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RESEARCHES REGARDING THE ANTIOXIDANT POTENTIAL OF SELECTED *Brassicaceae* VEGETABLES REPRESENTATIVE FOR HUMAN NUTRITION

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Abstract

Brassica vegetables belong to the Cruciferous family and include different kinds of cabbage (white, red), cauliflower and broccoli. These vegetables are recognised for their contribution to human health and nutrition. Brassica vegetables are extensively studied, analysed and characterised lately due to their antioxidant character and antioxidant capacity. The aim of the study was to investigate the antioxidant capacity of four white cabbage varieties ('De Buzău', 'Buzoiana', 'Măgura', 'De Işalniţa'). The content of total polyphenolics of fresh vegetables (Folin Ciocalteu procedure) was assessed as well as carotenoids and chlorophyll pigments (spectrophotometric methods) and vitamin C content (titrimetric assay using 2,6-dichlorphenol indophenol). The antioxidant activity of the vegetables was determined according to DPPH (2,2-diphenyl-1-picrylhydrazyl) protocol. The results showed the highest concentration of antioxidant compounds for Magura variety, while the lowest one was for De Isalnita variety. A study regarding stability of antioxidant capacity during storage for three months was performed too.

Key words*: Brassicaceae, cabbage, antioxidant capacity, polyphenolics, carotenoids, chlorophyll.*

INTRODUCTION

Recent research associated the reduced risk of cardiovascular disease with a rich diet in fruit and vegetables (Kaur & Kapoor, 2001). They are also an excellent source of antioxidants as polyphenolic compounds, carotenoid and chlorophyll pigments and vitamins (ascorbic acid, vitamin E, vitamin K) (Krinsky, 2001). The nutritional quality of vegetables has been extensively studied focusing on the role of diet in human health (Tiwari & Cummnis, 2013).

Brassica vegetables represent a group of horticulture species that are very important in human nutrition (Cartea & Velasco, 2008). Vegetables from the Brassicaceae family are known for their antioxidant properties that are scientifically correlated with lower risk of developing prostate cancer by 40%. Cruciferous are an excellent source of antioxidant and glucosinolate vitamins, being the precursors of a group of isocyanates that have been shown to be compounds with anticarcinogenic activity (Kris-Etherton et al., 2002).

White cabbage (*Brassica oleracea* var. *capitata* f. *alba*) is among the world's most commonly cultivated vegetables. Due to its affordable price and availability at local markets, white cabbage stands out as an important source of phytonutrients in the human diet. It may be stored raw for long periods of time and hence could be available throughout the year. Throughout history it has also been known as 'medicine for the poor' and has been used for the general improvement of health and the treatment of various inflammation and gastrointestinal (Hatfield, 2004; Cavender, 2006; Passalacqua et al., 2007). White cabbage is an inexpensive, very nutritive source of food, providing nutrients and health-promoting phytochemicals.

Phytochemicals have attracted much recent scientific attention and it is well known that white cabbage is a significant source of

glucosinolates, phenolic compounds, carotenoids and various vitamins. Several reviews have been published on the phytochemistry and health benefits of *Brassica* vegetables (Podsędek et al., 2006; Cartea et al., 2011; Jahangir et al. 2009; Björkman et al., 2011; Kapusta-Duch et al., 2012; Avato & Argentieri, 2015), but to the best of our knowledge nobody has focused specifically on white cabbage (*Brassica oleracea* var. *capitata* f. *alba*).

Polyphenolic compounds or "phenolics" are a complex group of compounds of plant origin. (Blasco Antonio J. et al., 2005). The interest of phenolic acids is continuously increasing because of their antioxidant properties among others (Cadenas E. & Packer, 1996). The "total phenolics" determination is very difficult because of their chemical complexity, difficult extraction from plant matrix, and the presence of complex interferences in food samples. Total phenolics in white cabbage has been reported by different authors (Kaulmann et al., 2014; Vicas et al., 2013; Deng et al., 2013) and the range of concentrations was between 9.3-1043.6 mg galic acid/100 g fresh weight.

Vitamins and carotenoids are essential compounds which promote human health and are responsible for accurate functioning of human metabolism and immune response. Considerable attention was focused on ascorbic acid (AA), known for its reductive properties and for its use on a wide scale as an antioxidant agent in foods; it is also important for therapeutic purposes and biological metabolism (Raoof, Ojani & Beitollahi, 2007). Due to its properties, vitamin C represents an important quality indicator of foodstuffs (Wawrzyniak, Ryniecki & Zembrzuski, 2005) and contributes to the antioxidant capacity of food (Glevitzky et al., 2008; Popa et al., 2010; Pisoschi et al., 2008; Pisoschi et al., 2010; Pisoschi et al., 2011). Vitamin C concentration in white cabbage ranges between 23.0-55.8 mg ascorbic acid/100 g fresh weight (Podsęsdek et al., 2006; Tiwuri & Cummins, 2013).

The determination of leaf pigment content is another important analytical tool in the field of plant physiology (Pompelli et al., 2012). Therefore, the chlorophyll (Chl) level is an accurate indicator of plant vigour and is routinely measured in physiological research. Carotenoids are synthesized by all plants and many microorganisms (bacteria and fungi), but not by animals, including humans, who therefore rely on dietary uptake. Due to the correlation of carotenoid intake and chronic diseases, methods allowing the rapid, accurate determination of carotenoids in these matrices are highly desired. Without prior separation carotenoids may be determined in plants together with chlorophylls, absorbing at similar wavelengths (Bieler et al., 2010). In white cabbage, the level of chlorophyll varies between 1.5 and 3.2 mg/100 g fresh weight and the level of carotenoids ranges between 0.01and 0.12 mg/100 g fresh weight.

According to the USDA National Nutrient Database for Standard Reference and dietary intake recommendations for adults (USDA), the white cabbage contains approximately 72 % of the recommended daily value (DV) for vitamin K, and 44 % of DV, 11 % of DV and 10 % of DV for vitamin C, folate and vitamin B6, respectively.

The variation in antioxidant content is caused by many factors such as geographical region, climate, variety, harvest maturity, growth conditions, soil condition and post-harvest conservation and processing method (Goncçalves et al., 2004).

The aim of the present work was the assessing of antioxidant capacity of white cabbage varieties (*Brassica oleracea* var. *capitata* f. *alba*) correlated with total phenolics, caretonoids and chlorophyll content and vitamin C level. The stability of these biochemical parameters was monitored during three months storage period.

MATERIALS AND METHODS

Materials

Four white cabbage varieties were analysed: 'De Buzău', 'Buzoiana', 'Măgura' and 'De Işalniţa'. All varieties were cultivated (Research and Development Station for Vegetables Buzău, Romania) in the same conditions, the same location, with the same agro-technical practices and harvested when reached the optimal maturity.

After harvesting the samples were transported and analysed at the Food Chemistry Laboratory. Then were selected the

inflorescences without infection or mechanical damage weighting about 2 kg each one.
Chemical substances 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,6 diclorphenol-indophenol were purchased from SIGMA-ALDRICH CHEMICAL CO. Meta-phosphoric acid, ethylendiaminetetraacetic acid, sodium hydrogen carbonate and sodium carbonate were purchased from ROTH. Folin-Ciocalteau reagent and ascorbic acid were purchased from MERCK. The organic solvents (methanol and acetone) were of analytical grade (MERCK).

Methods
Fresh samples were cleaned, cut and homogenized for optimum results. Methanol: water (1: 1, v/v) and acetone: water (80: 20, v/v) were used for extraction. Triplicates were prepared for each one.

Determination of total phenolics
The phenolics content was measured with Folin-Ciocalteau reagent (Singleton & Rossi, 1965) using gallic acid as standard. The samples were prepared by mixing 1 ml with 5 ml Folin-Ciocalteau reagent and 4 ml sodium bicarbonate (7.5% w/v). The solution was kept in the dark, at room temperature, for 20 min; the absorbance was measured at 752 nm with a Specord 210 spectrophotometer (Analytic Jena, Germany). Total phenolics content was expressed as mg gallic acid equivalents per 100 g fresh weight (mg GAE/100 g FW), calculated based on a calibration curve obtained with 1 mg/ml gallic acid solution.

Determination of ascorbic acid
The dye-titration method was used, according to AOAC procedure, 2006. Metaphosphoric acid extracts of vegetables were analysed by titration with 2,6-dichlorophenolindophenol reagent (DCIP). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidized to dehydroascorbic acid and the indophenol dye reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose pink colour in acid solution. The tests were carried out on white cabbage. Dehydroascorbic acid was not analysed in this study. The results were expressed in mg ascorbic acid/100 g fresh weight.

Determination of pigments
Carotenoid and chlorophyll pigments were extracted from 3 g fresh white cabbage using a mixture of acetone/water (80: 20, v/v/). The final mixture was vortexed (15 min., 2000 rpm, 20°C) and centrifuged (15 min., 3500 rpm. 20°C). The obtained extract was filtrated and the absorbance was recorded at 470, 646, 663 nm with Specord 210 spectrophotometer (Analytic Jena, Germany) as described by Lichtenthaler (1987). The results were expressed in µg /1 g fresh weight.

Determination of antioxidant capacity using DPPH protocol
The method is based on the color modification (from purple to yellow) of DPPH (2,2-difenyl-1-picrylhydrazyl) radical.
A modified protocol was used (A. Culetu et al., 2016) and consisted in extraction of the samples in methanol:water (1: 1, v/v). One ml of extract was treated with 6 ml DPPH. Following a 30 minutes rest in the dark, the absorbance at 517 nm was measured with a Specord 210 spectrophotometer (Analytic Jena, Germany).
The results were expressed in µmol Trolox/g fresh weight.

Stability of cabbage varieties
In order to determine the stability over time, cabbage samples were stored in the Vegetables-Fruits Section at a temperature of 20°C, for 3 months period. After two months and three months, the same parameters was re-analysed for the selected cabbage samples.

RESULTS AND DISCUSSIONS

Samples of the white cabbage varieties were assayed for antioxidant phytonutrients: phenolics, vitamin C, chlorophyll and carotenoid pigments. Total phenolics content (TP) ranged between 5.78 and 7.28 mg GAE/g FW, with highest value for ‘De Buzău’ cabbage variety (Figure 1). The obtained results showed rather similar level of TP for ‘De Buzău’, ‘Buzoiana’ and ‘Măgura’ varieties, while for ‘De Işalniţa’ variety this was about 20% lower.

It is difficult to compare these results with those of other authors because the reported values are spread over a wide range (9.3-1043.6 mg GAE/100 g FW). This high variability suggests that TP content may be influenced by a lot of factors, not only by the tested variety, geographic origin, agro-technical practices, harvest time, but also by extraction method and analytical determination parameters.

Regarding the ascorbic acid (AA) content, the highest value was registered for 'Măgura' cabbage variety with 42.85 mg/100 g FW (Figure 1). The obtained results are similar to those reported by other authors (Podsędsek et al., 2006; Tiwuri & Cummins, 2013).

The tested white cabbage varieties showed close values, the results obtained for the chlorophyll pigments being in the order: 'Buzoiana' variety > 'Măgura' variety > 'De Buzău' variety > De Işalniţa variety (Figure 1).

In terms of carotenoids, the highest value was recorded for 'Măgura' variety (2.73 µg/g) and the lowest for 'De Buzău' variety (1.7 µg/g) (Figure 1). The obtained results are close to previously published works of Fernandez-Leon et al. (2014).

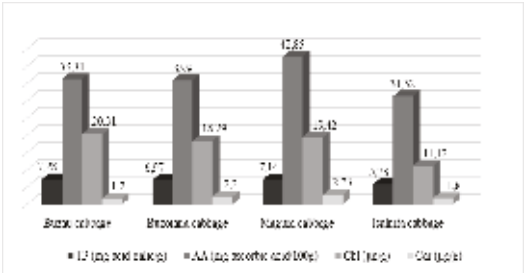

Figure 1. Variation of antioxidant parameters for different varieties of cabbage (TP - total phenolics; AA - ascorbic acid; Chl - chlorophyll; Car – carotenoid)

The antioxidant capacity, based on DPPH radical scavenging activity, assayed for cabbage varieties showed comparable results for 'De Buzău', 'Buzoiana' and 'Măgura' varieties, and about 25% lower for 'De Işalniţa' variety (Figure 2). Best correlation (r^2 0.899) was found between antioxidant capacity and the total phenolic content of the selected cabbage varieties. This suggests that total phenolic content may be used to predict the antioxidant activity of cabbage.

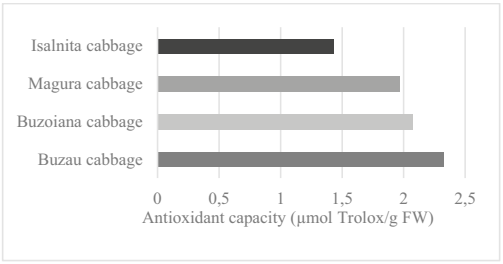

Figure 2. Antioxidant capacity of cabbage varieties using DPPH radical

The stability of antioxidants during cabbage storage was assayed for three months. After two months of storage an insignificant decrease of the amount of phenolic compounds was registered (Figure 3).

But the determinations made after three months showed 30% lower level of the total phenolics for all the tested cabbage varieties ($p<0.05$).

These results suggest that phenolic compounds were affected by abiotic factors (light, temperature, relative humidity) especially after 60 days storage.

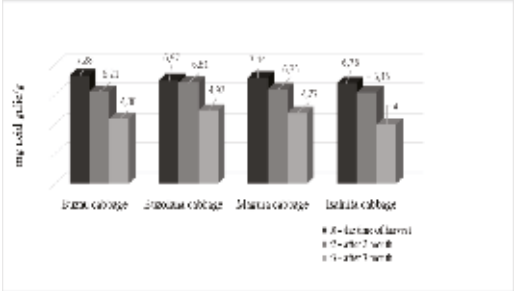

Figure 3. Stability of phenolics during storage

Analysing the stability of vitamin C during storage, the results presented in Figure 4 showed a significant decrease of the obtained values after two months ($p<0.05$).

The results confirm ascorbic acid sensitivity to degradation during handling and storage of fruits and vegetables (Balan et. al., 2016; Tiwuri & Cummins, 2013).

The loss of ascorbic acid is attributed to the conversion to dehydroascorbic acid and further on to 2,3-diketogulonic acid, favoured by exposure to oxygen, heavy metals, alkaline pH and high temperature.

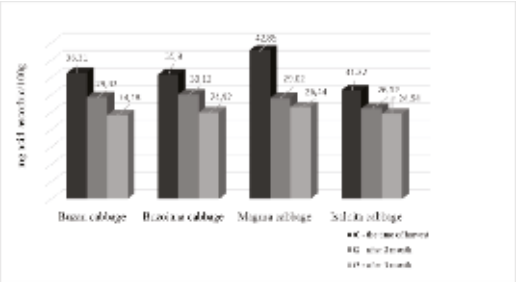

Figure 4. Stability of ascorbic acid during storage

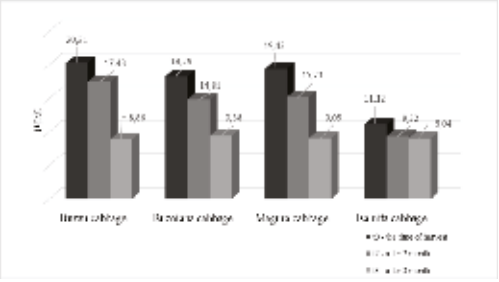

Figure 5. Stability of chlorophyll during storage

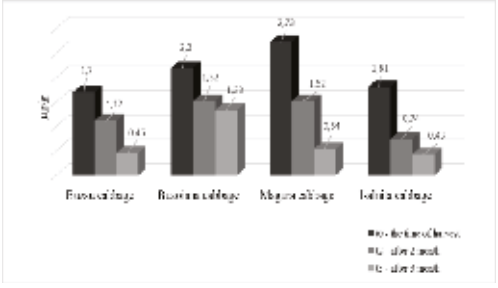

Figure 6. Stability of carotenoids during storage

With regard to chlorophyll and carotene pigments, it can be seen that they decreased over time, along with storage. Their degradation is due to the temperature and lack of natural light (Figures 5 and 6).

CONCLUSIONS

The antioxidants content in cabbage depends on the tested varieties. The highest concentration of antioxidant compounds was found in 'Măgura' variety, while the lowest one in 'Işalniţa' variety. Antioxidant capacity assayed with DPPH method was best correlated with total phenolic content. Cabbage storage for three months period at 20°C resulted in significant decrease of antioxidant compounds, especially vitamin C and chlorophyll and carotenoid pigments.

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