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ANTIOXIDANT ACTIVITY OF EXTRACTS OF WHITE CABBAGE AND SAUERKRAUT

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Key words: white cabbage, sauerkraut, antioxidant activity, antiradical properties, phenolic compounds

Phenolic compounds were extracted from white cabbage and sauerkraut using 80% aqueous methanol. The extract of sauerkraut was characterised by a higher content of total phenolics (8.25 mg/g) than that of white cabbage (5.72 mg/g). Phenolic compounds present in extracts showed antioxidant and antiradical properties investigated using the Total Antioxidant Capacity (TAC) method, DPPH radical scavenging activity and reducing power. The total antioxidant capacity of the sauerkraut extract (0.031 mmol Trolox/g) was stronger than that of white cabbage (0.025 mmol Trolox/g). The extract of white cabbage exhibited a slightly stronger ability to scavenge DPPH radical compared to that of sauerkraut. Its reducing power was also stronger. Results of the HPLC analysis indicate the metabolism of phenolic compounds during the fermentation of white cabbage.

INTRODUCTION

The phenolic compounds which occur in food of plant origin and natural health products possess such common biological properties as antioxidant activity, the ability to scavenge active oxygen species, the ability to scavenge electrophiles, the ability to inhibit nitrosation, the ability to chelate metals, the potential to produce hydrogen peroxide in the presence of certain metals, and the capability to modulate certain cellular enzyme activities [Shahidi & Ho, 2005]. The role of antioxidants in foods is to inhibit or control oxidation. The antioxidation process in foods involves a free radical chain mechanism *via* initiation, propagation, and termination steps. Antioxidants from natural sources are often present in combinations involving a number of various compounds. The mode of action of natural antioxidants involves multiple mechanisms of action, depending on the type and source of the material used [Shahidi, 2000].

Cruciferae vegetables have a high nutritional value and contain different antioxidant compounds [Miller *et al.*, 2000]. Their activity has been reported by several authors. Cao *et al.* [1996] using the automated oxygen radical absorbance capacity with three different reactive species (peroxyl and hydroxyl radicals generators, and Cu^{2+}) observed the antioxidant capacity of cabbage, broccoli, cauliflower, and Brussels sprouts to range from 0.3 to 8.9 μmol Trolox/g wet matter. Ten fresh cabbage genotypes representing fresh-market, processing, and storage types exhibited the total antioxidant capacity values varying from 108.4 to 176.1 mg vitamin C equivalents / 100 g [Kim *et al.*, 2004]. The extracts obtained from fresh, precooked, cooked, as well as precooked and then cooked broccoli displayed the antioxidant properties, including reducing power, ferrous ion chelating, DPPH radical-scavenging activity and an inhibitory effect against lipid peroxidation [Lin & Chang, 2005]. Antioxidant properties of the extracts from different parts of broccoli have been reported in Taiwan by Guo *et al.* [2001].

The purpose of this study was to evaluate extracts of white cabbage and sauerkraut for their radical scavenging properties and antioxidant activity.

MATERIAL AND METHODS

Chemicals. All solvents used were of analytical grade. Methanol, potassium persulfate, potassium ferricyanide, ferric chloride and trichloroacetic acid were acquired from the P.O.Ch. Company (Gliwice, Poland). Acetonitrile and acetic acid (HPLC grade) were obtained from Merck. Folin & Ciocalteau's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'azino-bis-(3-ethylbenzthizoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox), caffeic, *p*-coumaric, ferulic, sinapic acid, catechin, and quercetin were obtained from Sigma Ltd. (Poznań, Poland).

Material. White cabbage was obtained from a local supermarket in Olsztyn in 2004 (Poland).

Fermentation. Cabbage heads were cut in a shredder into approx. 2 mm thick strips. So prepared sample (kg) was mixed with NaCl (3%) and transferred to traditional stoneware pots to run fermentation. After 10 days, the

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excess of the formed juice was removed and cabbage was lyophilized.

Preparation of crude extract. From the lyophilized white cabbage and sauerkraut phenolic compounds were extracted according to Amarowicz *et al.* [1995a] with 80% (v/v) aqueous methanol at 80°C for 15 min at a solid to solvent ratio of 1:8 (w/v). Extraction was carried out in dark-colored flakes using a shaking water bath. The extraction was repeated twice more, supernatants combined and methanol evaporated under vacuum at 40°C in a rotary evaporator. The remaining water solution was lyophilized.

Total phenolics. The content of total phenolic compounds was estimated using the Folin & Ciocalteau's reagent [Naczk & Shahidi, 1989]. (+)-Catechin was used as a standard.

UV spectra. UV spectra of individual fractions were recorded using a Beckman DU 7500 diode array spectrophotometer.

Separation of phenolic acids from the extract. Phenolic acids (*i.e.* free and those liberated from soluble esters and soluble glycosides) were isolated from the extract according to Amarowicz and Weidner [2001]. An aqueous suspension of the extract (500 mg in 20 mL) was adjusted to pH 2 (6 mol/L HCl), and free phenolic acids were extracted 5 times into 20 mL of diethyl ether using a separatory funnel. The extract was evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized to pH 7 with 2 mol/L NaOH and then lyophilized. The residue was dissolved in 20 mL of 2 mol/L NaOH and hydrolyzed for 4 h at room temperature under nitrogen atmosphere. After acidification to pH 2 using 6 mol/L HCl, phenolic acids released from soluble esters were extracted from the hydrolyzates 5 times into 30 mL of diethyl ether using a separatory funnel. A 15 mL aliquot of 6 mol/L HCl was added to the aqueous phase and the solution obtained in this way was placed under nitrogen atmosphere and hydrolysed for 1 h in a boiling water bath. Phenolic acids released from soluble glycosides were separated from the hydrolyzates 5 times into 45 mL of diethyl ether. Then, ether was evaporated to dryness. The dry residues were dissolved in 2 mL methanol and filtered through a 0.45 μm filter. The samples obtained in this way were injected onto an HPLC column.

HPLC analysis of phenolic acids. Phenolic acids were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10ADvp pump, SCL-10Avp system controller and SPD-M10Avp photo-diode array detector. The separation of phenolic acids was carried out by a prepacked LiChrospher 100 RP-18 column (4×250 mm, 5 μm; Merck, Darmstad, Germany). The mobile phase water-acetonitrile-acetic acid (88:10:2; v/v/v) [Amarowicz & Weidner, 2001] was delivered at a rate of 1 mL/min. The detection was monitored at 320 nm.

Acidic hydrolysis of phenolics from the extract. Acidic hydrolysis of phenolic compounds from the extract was carried out according to Crozier *et al.* [1997]. Briefly, 50 mg of the extract was dissolved in 5 mL of solution of 1.2 mol/L HCl in 50% (v/v) aqueous methanol containing 0.2% (m/v) of TBHQ. The solution was heated at 90°C for 2 h. After hydrolysis, the sample was adjusted to 25 mL with distilled water.

Analysis of phenolics liberated after acidic hydrolysis. The same Shimadzu HPLC system was used for the analysis of phenolic constituents liberated after acidic hydrolysis. A sample was filtered through a 0.45 μm filter and injected on the prepacked LiChrospher 100 RP-18 column (4×250 mm, 5 μm). Elution for 50 min in a gradient system of 5–40% acetonitrile in water adjusted to pH 2.5 with TFA was used. The detector was set at 280 nm and 350 nm; the flow rate was 1 mL/min, injection volume was 20 μL.

Total antioxidant capacity. The total antioxidant capacity (TAC) in the extracts was determined according to the Trolox equivalent antioxidant activity (TEAC) assay described by Re *et al.* [1999] and expressed as mmol Trolox/g of extract.

TABLE 1. Characteristics of extracts of white cabbage and sauerkraut: content of total phenolics, total antioxidant activity, and UV spectral data.

Material	Total phenolics (mg/g extract)	Total antioxidant activity (mmol Trolox/g extract)	λ_{max} (nm)
White cabbage	5.72	0.025	290
Sauerkraut	8.25	0.031	273

TABLE 2. Content of total phenolics in white cabbage and sauerkraut and total antioxidant capacity of this material.

Material	Total phenolics (mg/g f.m.)	Total phenolics (mg/g d.m.)	Total antioxidant capacity (mmol Trolox/g f.m.)	Total antioxidant capacity (mmol Trolox/g d.m.)
White cabbage	0.36	3.31	0.002	0.015
Sauerkraut	0.44	4.06	0.002	0.015

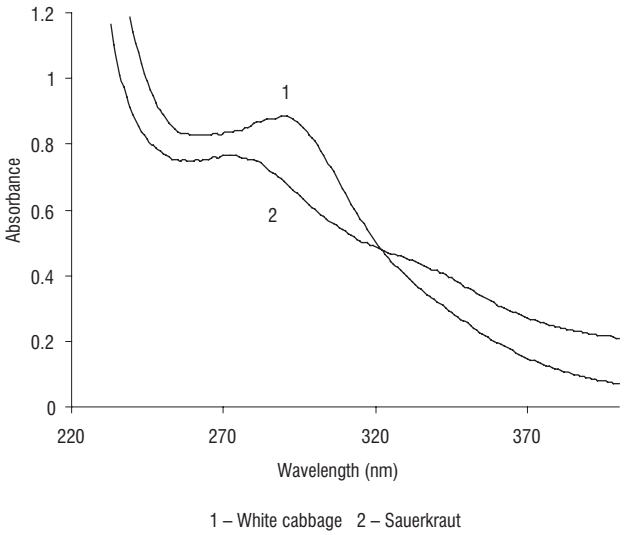

FIGURE 1. UV spectra of white cabbage and sauerkraut extracts.

Antiradical activity. The method described by Amarowicz *et al.* [2002] was used in order to assess the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts. Briefly, a 0.1 mL of methanolic solution containing from 0.80 to 4.0 mg of extract was mixed with 2 mL of distilled water and then 0.25 mL 1 mmol/L methanolic solution of DPPH radical was added. The mixture was vortexed thoroughly for 1 min. Finally, the absorbance of the mixture after standing at ambient temperature for 20 min was read at 517 nm.

Reducing power. Reducing power of phenolic compounds was determined as described by Oyaizu [1986]. The suspension of extracts (2–10 mg) in 1 mL of distilled water was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Following this, 2.5 mL of trichloroacetic acid (TCA) was added and the mixture was then centrifuged at 1750g for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ and the absorbance of the mixture was read at 700 nm.

Statistical analysis. Each extract was considered as a "treatment". All measurements were replicated three times for each treatment and their means are reported.

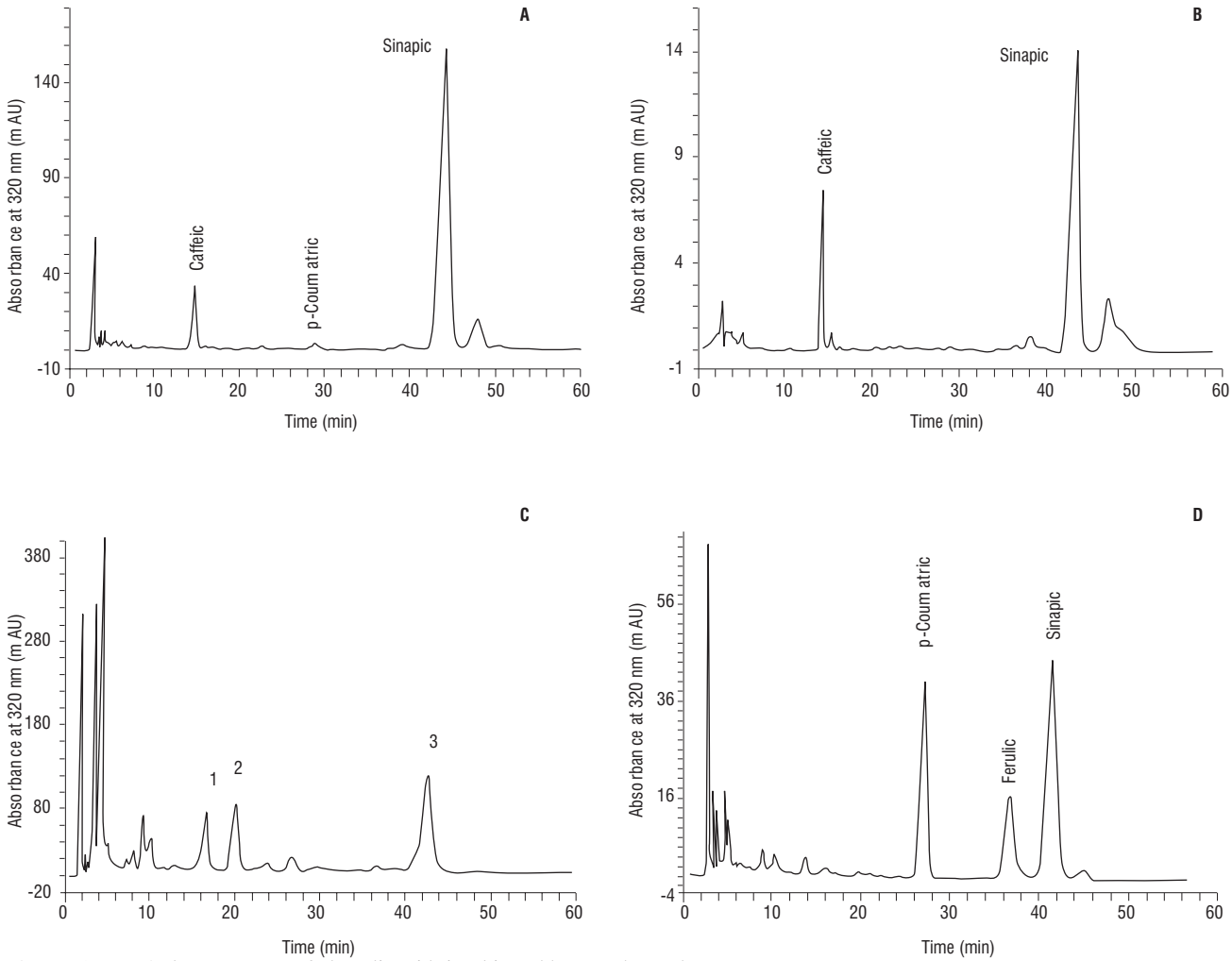

FIGURE 2. HPLC chromatogram of phenolic acids in white cabbage and sauerkraut extract;
A – phenolic acids of white cabbage extract liberated from esters; B – phenolic acids of sauerkraut extract liberated from esters; C – phenolic acids of white cabbage extract liberated from glycosides; D – phenolic acids of sauerkraut extract liberated from glycosides.

TABLE 3. Content of phenolic acids (μg/g) in extracts of white cabbage and sauerkraut.

Material	Form of phenolic acids	Caffeic	*p*-Coumaric	Ferulic	Sinapic
White cabbage	Free phenolic acids	–	–	–	–
	Esterified phenolic acids	23	3	–	278
	Glycosides phenolic acids	–	–	–	–
Sauerkraut	Free phenolic acids	–	–	–	–
	Esterified phenolic acids	3	–	–	20
	Glycosides phenolic acids	–	30	23	84

RESULTS AND DISCUSSION

The contents of total phenolics in the extracts of white cabbage and sauerkraut are presented in Table 1. The extract of sauerkraut was characterised by a higher content of total phenolics (8.25 mg/g) than that of white cabbage (5.72 mg/g). This difference caused that the total antioxidant capacity of the extract of sauerkraut (0.031 mmol Trolox/g) was stronger than that of white cabbage (0.025 mmol Trolox/g) (Table 1). The same tendency for the content of total phenolics was noted when the results were calculated and reported according to fresh and dry matter of the material examined (Table 2). The total antioxidant capacity of fresh and dry matter of white cabbage and sauerkraut were alike (Table 2). The higher content of the total phenolics noted in the sauerkraut extract may be caused by the action of bacterial enzymes liberating phenolics bounded to the cell walls as the esters and glycosides [Andreasen *et al.*, 1999].

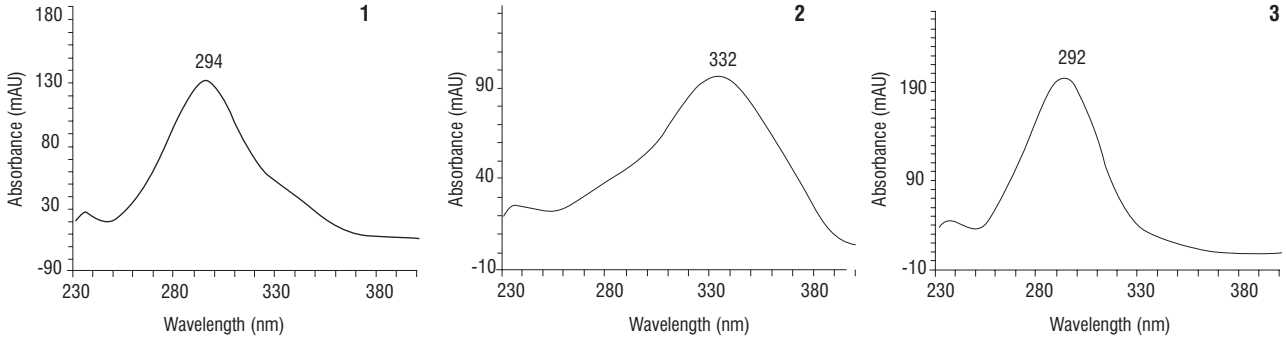

FIGURE 3. UV spectra of phenolic compounds separated from the white cabbage extract after acid hydrolysis using HPLC method; 1-3 – numbers like in FIGURE 2.

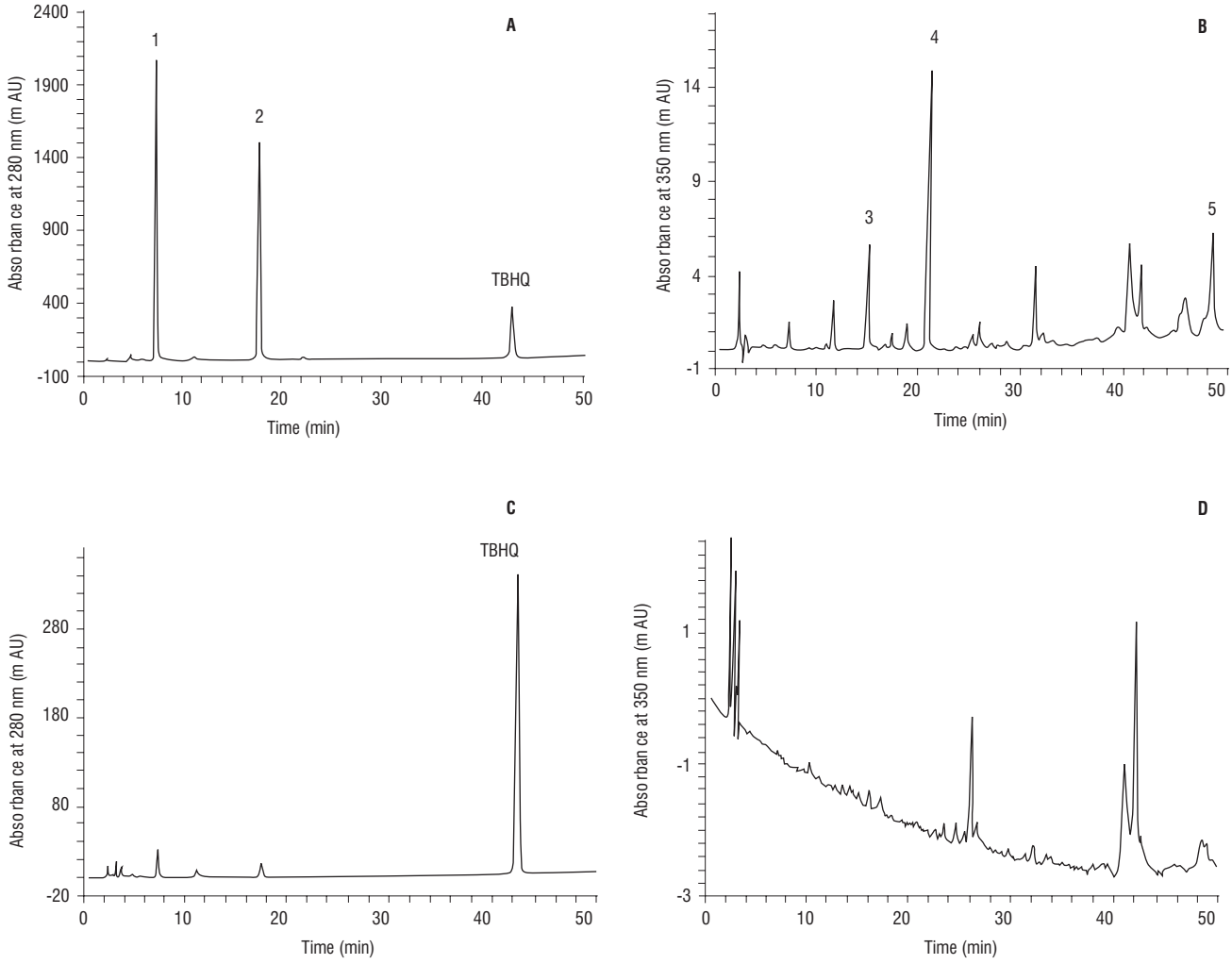

FIGURE 4. HPLC chromatogram of gradient separation of phenolic compounds from white cabbage and sauerkraut extracts after acid hydrolysis; A – phenolic compounds of the white cabbage extract detected at 280 nm; B – phenolic compounds of the white cabbage extract detected at 350 nm; C - phenolic compounds of the sauerkraut extract detected at 280 nm; D – phenolic compounds of the sauerkraut extract detected at 350 nm.

The contents of total phenolics in extracts of white cabbage and sauerkraut were low. Much higher results (22.6–89.7 mg/g) were determined in extracts of leguminous seeds [Amarowicz *et al.*, 2002; Karamać *et al.*, 2002, 2004] and oilseeds [Amarowicz *et al.*, 1995b]. In a study carried out by Ismail *et al.* [2004] the total phenolic content of the cabbage extract was 11.1 mg/g. Other authors reported in fresh white cabbage 0.4 mg total phenolics/g f.m. [Hassimotto *et al.*, 2005], 0.36 mg total phenolics/g f.m. [Chu *et al.*, 2002], 1.10-1.53 mg total phenolics/g f.m. [Kim *et al.*, 2004].

Total antioxidant activity of white cabbage and sauerkraut obtained in this study was much lower than that reported for leguminous seeds [Amarowicz *et al.*, 2004,] and almond extract [Amarowicz *et al.*, 2005]. For methanolic extracts of wheat, barley, rye and oat Zieliński and Kozłowska [2000] reported higher values of TAA (0.054–0.222 mmol Trolox/g) than those obtained in this study. The total antioxidant capacity of cabbage determined using the oxygen radical absorbance capacity (RAC) assay with three different reactive species (AAPH, Cu^{2+}, Cu^{2+}- H_2O_2) was 0.003, 0.0015, 0.0003 mmol Trolox/g f.m., and 0.032, 0.0158, 0.0034 mmol Trolox/g d.m., respectively [Cao *et al.*, 1996].

The influence of fermentation on white cabbage phenolic compounds was observed using UV spectrometry (Figure 1). The spectrum of the white cabbage extract showed the maximum at 290 nm whereas the spectrum of the sauerkraut extract was characterized by an absorption band at shorter wavelength of 273 nm (Table 1). The shoulder at 322 nm was recorded at the UV spectrum of sauerkraut.

Results of phenolic acids analysis are reported in Table 3 and presented in Figure 2. The absence of free phenolic acids was noted in both extracts. Only esterified acids were observed in white cabbage extract. Sinapic acid was the dominating phenolic acid in this extract (278 μg/g). The presence of caffeic and sinapic acids was noted in the extract of sauerkraut – sinapic acid was the dominating one (84 μg/g). Herrmann [1989] reported that six varieties of white cabbage contained sinapoylglucose and feruolylglucose.

The acidic hydrolysis of white cabbage extract was found to liberate three phnenolic compounds from the glucosides (1–3 in Figure 2 C). According to UV spectra data (λ_{max} 332 nm), compound 2 belongs probably to phenolic acids. Compounds 1 and 3 were characterised by absorption bands at shorter wavelength (λ_{max} 292 nm) and were absent on the chromatogram of phenolic acids of sauerkraut after acidic hydrolysis (Figure 3). Peaks originated from *p*-coumaric, ferulic, and sinapic acids were observed on the chromatogram of the extract of sauerkraut after acidic hydrolysis (Figure 2 D).

Several phenolic constituents were noted on chro-

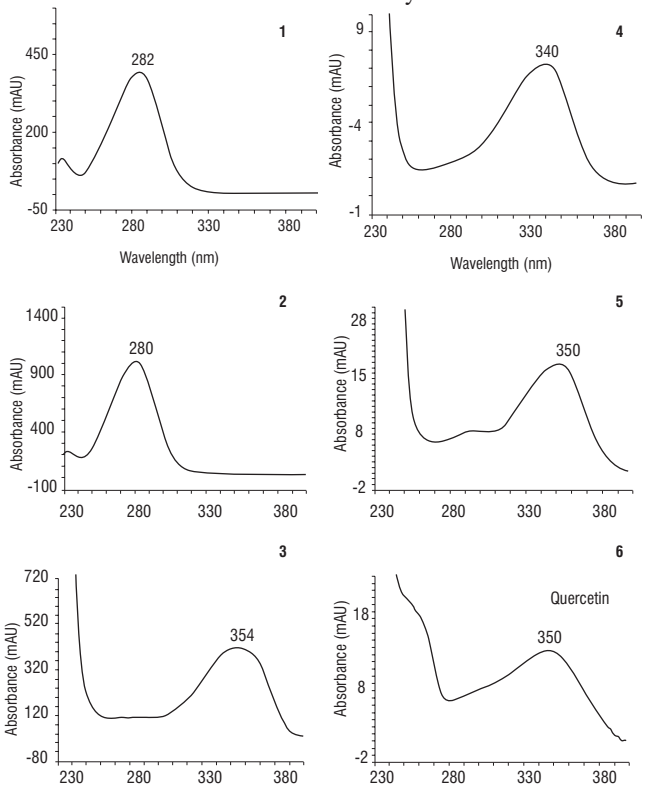

FIGURE 5. UV spectra of phenolic compounds separated from the white cabbage extract after acid hydrolysis using HPLC method in gradient system; 1-5 – numbers like in FIGURE 4.

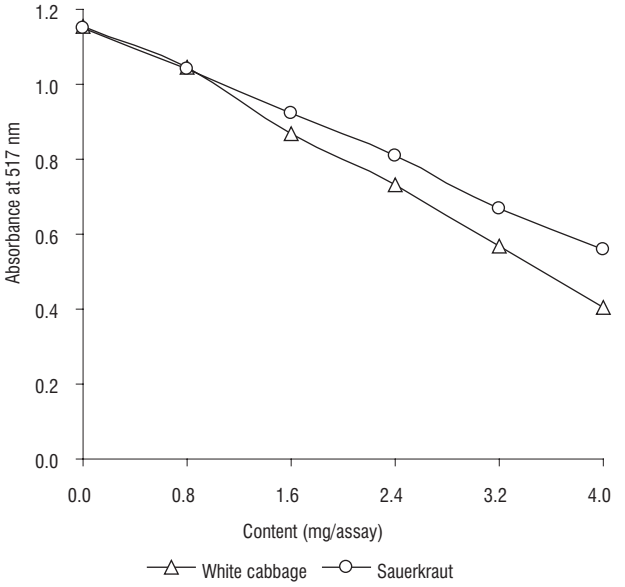

FIGURE 6. Scavenging effect of white cabbage and sauerkraut extracts on DPPH radical.

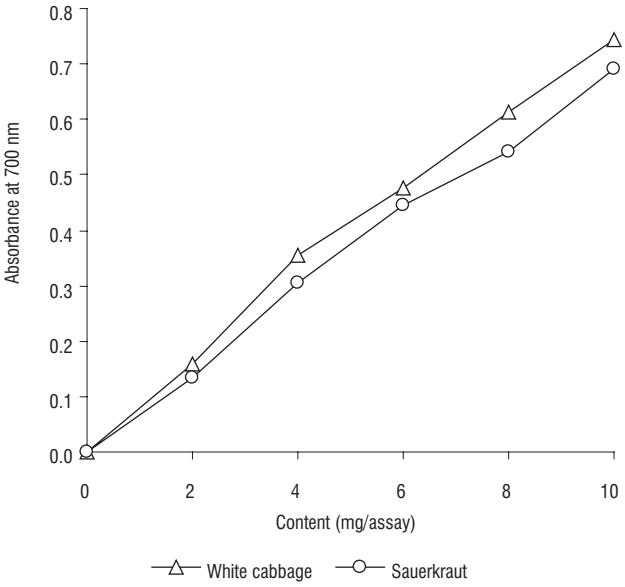

FIGURE 7. Reducing power of white cabbage and sauerkraut extracts.

matograms from extracts of white cabbage samples after acidic hydrolysis (Figure 4 A and B). The chromatogram of the extract of white cabbage recorded at 280 nm (Figure 4 A) was characterised by peaks 1 and 2 with retention times of 7.84 and 17.92 min, respectively. The presence of peaks 3, 4 and 5 with retention times of 15.05, 20.87, and 49.23 min was noted when the detector was set at 350 nm. UV spectra of compound 1 and 2 possessed their maxima at 282 and 280 nm (Figure 5). The maxima of the spectra of compounds 3, 4, 5 were recorded at 354, 340 and 350 nm, respectively (Figure 5). The similarity of the sepectra of compounds 3–5 to that of quercetin (λ_{max} 350 nm) indicates that they belong to flavanols. Peaks 1–5 disappeared on the chromatograms originated from the sauerkraut extract (Figure 4 C and D), which can be an evidence of the strong metabolism of flavonoids of white cabbage during fermentation.

Figure 6 depicts the antiradical activity of both extracts against DPPH radical. The extract of white cabbage exhibited a slightly stronger ability to scavenge DPPH radical, especially when a higher content of extracts was added to the model solution of free radical. The extract of white cabbage possessed also the stronger reducing power (Figure 7).

The antiradical activity of the extracts examined in this study and their reducing power were low. Similar results were reported for extracts of cereal grains [Amarowicz *et al.*, 2002; Karamać *et al.*, 2002, 2004]. The contents of total phenolics in cereal extracts were similar to those of white cabbage and sauerkraut. The strongest results of the above-mentioned test were found in the case of leguminous and oil seeds [Amarowicz *et al.*, 2004, 1995b]. The antiradical activity assayed using DPPH radical was reported for extracts of broccoli [Guo *et al.*, 2001; Miller *et al.*, 2000; Lin & Chang, 2005], red cabbage and Brussels sprouts [Miller *et al.*, 2000], cauliflower by-products [Llorach *et al.*, 2003]. Reducing power of broccoli extracts was used as a method for antioxidant activity investigation by Guo *et al.* [2001] and Lin and Chang [2005].

CONCLUSIONS

The research demonstrates that fermentation of white cabbage influences its phenolic constituents but only a bit modifies their antioxidant and antiradical properties measured using TAC, scavenging DPPH, and reducing power methods.

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AKTYWNOŚĆ PRZECIWUTLENIAJĄCA EKSTRAKTÓW KAPUSTY BIAŁEJ I KAPUSTY KISZONEJ

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Ekstrakty związków fenolowych uzyskano z białej kapusty i kapusty kiszonej stosując 80% wodny roztwór metanolu. Ekstrakt z kapusty kiszonej charakteryzował się wyższą zawartością związków fenolowych ogółem (8.25 mg/g) niż ekstrakt z kapusty białej (5.72 mg/g). Związki fenolowe obecne w ekstraktach wykazywały właściwości przeciwutleniające i przciwrodnikowe badane metodą całkowitej pojemności przeciwutleniającej (TAC), testem zmiatania wolnego rodnika DPPH i siłą redukującą. TAC ekstraktu z kapusty kiszonej wynosiła 0.031 mmol Trolox/g i była wyższa od wartości uzyskanej w przypadku kapusty białej (0.025 mmol Trolox/g). Ekstrakt z kapusty białej wykazywał nieznacznie silniejszą aktywność przeciwrodnikową wobec DPPH i siłę redukującą. Wyniki analizy HPLC wskazują metabolizm związków fenolowych zachodzący podczas fermentacji kapusty.