ANTIOXIDANTS OF VEGETABLE-POTENTIAL NATURAL PRESERVATIVES

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Summary

Cabbage (Brassica oleracea var. capitata forma rubra) was cultivated under controlled plastic-covered greenhouse conditions using standard production methods. During harvest maturity, cabbagehead sampling was performed for chemical analysis. The objective of this study was to investigate the correlation between total phenolic content and antioxidant activity of ethanol extracts of cabbage. Total phenols were evaluated by the Folin-Ciocalteu spectrophotometric method. Antioxidant activity, defined as the DPPH radical neutralizing ability, was also determined by spectrophotometry. Results show that the total phenolic content was higher in cabbage macerate (E_1) (0.0577±0.0001 g GAE/100g sample) than in (E_2) ultrasonic extract (0.0811±0.0001 g GAE/100g sample). High values of antioxidant activity were identified (91.67 % for E_1 and 92.67 % for E_2).

Key words: cabbage, antioxidant, extract

Introduction

Different parts of plants (roots, leaves, flowers, fruit, stem, bark) have been successfully used to treat numerous diseases (Barros et al., 2007). Owing to their antioxidant activity, they can influence a number of physiological processes, thus protecting the organism from the damaging effect of free radicals and inhibiting the development of unwanted microorganisms (Velioglu et al.,1998). However, synthetic antioxidants, such as butylated hydroxyltoluene (BHT) and butylated hydroxyanisole (BHA), known for their ability to terminate the chain reaction of lipid peroxidation, have been proven to be carcinogenic and to cause liver damage (Kahl and Kappus, 1993) . The use of plants in the food industry in place of synthetic preservatives, antioxidants or other food additives has significantly increased over the last few years due to their ability to produce biologically active substances (Hinneburg et al.,2006).

Experimental Part

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). The plant material used in the experiment included dried red cabbage grown under plastic-covered greenhouse conditions in Cacak.

Spectrophotometric measurements were performed using an MA9523-SPEKOL 211 UV-VIS spectrophotometer (ISKRA, Horjul, Slovenia).

Dried cabbage leaves (20 g) were macerated in a mixture of 95% ethanol (250 ml) and 0.5% glacial acetic acid at room temperature for 24 hours. The resulting macerate was filtered and the maceration procedure was repeated once. The extracts obtained were combined and concentrated until dry in a rotary vacuum evaporator to produce the (E_1) extract (Hettiarachchy et al.,1996). The (E_2) extract was obtained by ultrasound-assisted extraction using a Brason B-220 ultrasonic bath (Smith-Kline Company, USA).

The typical procedure involved ultrasound-assisted extraction of crushed plant material with 95% ethanol over a period of 1 hour.

Total phenols were estimated using the Folin-Ciocalteu method (Brighent et al.,2007). Plant extracts were diluted to a concentration of 1 mg/mL, and aliquots of 0.5 mL were

mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted tenfold with distilled water) and 2 mL of $NaHCO_3$ (7.5%). After heating for 15 min at 45˚C, the absorbance was measured at 765 nm in a spectrophotometer against blank sample. Total phenols were determined as gallic acid equivalents (mg GA/g extract), and the values are presented as means of triplicate analyses (PH. JUG. IV,1984).

The method used by (Yen et al., 2002). was adopted with suitable modifications from (Zheng and Wang, 2001). DPPH (8 mg) was dissolved in C_2H_5OH (100 mL) to obtain a concentration of 80 µg/mL. Serial dilutions were carried out with the stock solutions (1 mg/mL) of the extracts. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 510 nm. Ascorbic acid (AA), gallic acid (GA) and butylated hydroxytoluene (BHT) were used as reference standards and dissolved in methanol to make a stock solution at the same concentration

(1 mg/mL). Control sample was prepared containing the same volume without test compounds or reference antioxidants. Ninety-five percent ethanol was used as blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\% \text{ inhibition} = [(A_c - A_s) / A_c] 100.$$

Results and Discussions

The total phenolic content of the (E_1) ethanol extract was 0.0577±0.0001 g GAE/100g of sample, and that of the (E_2) extract 0.0811±0.0001 g GAE/100g of sample. The results obtained were calculated as average values of five parallel measurements. High values of antioxidant activity were identified, being 92.67 % and 91.67 % for the E_1 and E_2 extracts, respectively. Figures 1. and 2. show graphic presentation of the antioxidant activity of the E_1 and E_2 extracts.

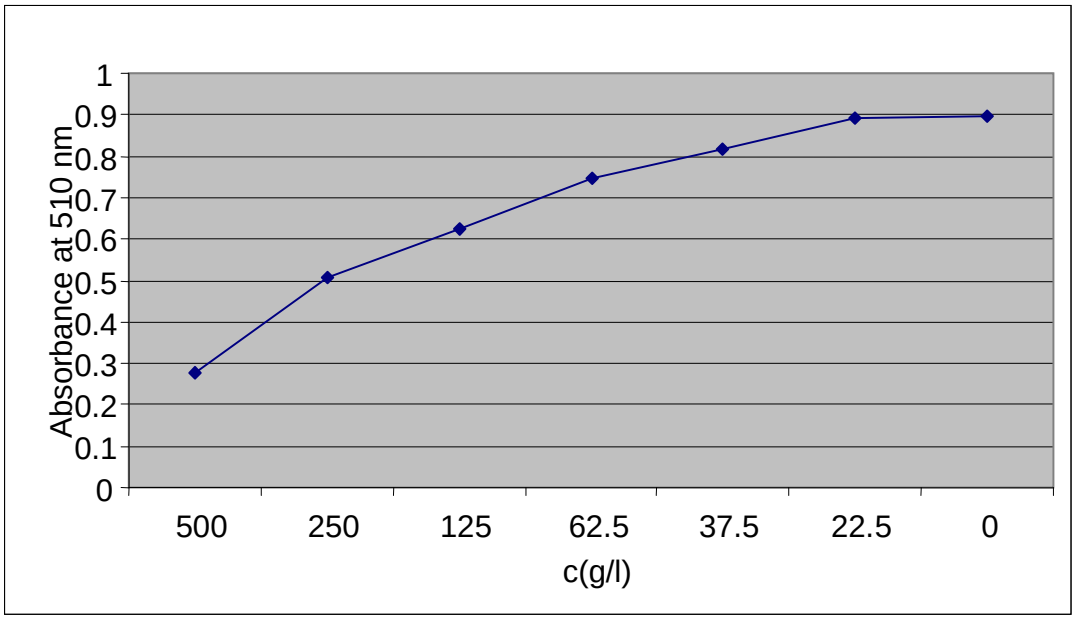

Fig. 1. Antioxidant activity of the E_1 extract

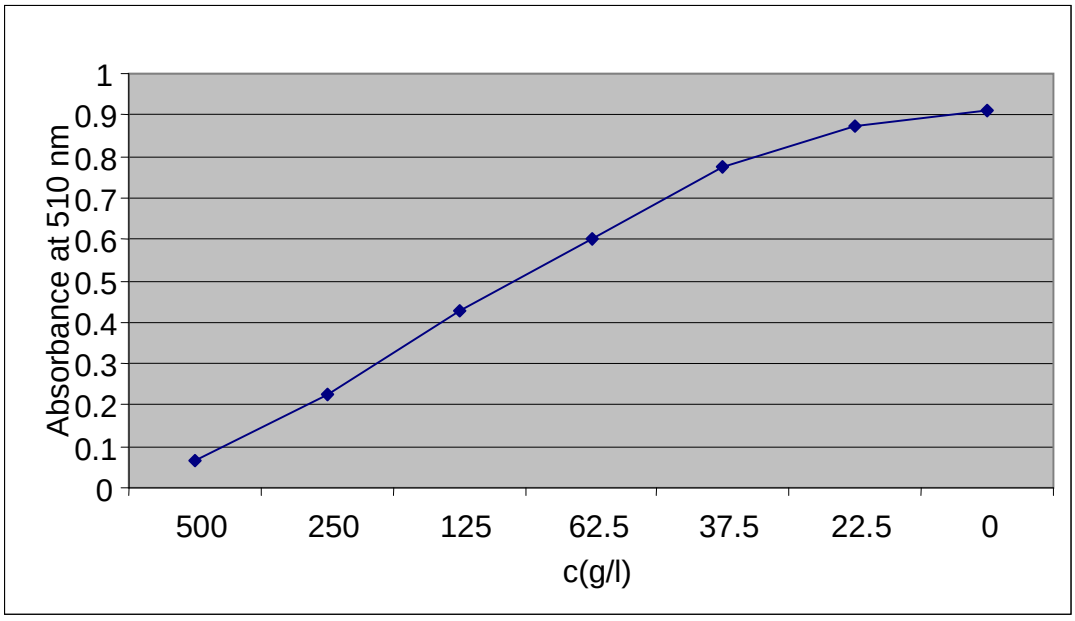

Fig. 2. Antioxidant activity of the E_2 extract

The DPPH capacity values were calculated relative to rutin, trolox and quercetin. (Table 1). The values were calculated based on the graph representing dependence between standard concentration (mg/g DPPH) in the X-axis and capacity of DPPH radicals (%) in the Y-axis.

Rutin c= (% DPPH- 1,29481) / 0,37472

Troloks c= (% DPPH- 0,24856) / 0,38094

Quercetin c= (% DPPH- 12,09185) / 5,20323

Extract	mg/g DPPH	standard
Ethanol E_1	0.24385	Rutin
Ethanol E_2	0.24118	Rutin
Ethanol E_1	0.24261	Trolox
Ethanol E_2	0.23999	Trolox
Ethanol E_1	0.01549	Quercetin
Ethanol E_2	0.01529	Quercetin

Table 1. DPPH mg/g relative to rutin, trolox and quercetin

Conclusions

The importance of this study lies in a preliminary examination of whether cabbage can be used as a source of natural preservatives in the food industry. Results show significant antioxidant activity in all the vegetable extracts tested. Moreover, the E_1 and E_2 extracts were found to have significant antioxidant activity which correlated with the total phenolic content. Total phenols, hence and antioxidant activity, are dependent upon

the method and time of extraction, which is most likely due to their instability. Therefore, this fact should be considered when selecting and obtaining natural antioxidants.

Acknowledgments

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