Antioxidant Enzyme Activities of some *Brassica* Species

Rodica SOARE¹, Maria DINU², Cristina BĂBEANU^{3*} and Mihaela POPESCU⁴

¹University of Craiova, Faculty of Agriculture, 19 Libertatii St, 200583, Craiova, Romania

²University of Craiova, Faculty of Horticulture, 13, A.I. Cuza St, Craiova, Romania

³University of Craiova, Faculty of Science, 13, A.I. Cuza St, Craiova, Romania,

⁴University of Medicine and Pharmacy in Craiova, 2 Petru Rareș St, 200349, Romania

*Corresponding author, e-mail: cbabeanu@yahoo.com

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ABSTRACT

This paper set out to comparatively study five species: white cabbage (*Brassica oleracea* L. var. *capitata* alba Alef.), red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* Alef.), Kale (*Brassica oleracea* L. var. *Acephala*), cauliflower (*Brassica oleracea* var. *botrytis*) and broccoli (*Brassica oleracea* var. *cymosa*) in order to identify those with high enzymatic and antioxidant activities. The enzymatic activity of superoxide dismutase (SOD), catalase (CAT) and soluble peroxidase (POX) as well as the antioxidant activity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation were determined. Total superoxide dismutase activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium. Total soluble peroxidase was assayed by measuring the increase in A_{436} due to the guaiacol oxidation and the catalase activity was assayed through the colorimetric method. The capacity of extracts to scavenge the ABTS radical cation was assessed colorimetric using Trolox as a standard. The obtained results show that studied enzymatic activities and the antioxidant activity against ABTS vary depending on the analyzed species. So, among the studied *Brassicaceae* species, it emphasize red cabbage with the highest enzymatic activity (CAT 22.54 mM H_2O_2 /min/g and POX 187.2 mM ΔA /1min/1g f.w.) and kale with highest antioxidant activity, of 767 μ mol TE/100g f.w. The results of this study recommend introducing the studied varieties in diet due to the rich antioxidant properties.

Keywords: *catalase, peroxidase, superoxide dismutase, Brassicaceae*

INTRODUCTION

At present, a priority in the human diet, both nationally and internationally, is to identify natural sources of antioxidants. *Brassicaceae* vegetables are source of dietary nutrients and antioxidants (Apahidean *et al.*, 2010; Sing *et al.*, 2010). Those vegetables exhibit a rich content of phytochemicals such as polyphenols, anthocyanins, flavonoids, terpenes, glucosinolates or sulphur-containing compounds, S-methylcysteine sulfoxide, coumarins, vitamins, soluble sugars, minerals and antioxidant enzymes (Samec *et al.*, 2017; Soare *et al.* 2016; Podsdek, 2005).

The level of chemical composition vary depending on crop types, species, cultivars and

their different parts (Bhandari and Jung, 2015). Lately, consumption of vegetables containing significant amounts of phytochemicals has been associated with reduced risk of developing diseases and decreased effects of aging (Gul *et al.*, 2013, Kaulmann *et al.*, 2014). Particular attention has been paid to plants rich in compounds with antioxidant activity or other health-promoting properties which scavenge and inhibit the reactions of free radicals and protect the cell against oxidative damage. Free radicals that occur normally during aerobic metabolism are highly reactive molecules, and if they are formed in excess, they play a causal role in the pathology of many diseases. The enhanced reactive oxygen species

(ROS) production is scavenging by a versatile antioxidant system that modulates intracellular ROS concentration and sets the redox-status of the cell (Cruz de Carvalho, 2008). The major scavenging mechanisms include enzymatic antioxidant systems (superoxide dismutase-SOD, ascorbate peroxidase-APX, glutathione reductase (GR), catalase (CAT) and non-enzymatic antioxidants: glutathione, ascorbic acid, tocopherol, carotenoids and phenolic compounds.

Vegetables of *Brassica* are used raw in the form of salads, juices, dehydrated or frozen in an assortment of preparations much appreciated by consumers. *Brassicaceae* vegetables are an abundant source of health-promoting substances, which reduce the risk of diseases (Soare *et al.*, 2016; Leja *et al.*, 2010). Among these, the most important in terms of diet is the antioxidant property.

The crude extract and different fractions of *Brassica rapa* L. merits considerations to be used as future components in a range of phytochemicals composition and/or different pharmaceutical usage (Gul *et al.*, 2013). Many studies report on the relationship between the consumption of *Brassica* vegetables and the protection against many chronic diseases, cardiovascular and cerebrovascular diseases, neurological diseases, diabetes, cancer, hypertension or obesity (Kapusta-Duch *et al.*, 2012).

The mechanism of preventive action of cruciferous vegetables is still not clarified. Many studies have shown that phytochemicals present in these plants are able to prevent oxidative stress, induce detoxification enzymes, stimulate the immune system and inhibit tumor cell growth, malignant transformation and carcinogenic mutations (Kapusta-Duch *et al.*, 2012; Podsedek, 2005). However, not all *Brassica* species have equal nutritional composition, in the phytochemical profiles of species and varieties there are differences among these. In this respect, the objective of this study was to evaluate and compare the enzymatic antioxidant activity and total antioxidant capacity of five *Brassica* vegetables cultivated in the pedoclimatic conditions in southern Romania: white and red cabbage, kale, broccoli and cauliflower and identifying the variety as a good source of natural bioactive compounds. In order to achieve this, enzymatic activity of catalase, peroxidase and superoxide dismutase, as

well as antioxidant activity against ABTS radical cation were determined.

MATERIAL AND METHODS

The biological material was represented by five species of *Brassica*: white cabbage (*Brassica oleracea* L. var. *capitata* f. *alba* Alef.), red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* Alef.), kale (*Brassica oleracea* L. var. *Acephala*), cauliflower (*Brassica oleracea* var. *botrytis*) and broccoli (*Brassica oleracea* var. *cymosa*). These species were grown in southern Romania, on a chernozem soil, in the field. To achieve the objective of this work, samples were taken from each species, edible parts, then brought to the laboratory and homogenized.

Chemicals and Reagent

Sigma Aldrich supplied the used methanol for the extraction, L-ascorbic acid, methionine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). Nitro-blue tetrazolium chloride (NBT) and riboflavin were purchased from Carl Roth. All the other used chemicals were of analytical grade.

Methods

For antioxidant enzymes extraction, fresh tissue was homogenised with 0,1M phosphate buffer (pH 7.5) containing 0,1mM EDTA. The homogenates were centrifuged for 20 min at 6000 r.p.m. and the supernatants were used for enzyme assays. In addition for antioxidant activity determinations was prepared an extract in 80% aqueous methanol (1:10 w/v) at 24°C for 16 h.

The Superoxide dismutase (EC 1.15.1.1) activity (SOD) was assayed by measuring ability of sample extract to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Babeanu *et al.*, 2017). SOD activity was expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of NBT.

Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) activity (POX) was assayed by measuring the increase in A_{470} due to guaiacol oxidation to tetraguaiacol on addition of H_2O_2 and their activity was expressed as $\Delta A/\text{min}/1g$ f.w. (Babeanu *et al.*, 2017).

Catalase activity (E.C.1.11.1.6) CAT was assayed through the spectrophotometric method at 240 nm and expressed as $\text{mM } H_2O_2/\text{min}/g$ f.w. at 25°C.

Tab. 1. The activity of antioxidant enzymes in species of *Brassica*

Species	CAT mM H ₂ O ₂ /min/g	POX ΔA/1min/1g f.w	SOD IC50%(mg)
White cabbage	17.8 4±0.66b	66.25 ± 0.95c	28.35 ± 0.85b
Red cabbage	22.54 ± 2.44b	187.2 ± 10.8a	3.85 ± 0.95d
Kale	33.6 ± 3.4a	54.6 ± 4.6c	32.4 ± 2.3a
Cauliflower	7.34 ± 0.57c	10.6 ± 1.1d	13.9 ± 0.3c
Broccoli	36.18 ± 0.98a	121.2 ± 13.8b	4.83 ± 0.87d
LSD ≤0.05%	5.81	24.11	3.67

Note: Values represent the mean and SE.

Values in the same column followed by different letters are significantly different at $p < 0.05$

ABTS radical cation scavenging activity: the capacity of extracts to reduce the radical cation ABTS was measured colorimetric at 734 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer. A blank reagent was used to study stability of ABTS over the test time. The scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0 - (A_1 - A_s)]/A_0 \times 100$, where A_0 is the absorbance of ABTS alone, A_1 is the absorbance of ABTS + extract and A_s is the absorbance of the extract only. The trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The results were expressed as μM trolox equivalents (TE) per 1g f.w. (Babeanu *et al.*, 2017).

Statistics

The analysis of variance was performed on data for differences between and within the varieties using the ANOVA. Mean separations were determined by least significant difference (LSD) at $p \leq 0.05\%$ and the Pearson correlation coefficients were calculated.

RESULTS AND DISCUSSIONS

The obtained results show that the activity of antioxidant enzymes varies with the investigated variety.

SOD is an enzyme localized in cytosol, chloroplast, mitochondria and peroxisomes and catalyses the dismutation of the superoxide anion into oxygen and hydrogen peroxide. The results obtained for superoxide dismutase activity, expressed as IC 50% (mg), vary between 3.85 mg (red cabbage) and 32.4 mg (kale) (Tab. 1). A low IC 50% expressed as the amount of sample (mg) which caused 50% inhibition of photochemical reduction of nitroblue tetrazolium correspond with high superoxide dismutase enzymatic

activity. In studies, there is little data on the activity of antioxidant enzymes in the investigated species.

In a study that aimed to determine the variability and inheritance of antioxidant enzymes in cabbage, authors report for 33 genotypes of white cabbage a superoxide dismutase activity range between 4.86 and 7.43 units of SOD $\text{min}^{-1}\text{g}^{-1}$ f.w. and for three genotypes of red cabbage the superoxide dismutase activity range between 7.09 and 7.63 units of SOD $\text{min}^{-1}\text{g}^{-1}$ f.w. (Singh *et al.*, 2010).

Some authors reports for superoxide dismutase activity of broccoli at different storage temperatures values between 110 units/g f.w at 5 °C and 130 units/g f.w at 20 °C (Zang *et al.*, 2009). Other authors observes for two broccoli cultivars kept four days at 13 °C superoxide dismutase activity 30-55 units/mg protein (Emperor cultivar) and 50-60 units/mg protein (Greenbelt cultivar) and supposes that the antioxidant protection offered by SOD is important to retention of green color in broccoli (Toivonen and Sweeney, 1998). In another study for broccoli and red cabbage purchased from a market is presented superoxide dismutase activity at 38 U/g respectively 35 U/g (Erguder *et al.*, 2007).

Peroxidase (POX) is non-heme iron protein that is mostly located in the cell wall. This enzyme has been recognized to be involved in the control of development, lignification, pathogen defense and the catabolism of growth regulators (Van Huystee, 1987). The results obtained for peroxidase activity are shown in table 1. In the case of peroxidase the enzymatic activity ranges from 10.6 $\Delta\text{A}/1\text{min}/1\text{g}$ f.w. to 187.2 $\Delta\text{A}/1\text{min}/1\text{g}$ f.w. The highest value for peroxidase activity was determined at red cabbage (187.2 $\Delta\text{A}/1\text{min}/1\text{g}$ f.w.) followed by broccoli (121.2 $\Delta\text{A}/1\text{min}/1\text{g}$

Fig. 1. The ABTS radical cation scavenging activity of investigated *Brassica* vegetables
Values in the same column followed by different letters are significantly different at $p < 0.05$

f.w.), white cabbage (66.25 $\Delta A/1\text{min}/1\text{g f.w.}$), kale (54.6 $\Delta A/1\text{min}/1\text{g f.w.}$) and cauliflower (10.6 $\Delta A/1\text{min}/1\text{g f.w.}$).

Catalase, absent in chloroplast is localized in mitochondria and peroxisomes. This enzyme catalyses the reduction of H_2O_2 generated during photosynthesis to H_2O and molecular oxygen. Catalase and peroxidases are among the most studied enzymes in fruits and vegetables. Catalase and peroxidase have different affinities for reactive oxygen species and appear to have different cellular roles in H_2O_2 scavenging. Catalase does not need a reductant to scavenge H_2O_2 making it reducing power-free, whereas peroxidase needs a reductant. Catalase has a lower affinity for H_2O_2 (mM range) than peroxidase (μM range) (Cruz de Carvahlo, 2008). Peroxidase may function as a fine regulator of intracellular reactive oxygen species at steady-state levels, possibly for signaling purposes, whereas catalase might function as a bulk remover of excess reactive oxygen species production under stress conditions (Podsedeck, 2007).

In the present study, the highest value for catalase activity (CAT) was determined at broccoli 36.18 $\text{mM H}_2\text{O}_2/\text{min}/\text{g}$ followed by kale 33.6 $\text{mM H}_2\text{O}_2/\text{min}/\text{g}$, red cabbage 22.54 $\text{mM H}_2\text{O}_2/\text{min}/\text{g}$, white cabbage 17.84 $\text{mM H}_2\text{O}_2/\text{min}/\text{g}$ and cauliflower 7.34 $\text{mM H}_2\text{O}_2/\text{min}/\text{g}$ (Tab. 1).

In many research reports, *Brassica* vegetables have been studied in terms of antioxidant activity using different antioxidant systems and methods. The variation of the antioxidant potential depends

on the stage of maturity of the plant or the parts of the analyzed plant, the culture method, the environmental conditions, and also by post-harvest storage, industrial processing, and different cooking (Soare *et al.*, 2016; Soengas *et al.*, 2011; Posedek 2007). The enzymatic activities and the antioxidant activity against ABTS vary depending on the analyzed cultivar (Babeanu *et al.*, 2017).

The antioxidant activity of the studied *Brassica* vegetables was determined by the ability of extracts to reduce the ABTS radical cation. The results obtained are shown in figure 1.

All results showed significant ABTS radical cation scavenging activity. The values of ABTS radical cation scavenging activity for samples ranged from 129.7 $\mu\text{mol TE}/100\text{g f.w.}$ (white cabbage) to 767.0 $\mu\text{mol TE}/100\text{g f.w.}$ (kale) and decreased as follows: kale, red cabbage, broccoli, cauliflower, white cabbage. Our results are in agreement with literature data for investigated *Brassica* vegetables which are reported to have high antioxidant activities. In a study that aimed to determine the antioxidant activity of ten Mauritan vegetables, the authors reports for white cabbage antioxidant activity performed in terms of ABTS radical scavenging ability 1.73 $\mu\text{M Trollox equivalent}/\text{g f.w.}$, for cauliflower 2.09 $\mu\text{M Trollox equivalent}/\text{g f.w.}$ and for broccoli 2.85 $\mu\text{M Trollox equivalent}/\text{g f.w.}$ (Bahorun *et al.*, 2004).

According to other authors the antioxidant activity registered at *Brassica* species the following values: 17.7 $\mu\text{M TE}/\text{g}$ at kale, 8.9 μM

Tab. 2. Correlations between chemical compounds analyzed for *Brassica*

Chemical compound	CAT (mM H ₂ O ₂ /min/g)	POX (ΔA/1min/1g f.w)	SOD IC50%(mg)
CAT (mM H ₂ O ₂ /min/g)	1		
POX (ΔA/1min/1g f.w)	0.397	1	
SOD IC50% (mg)	1.158	-0.623	1
ABTS (μmol TE/100g f.w) <i>p</i> <0.05	0.500	0.169	0.662

TE/g at broccoli, 3.8 μM TE/g at cauliflower, 3 μM TE/g at cabbage (Cao *et al.*, 1996) and in hydrophilic extract: 22.32 μM TE/g at red cabbage, 14.18 μM TE/g at broccoli, 13.39 μM TE/g at white cabbage, 6.1 μM TE/g at cauliflower (Wu *et al.*, 2004). In a study of 27 *Brassica* varieties, in which they estimated antioxidant capacity authors showed that the highest value was in kale (381mg VCE/100g) (Kaulmann *et al.*, 2014).

The obtained results are difficult to compare with other literature data due to different measurement units and extraction mode. Heimler *et al.*, 2006 studies seven local edible *Brassicaceae* and reports that the antioxidant activity expressed in EC50% (mg sample/mg DPPH.) varies as in the following order: broccoli, kale, white cabbage, cauliflower. Haytowitz and Bhagwat 2010 report the total oxygen radical absorbance capacity (ORAC) for fresh cabbage is between 498 and 1784 μmol TE/100 g which ranks white cabbage as vegetable with moderate antioxidant activity. In most studies, high antioxidant activity is presented in kale, red cabbage and broccoli.

In terms of correlations between the chemical compounds analysed in *Brassicaceae* are in all cases positive except for POX and SOD, which are negative (-0.623). The results obtained for the activity of antioxidant enzymes does not correlate significantly with total antioxidant activity measured by the ABTS radical cation method suggesting that the latter is due to the presence of other compounds with antioxidant properties such as: ascorbic acid, polyphenols, carotenoids (Tab. 2). Soare *et al.*, (2015) showed that the antioxidant activity measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method is correlated positively significantly with the content of polyphenols and flavonoids.

CONCLUSIONS

The obtained results show that studied enzymatic activities and the antioxidant activity against ABTS vary depending on the analyzed species. For all species a high antioxidant enzymatic activity was reported and as a result it is recommended the consumption of these *Brassica* species.

The superoxide dismutase activity and peroxidase registered higher values in red cabbage, the highest value for catalase activity was determined in broccoli, and the highest value for antioxidant activity in kale.

The results of this study recommend introducing the investigated species in diet due to the rich antioxidant enzymatic properties. Also, the cultivation of *Brassica* species such as Broccoli or Kale, is an opportunity for native farmers to diversify the range of vegetables and promote them as nutraceuticals foods.

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