoli. Study review.* Journal of Food Composition and Analysis, 16, p. 323–330.

10. **Kim D.O., Padilla-Zakour O. I., Griffiths P. D., 2004** - *Flavonoids and Antioxidant Capacity of Various Cabbage Genotypes at Juvenile Stage*. Journal of Food Science 69 (9), p. 685-689.
11. **Kurilich A.C., Tsau G.J., Brown A., Howard L., Klein B. P., Jeffery E. H. et al., 1999 -** *Carotene, tocopherol, and ascorbate contents in subspecies of Brassica oleracea*. Journal of Agriculture and Food Chemistry, 47, p. 1576–1581.
12. **Kurilich A. C. et al., 2002** - *Antioxidant Capacity of Different Broccoli (Brassica oleracea) Genotypes Using the Oxygen Radical Absorbance Capacity (ORAC) Assay.* Journal of Agricultural and Food Chemistry 50, p. 5053-5057.
13. **Kyung K.H., Fleming H.P., 1994 -** *S-Methyl-L-Cysteine sulfoxide as the precursor of methyl methanethiolsulfinate, the principal antibacterial compound in cabbage*. Journal of Food Science, 59, p. 350–355.
14. **Tadolini B., Juliano C., Piu L.; Franconi F., Cabrini L., 2000 -** *Resveratrol inhibition of lipid peroxidation.* Free Radical Res., 33, p. 105–114.
15. **Wu X., Beecher G. R., Holden J. M., Haytowitz D. B., Gebhardt S. E., Prior R. L., 2004** - *Lipophilic and hydrophilic antioxidant capacities of common foods in the United States*. J. Agric. Food Chem., 52, p. 4026–4037.
16. **Zhou K., Yu L., 2006 -** *Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado*. LWT-Food Science and Technology, 39, p. 1155–1162.