Original article

Antioxidant capacity and content of *Brassica oleracea* dietary antioxidants

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Summary This study has evaluated the antioxidant capacity and content of both water- and lipid-soluble antioxidants present in red and white cabbages, savoy cabbage and Brussels sprouts. Total phenolic and vitamin C contents varied from 21 to 171 mg per 100 g and from 18 to 129 mg per 100 g, respectively. Levels of carotenoids ranged from 0.009 to 1.16 mg per 100 g, while α-tocopherol levels from 0.008 to 0.82 mg per 100 g. Red cabbage and Brussels sprouts were the most rich sources of dietary antioxidants, while their content was the lowest in white cabbage. Water-soluble antioxidants were the main antioxidant compounds in the vegetables tested, and their contribution to the trolox equivalent antioxidant capacity values was near 99%. Among phenolic compounds, anthocyanins were the main constituents in red cabbage, but hydroxycinnamic acids predominated in other vegetables. Phenolic extracts were capable of scavenging $O_2^{\bullet-}$, DPPH$^\bullet$ and ABTS$^{\bullet+}$ radicals and inhibited a lipid peroxidation in a linoleic acid emulsion.

Keywords Antioxidant content, Brassica vegetables, phenolic activities, phenolic profiles, total trolox equivalent antioxidant capacity.

Introduction

Subspecies of *Brassica oleracea*, including white and red cabbages (var. *capitata*), broccoli and cauliflower (var. *bortrytis*), savoy cabbage (var. *sabauda*) and Brussels sprouts (var. *gemmifera*), belong to Cruciferous family. Brassica vegetables contain many bioactive compounds, especially organosulphur phytochemicals possessing anticarcinogenic activity and other phytochemicals, which are known to possess antioxidant activity. Dietary antioxidants present in these vegetables, such as water-soluble vitamin C and phenolic compounds, as well as lipid-soluble vitamin E and carotenoids, contribute to both the first and the second defence lines against oxidative stress (Lampi *et al*., 2002; Krinsky, 2001; Czeczot, 2000; Davey *et al*., 2000). As a result, they may protect humans from chronic diseases, such as cancer and cardiovascular disease. Among Brassica vegetables, kale, Brussels sprouts and broccoli are the most rich sources of antioxidant vitamins and carotenoids (Pfendt *et al*., 2003; Davey *et al*., 2000; Kurilich *et al*., 1999; Muller, 1997), while Chinese cabbage, broccoli and red cabbage are rich in phenolics (Bahorun *et al*., 2004; Wu *et al*., 2004; Chu *et al*., 2002). The content of natural antioxidants among Brassica vegetables varies significantly between and within their subspecies because of different maturity at harvest and conditions of growing, soil state and postharvest storage. For example, vitamin C levels in broccoli ranged from 41 to 146 mg per 100 g, total phenols from 31 to 337 mg per 100 g and carotenoids from 0.37 to 5.42 mg per 100 g (Bahorun *et al*., 2004; De Sa & Rodriguez-Amaya, 2004; Franke *et al*., 2004; Wu *et al*., 2004; Zhang & Hamauzu, 2004; Chu *et al*., 2002; Vallejo *et al*., 2002; Holden *et al*., 1999; Kurilich *et al*., 1999).

Antioxidant capacity of Brassica vegetables depends on antioxidant levels and its composition. In order to determine the total antioxidant capacity of the vegetables, the activity of both water- and lipid-soluble antioxidants must be considered. However, until now, only a limited number of studies have simultaneously estimated these different groups of antioxidants (Wu *et al*., 2004; Roberts & Gordon, 2003; Kurilich *et al*., 2002). In addition, in the studies published on the antioxidant activity of Brassica vegetables, different extraction procedures and several methods measuring this activity have been discussed. For this reason, a comparison of the results is very difficult. A lot of studies have been made to evaluate the antioxidant potential of hydrophilic antioxidants, which are extracted from food matrix with water (Honer & Cervellati,

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2002; Kurilich *et al*., 2002), or water solution of methanol, acetone with or without acetic or metaphosphoric acid (Wu *et al*., 2004; Roberts & Gordon, 2003; Ou *et al*., 2002). On the contrary, lipid-soluble antioxidants from Brassica vegetables are easily extracted with the use of acetone, hexane or hexane/dichloromethane (Wu *et al*., 2004; Roberts & Gordon, 2003; Kurilich *et al*., 2002).

Levels of vitamins C and E, carotenoids and phenolic compounds in subspecies of *B. oleracea* have been quantified in some cases, but no study on all of these antioxidants and their antioxidant capacities have been reported so far. In this study, we assayed content and antioxidant activity of water- and lipid-soluble antioxidants in the edible portions of vegetables from *B. oleracea* group including var. *capitata* (white and red cabbages), var. *sabauda* (savoy cabbage) and var. *gemmifera* (Brussels sprouts). Because of different mechanisms of dietary antioxidant actions, several methods are recommended to evaluate the antioxidant capacity of food. We have used four methods in our studies: a lipid peroxidation in a linoleic acid emulsion, an enzymatic method for superoxide anion radical ($O_2^{\cdot-}$) and chemicals methods for the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\cdot}$) and for the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical monocation (ABTS$^{\cdot+}$).

Materials and methods

Chemicals

Chlorogenic acid, gallic acid, rutin, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), DPPH$^{\cdot}$, ABTS, sodium acetate, ascorbic acid, dithiothreitol, tetrazolium blue (NBT), hypoxanthine (HPX), xanthine oxidase (XOD), Tween 20 and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich Chemicals Co. α-Tocopherol and linoleic acid were purchased from ICN Pharmaceuticals Inc. Cyanidin-3-glucoside was purchased from Extrasynthese SAS. HPLC grade methanol, acetonitrile and acetic acid were purchased from J.T. Baker. All other chemicals were reagent grade products purchased from POCh (Gliwice, Poland).

Plant materials

White cabbages were harvested in June (Almanag and Tukana) or in October (Vestri). Brussels sprouts were harvested in November (Ajax and Filemon) and red cabbage (Koda and Kissendrup) like savoy cabbage (Langedijker and 60F/100) in October. White cabbage and Brussels sprouts samples were collected from commercial gardens near ódź (central region of Poland), while samples of savoy and red cabbages were obtained from farms of the PlantiCo Horticulture Breeding and Seed Production Ltd, Goebiew. Samples of each vegetable (1 kg) were lyophilised and ground into powder to preserve the antioxidant content. Freeze-dried vegetables were stored at −20 °C for further analysis. Only ascorbic acid and dry-matter contents were analysed directly from fresh sample.

Dry-matter determination

Dry-matter content was determined by drying (2 g sample) at 105 °C to constant weight.

Ascorbic acid extraction and analysis

The extraction method used was a modification of the method described by Howard *et al*. (1999). Vegetables (10 g) were homogenised with 25 mL of 1% metaphosphoric acid and then extracted at room temperature for 15 min, centrifuged at 4000 r.p.m. for 10 min and the residue was reextracted with 10 mL of extracting solution and centrifuged. The combined supernatants were diluted to 50 mL with 1% metaphosphoric acid. One millilitre of the sample and 1 mL of 5% dithiothreitol were mixed and allowed to stand for 18 h at room temperature in the dark. After dilution with metaphosphoric acid, the samples were analysed using Knauer HPLC equipped with Eurospher-100 C-18 column (25 cm × 4.6 mm; 5 μm) fitted with the same guard column. HPLC method was adapted from Gliszczynska-Swiglo & Tyrakowska (2003). For detection of ascorbic acid, a gradient of methanol (solvent A) per 5 mmol L^{-1} KH_2PO_4, pH 2.6 (solvent B), was used according to the following program: linear increment starting with 5% A to 22% in 6 min and then return to the initial conditions within next 9 min with the flow rate 1 mL min^{-1}. The eluate was detected with UV-Vis detector set at 245 nm. Ascorbic acid was identified by comparing its retention time with that of an ascorbic acid standard.

Carotenoids and α-tocopherol extraction and analysis

Lyophilised vegetables (2 g) were extracted using hexane until organic layer was colourless. Mixed organic layers were measured at 450 nm, and total carotenoids content was expressed as milligram of β-carotene per 100 g of fresh weight (FW) of vegetables. Then, the hexane extract was split into two parts and evaporated to dryness on a rotary evaporator with a water bath set at 30 °C. The first part was redissolved in 1 mL of hexane (lipid-soluble extract) and used to measure antioxidant activity. The other part was reconstituted with 0.5 mL of THF for α-tocopherol analysis. α-Tocopherol concentration was quantified using HPLC Knauer system equipped with UV-Vis detector and a Eurospher-100 C-18 column (25 cm × 4.6 mm; 5 μm). Elution (1 mL min^{-1}) was

performed under isocratic conditions using a mixture of acetonitrile/methanol/THF at 52:40:8 (v/v/v) (Kurilich *et al.*, 1999). Absorbance was measured at 290 nm, and α-tocopherol identification was based on retention time of α-tocopherol standard.

Phenolics extraction and determination

Lyophilised vegetables (2 g) were extracted twice with 50 mL of 70% MeOH (v/v) at room temperature for 15 min by shaking (Vallejo *et al.*, 2002). The mixture was then centrifuged at 4000 r.p.m. for 15 min, and the supernatant was evaporated under reduced pressure ($T < 40$ °C). The aqueous extracts were diluted to 25 mL with water (water phenolic extract), and analysed in order to quantify total phenolics content and to determine phenolic profiles. Total phenolics were analysed spectrophotometrically by the Folin–Ciocalteu procedure (Peri & Pompei, 1972). Briefly, 10 mL of water, 0.1–0.6 mL of sample, 0.5 mL of Folin–Ciocalteu reagent and 5 mL of 20% Na_2CO_3 were mixed and diluted to 50 mL with water. After 20 min incubation in the dark, the absorbance was measured at 700 nm. The amount of total phenolics in the extract was expressed as gallic acid equivalents (GAE), milligrams per 100 g of FW.

Phenolic profiles were determined using HPLC Knauer system equipped with UV-Vis detector and a Eurospher-100 C-18 column (25 cm × 4.6 mm; 5 μm). The binary mobile phase according to Tsao & Yang (2003) consisted of 6% acetic acid in 2 mmol L^{-1} sodium acetate (solvent A) and acetonitrile (solvent B). The flow rate was 1 mL min^{-1} and the total run time was 70 min. The system was run with a gradient program: 0–15% B in 45 min, 15–30% B in 15 min, 30–50% B in 5 min and 50–100% B in 5 min. Based on the wavelength in which the maximum of UV-Vis absorption was observed, the phenolics were divided into four groups. The hydroxybenzoic acid derivatives were quantified at 280 nm and expressed as GAE, hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid equivalents, flavonols at 360 nm as rutin equivalents and anthocyanins at 520 nm as cyanidin-3-glucoside equivalents.

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation scavenging activity

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical monocation scavenging activity was determined following a procedure described by Re *et al.* (1999). $ABTS^{\bullet+}$ was produced by reacting 7 mmol L^{-1} ABTS water solution with 2.45 mmol L^{-1} potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Water phenolic extract or lipid-soluble extract (20 μL) was mixed with 1 mL of diluted $ABTS^{\bullet+}$ solution and its absorption was measured at 734 nm after 6 min at 30 °C. Trolox was used as a standard and the capacity of free radical scavenging was expressed as micromoles of Trolox per 1 g of FW of a vegetable (TEAC – trolox equivalent antioxidant capacity).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity was determined using a method of Kim *et al.* (2002). Water phenolic extract (0.1 mL) was mixed with 2.9 mL 100 μmol L^{-1} $DPPH^{\bullet}$ in 80% aqueous methanol and stored at ambient temperature in the dark for 30 min. The decrease in absorbance of the resulting solutions was measured at 517 nm. Ascorbic acid (vitamin C) was used as a standard and the capacity of free radical scavenging was expressed as micromoles of vitamin C per 1 g of FW of a vegetable (VCEAC – vitamin C equivalent antioxidant capacity).

Superoxide anion scavenging activity

Effect of water phenolic extract on $O_2^{\bullet-}$ generated by the hypoxanthine/xanthine oxidase system was determined according to Saint-Cricq de Gaulejac *et al.* (1999). A tetrazolium blue solution (NBT, 1 mmol L^{-1}), hypoxanthine solution (HPX, 5 mmol L^{-1}) and xanthine oxidase solution (XOD, 1.67 U in 1 mL) were prepared in a sodium phosphate buffer (SPB, 0.05 mol L^{-1}, pH 7.4). Water phenolic extract (0.1 mL) was mixed with 2.2 mL of SPB, 0.1 mL NBT, 0.5 mL HPX and 0.1 mL XOD. The blank sample included 2.4 mL of SPB, 0.1 mL NBT and 0.5 mL HPX. A control containing of 2.3 mL of SPB, 0.1 mL NBT, 0.5 mL HPX and 0.1 mL XOD has shown the highest concentration of $O_2^{\bullet-}$. The decrease in absorbance of samples was determined at 560 nm every minute for a 10-min period. Antiradical activity was defined as the amount of vegetables required to lower the initial $O_2^{\bullet-}$ concentration by 50% (IC_{50}).

Antioxidant activity in a linoleic acid system

Antioxidant activity of water phenolic extract was measured as their ability to inhibit a linoleic acid peroxidation according to Szwajgier & Targoński (2000). Linoleic acid and Tween 20 (equal volume to the linoleic acid) were added to 50 mmol L^{-1} phosphate buffer (pH 7.6) so that the final concentration of the acid was 30 mmol L^{-1}. Emulsion was prepared daily for experiments. Water phenolic extract (50 μL) was added to 5 mL of the linoleic acid emulsion and placed in darkness at 37 °C to reach a propagation phase for each sample. The reaction was stopped by the addition 0.1 mL of 90.8 mmol L^{-1} BHT alcoholic solution. The solution obtained in this way (0.5 mL) was mixed with

0.67% thiobarbituric acid (0.5 mL) and 0.05 mol L^{-1} HCl (1.5 mL) and heated for 30 min in a water bath at 95 °C (according to the method described by Tamura & Yamagami, 1994). The absorbance of the thiobarbituric-acid-reactive substances was measured at 535 nm. The antioxidant activity was expressed as the inhibition time (T_{inh}), which was estimated as the point of intersection between the tangents to the inhibition- and propagation-phase curves.

Results and discussion

Antioxidant content and total scavenging activity

The quantitative analysis of antioxidant vitamins (ascorbic acid and α-tocopherol), carotenoids and phenolics is presented in Table 1. The results show that water-soluble antioxidants (phenolics and ascorbic acid) were the main antioxidant compounds in all the *B. oleracea* vegetables tested. Brussels sprouts and red cabbages were the most rich sources of these antioxidants, while their content was the lowest in white cabbages. The results of the present study are generally in agreement with other reports. It was previously reported that total phenols content for red cabbage grown in the United States was 254 mg per 100 g FW (Wu *et al*., 2004), which is 1.7-fold higher than that obtained in this study. Bahorun *et al*. (2004) reported that white cabbage from Mauritius had 15.3 mg total phenols per 100 g FW, that is, about 40% lower as compared with white cabbage cultivated in Poland. According to literature data, an ascorbic acid content in Brussels sprouts varied from 76 to 192 mg per 100 g FW (Pfendt *et al*., 2003; Czarniecka-Skubina, 2002; Davey *et al*., 2000) and from 19 to 47 mg per 100 g FW in white cabbage (Bahorun *et al*., 2004; Pfendt *et al*., 2003,2003; Chu *et al*., 2002; Davey *et al*., 2000). Sum up, the levels of phenolics in the vegetables investigated were in the following order: red cabbage > Brussels sprouts > savoy cabbage > white cabbage, and the order of the levels of ascorbic acid was Brussels sprouts > red cabbage > savoy cabbage > white cabbage.

In contrast to water-soluble antioxidants, lipophilic antioxidant content was generally below 1 mg per 100 g FW of the vegetables tested (Table 1). The levels of lipid-soluble antioxidants (carotenoids and α-tocopherol) were the highest in Brussels sprouts. High content of α-tocopherol was also observed in savoy cabbage Langedijker. According to literature data, the amount of carotenoids varied from 0.77 to 4.83 mg per 100 g FW for Brussels sprouts, from 0.01 to 0.86 for white cabbage and from 0.05 to 0.20 for red cabbage (Murkovic *et al*., 2000; Holden *et al*., 1999; Kurilich *et al*., 1999; Muller, 1997; Heinonen *et al*., 1989). Among carotenoids, lutein and zeaxanthin are predominant compounds in these vegetables. According to Piironen *et al*. (1986), Brussels sprouts contained 0.40 mg of tocopherol and tocotrienol per 100 g FW, but red and white cabbages had only 0.05 and 0.04 mg of vitamin E per 100 g FW, respectively. These values were lower than the contents obtained in our study for Brussels sprouts and red cabbage (Table 1) as well as those reported by Kurilich *et al*. (1999), because mean value for Brussels sprouts was 0.87 mg per 100 g FW and for cabbage 0.17 mg per 100 g FW.

The antioxidant activity of water phenolic extract and lipid-soluble extract was determined by the assay that uses the ABTS as a source of stable synthetic radical cation. This simple method is an electron-transfer-based assay and gives scavenging capacity, which has been expressed relative to Trolox (TEAC) (Re *et al*., 1999). In order to determine the total antioxidant capacity of the vegetables tested, the activity of vitamin C was also

Table 1 Content of natural antioxidants of Brassica vegetables

		Mean concentration (mg/100 g FW ± SD; $n = 3$)				
Vegetable	**Cultivar**	**Dry weight (%)**	**Total phenolics[a]**	**Ascorbic acid**	**α-Tocopherol**	**Total carotenoids[b]**
Red cabbage	Kissendrup	11.48 ± 0.58	171.36 ± 13.77	62.00 ± 2.74	0.061 ± 0.003	0.016 ± 0.002
	Koda	10.42 ± 0.21	134.73 ± 3.35	72.56 ± 7.99	0.111 ± 0.008	0.013 ± 0.001
Brussels sprouts	Ajax	18.10 ± 0.04	140.13 ± 5.67	127.77 ± 7.82	0.545 ± 0.010	1.090 ± 0.050
	Filemon	20.64 ± 0.25	133.46 ± 6.43	129.27 ± 2.96	0.823 ± 0.011	1.160 ± 0.030
White cabbage	Almanag	8.09 ± 0.10	29.70 ± 0.66	25.46 ± 0.98	0.008 ± 0.001	0.042 ± 0.001
	Tukana	6.51 ± 0.14	20.81 ± 0.79	18.00 ± 0.60	0.009 ± 0.003	0.051 ± 0.003
	Vestri	7.99 ± 0.29	23.32 ± 0.47	35.64 ± 0.57	0.022 ± 0.005	0.009 ± 0.001
Savoy cabbage	Langedijker	10.90 ± 0.13	54.31 ± 1.60	51.66 ± 0.31	0.782 ± 0.091	0.048 ± 0.003
	60F/100	10.23 ± 0.10	47.62 ± 0.70	49.81 ± 0.88	0.011 ± 0.004	0.122 ± 0.008

[a]Total phenolics value is expressed as gallic acid.

[b]Total carotenoids value is expressed as β-carotene.

Table 2 TEAC values (μmol Trolox equivalents/g of fresh weight) of Brassica vegetable extracts

		TEAC (μmol Trolox/g)			
Vegetable	**Cultivar**	**Water phenolic extract**	**Ascorbic acid**	**Lipid-soluble extract**	**Total**
Red cabbage	Kissendrup	12.64 ± 0.21	3.34 ± 0.03	0.007 ± 0.002	15.99
	Koda	9.81 ± 0.45	3.91 ± 0.13	0.005 ± 0.001	13.72
Brussels sprouts	Ajax	7.04 ± 0.15	6.89 ± 0.07	0.064 ± 0.004	13.99
	Filemon	5.85 ± 0.24	6.97 ± 0.20	0.095 ± 0.014	12.92
White cabbage	Almanag	1.81 ± 0.12	1.35 ± 0.05	0.007 ± 0.001	3.17
	Tukana	1.46 ± 0.03	0.97 ± 0.03	0.009 ± 0.002	2.44
	Vestri	1.34 ± 0.07	1.92 ± 0.04	0.003 ± 0.001	3.26
Savoy cabbage	Langedijker	3.74 ± 0.10	2.79 ± 0.02	0.024 ± 0.002	6.55
	60F/100	2.89 ± 0.06	2.69 ± 0.05	0.021 ± 0.001	5.60

The values are expressed as mean ± SD, $n \geq 4$.

considered. The vitamin C activity as TEAC was calculated on the basis of its concentration in vegetables and TEAC value which was for ascorbic acid 0.95 mm. We could not use ascorbic acid extract (see Materials and Methods), because it contained phenolic antioxidants that are also extracted with metaphosphoric acid. Values of antioxidant activities measured by the ABTS method are shown in Table 2. The rank order based on the total TEAC mean values is: red cabbage > Brussels sprouts > savoy cabbage > white cabbage. Red cabbage and Brussels sprouts showed comparable activity, which was 5- to 4.5-fold and 2.4- to 2.2-fold higher than that for white and savoy cabbages, respectively. Total antioxidant capacity (expressed as TEAC value) was very closed to that one obtained for hydrophilic antioxidants (phenolic compounds + vitamin C), because the contributions of lipid-soluble extracts activity were below 1%. Such low scavenging capacity of lipid-soluble extract could be related to the low level of carotenoids and vitamin E in the vegetables tested. Furthermore, ABTS assay is carried out in methanol, what may not accurately reflect the antioxidant capacity of lipophilic compounds. Our results are different than that obtained by Wu *et al.* in ORAC assay (2004). They noticed that red cabbage only 1.6-fold better scavenged peroxyl radicals than common cabbage, which is probably caused by similar levels of phenolics in these both vegetables. However, the authors similarly to our results, have shown that the lipophilic extracts of common and red cabbage were responsible for 1.5% and 0.9% of the total antioxidant capacity, respectively.

In addition to antioxidant vitamins, carotenoids and polyphenols, Brassica vegetables contain a large group of glucosinolates, which according to Plumb *et al.* (1996) possess rather low antioxidant activity, but the products of their hydrolysis can activate transcription of some phase II enzymes and in this way can protect human cells against cancer (Keum *et al.*, 2004; Talalay & Fahey, 2001).

Phenolic compounds of *Brassica oleracea* vegetables and their antioxidant activity

Phenolic compounds were the main dietary antioxidant in red cabbage and very important constituent in other tested Brassica vegetables (Table 1). In the present

Table 3 Antioxidant activity of water phenolic fraction of Brassica vegetables in different antioxidant assay systems

					Linoleic acid emulsion, T_{inh} (days)	
Vegetable	**Cultivar**	***ABTS*$^{\bullet+}$**	**DPPH$^{\bullet}$**	**$O_2^{\bullet-}$**	**2 g vegetable per litre**	**0.5 g vegetable per litre**
Red cabbage	Kissendrup	12.64 ± 0.21	9.19 ± 0.74	2.80 ± 0.02	8.78	4.28
	Koda	9.81 ± 0.45	6.76 ± 0.46	2.73 ± 0.09	>10	4.61
Brussels sprouts	Ajax	7.04 ± 0.15	5.98 ± 0.21	2.60 ± 0.05	8.87	4.90
	Filemon	5.85 ± 0.24	3.90 ± 0.23	2.31 ± 0.14	8.69	4.70
White cabbage	Almanag	1.81 ± 0.12	1.00 ± 0.04	4.35 ± 0.21	5.65	2.66
	Tukana	1.46 ± 0.03	0.77 ± 0.05	10.07 ± 0.92	4.43	1.93
	Vestri	1.34 ± 0.07	0.90 ± 0.07	7.40 ± 0.35	4.75	1.87
Savoy cabbage	Langedijker	3.74 ± 0.10	1.68 ± 0.02	6.25 ± 0.21	4.22	1.42
	60F/100	2.89 ± 0.06	1.38 ± 0.02	5.55 ± 0.11	4.94	1.88

The values are expressed as mean ± SD, $n \geq 3$.

study, the antioxidant activity of water phenolic extract was evaluated by using four different assays: a lipid peroxidation in a linoleic acid emulsion, an enzymatic method for $O_2^{\bullet-}$, and chemical methods for the stable DPPH$^{\bullet}$ and for the ABTS$^{\bullet+}$. The phenolic extracts showing highest activities in all systems were obtained from red cabbage (Table 3). Our data reveal that the TEAC and VCEAC values for water phenolic extract correlate well. The rank order based on these values is as follows: red cabbage > Brussels sprouts > savoy cabbage > white cabbage. The TEAC values for all samples were higher than the corresponding VCEAC values. Moreover, content of phenolics was highly correlated both with TEAC ($R^2 = 0.88$) or VCEAC ($R^2 = 0.91$) values. The methods mentioned above use the DPPH$^{\bullet}$ radical and ABTS$^{\bullet+}$ radical cation, which are artificial radicals and are not found in any biological system.

Therefore, we have also used the enzymatic method for $O_2^{\bullet-}$. A number of reports indicate that increased production of superoxide anions combined with decreased antioxidant capacity of vascular tissue may contribute to development of vascular diseases including atherosclerosis, hypertension and vascular complication of diabetes (Katusic, 1996). The amounts of vegetables causing 50% decrease of $O_2^{\bullet-}$ (IC_{50} values) are listed in Table 3. We reported that all tested phenolics of

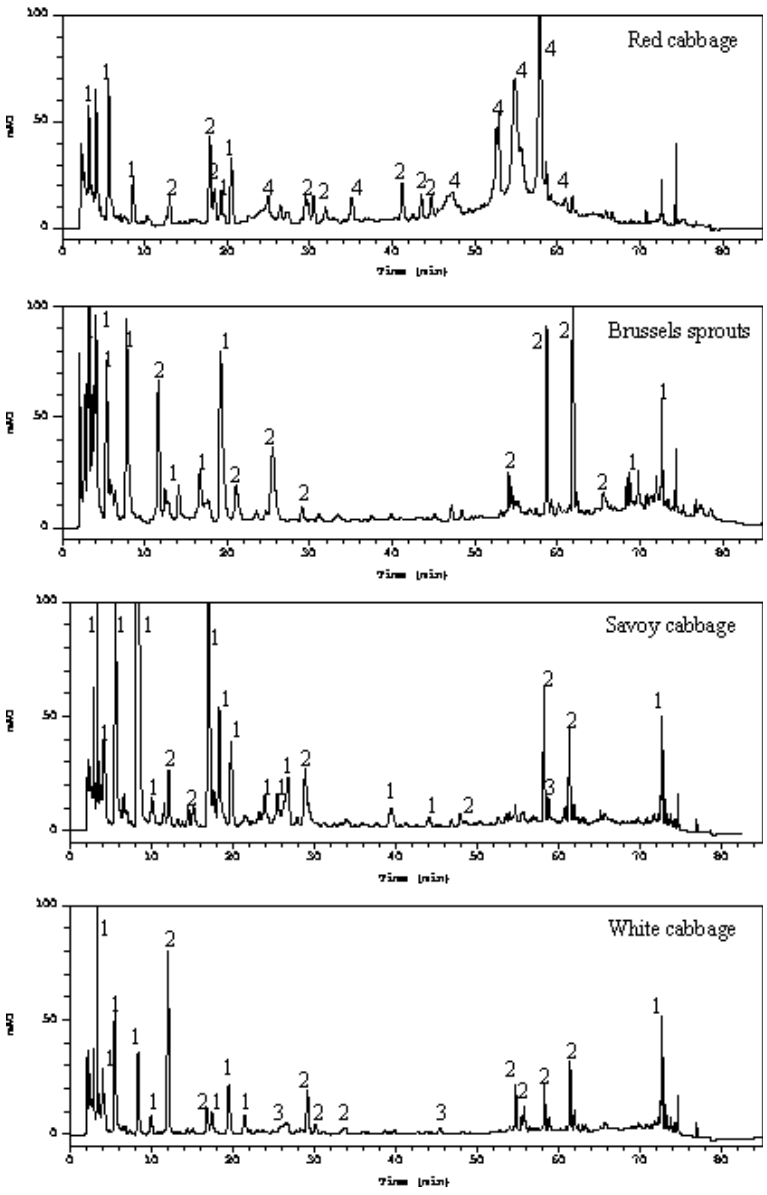

Figure 1 HPLC profiles of Brassica vegetables phenolic compounds at 280 nm wavelength. 1 – hydroxybenzoic acid derivatives; 2 – hydroxycinnamic acid derivatives; 3 – flavonols; 4 – anthocyanins.

Brassica vegetables were capable of scavenging superoxide anion radical, and the scavenging effect of these vegetables decreased in the following order: Brussels sprouts > red cabbage > savoy cabbage > white cabbage. We also noticed significant differences (2.3-fold) of IC_{50} values for three cultivars of white cabbage. This fact may be caused by different phenolic profiles, because total phenolic contents for these white cabbage cultivars varied insignificantly from 20.81 to 29.7 mg per 100 g.

Phenolic compounds may also reduce the level of oxidative stress by inhibition of lipid peroxidation. Antioxidant activity of water phenolic extracts in the linoleic acid emulsion, assayed by the thiobarbituric-acid-based method, is shown in Table 3. The inhibition time (T_{inh}) increased with the concentration of vegetable extract in the emulsion. A fourfold (from 0.5 to 2 g L^{-1}) increase of the concentration caused about twofold higher the T_{inh} values in term of red and white cabbages or Brussels sprouts and threefold in savoy cabbage. We have noticed that the T_{inh} values for linoleic acid emulsion containing the red cabbage and Brussels sprouts phenolics extract were comparable. The highest differences were observed for different cultivars of white cabbage. Similar relationship was observed for hypoxanthine–xanthine oxidase enzymatic system, which

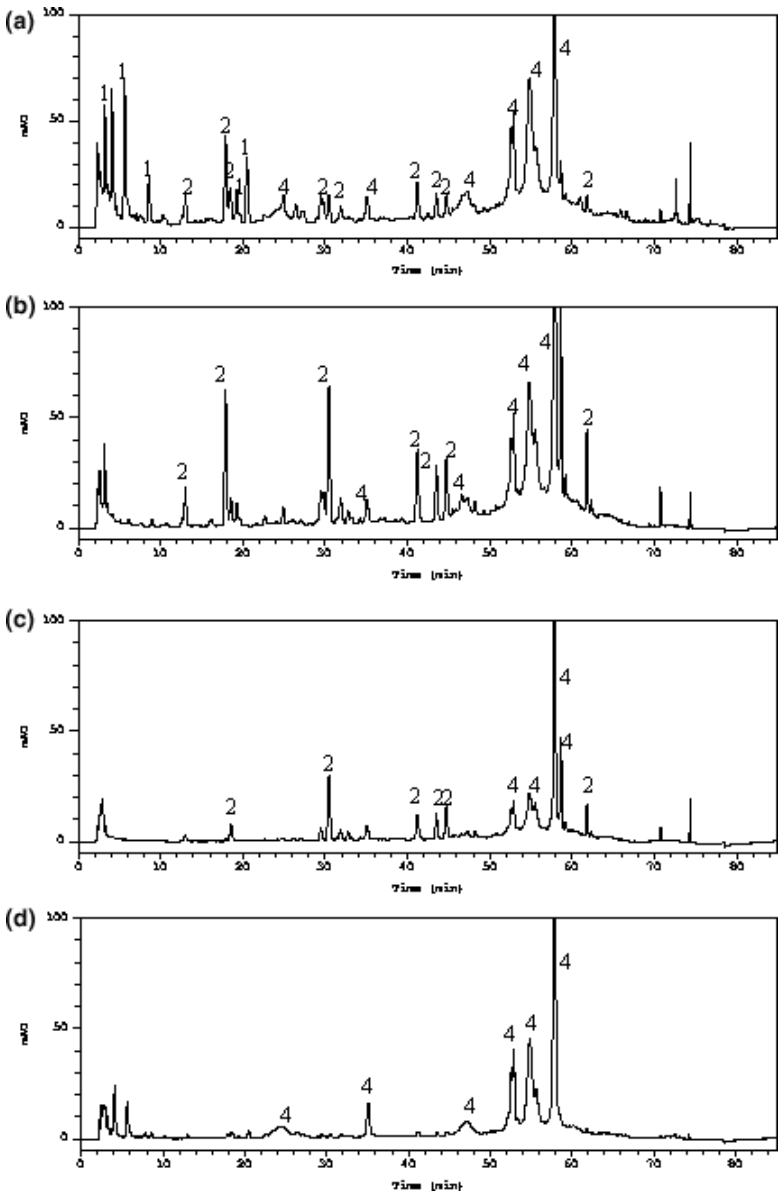

Figure 2 HPLC profiles of red cabbage phenolic compounds at four different wavelengths: (a) 280 nm; (b) 320 nm; (c) 360 nm and (d) 520 nm. 1 – hydroxybenzoic acid derivatives; 2 – hydroxycinnamic acid derivatives; 3 – flavonols; 4 – anthocyanins.

Table 4 Hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and anthocyanins contents of Brassica vegetables

		Mean concentration (mg per 100 g FW ± SD; $n = 2$)				
Vegetable	**Cultivars**	**Hydroxybenzoic acids[a]**	**Hydroxycinnamic acids[b]**	**Flavonols[c]**	**Anthocyanins[d]**	**Total**
Red cabbage	Kissendrup	19.92 ± 1.31	45.13 ± 3.21	0	76.16 ± 3.51	141.21
	Koda	15.52 ± 1.10	33.96 ± 4.22	0	40.53 ± 2.51	90.01
Brussels sprouts	Ajax	33.90 ± 2.11	44.55 ± 2.46	0	0	78.45
	Filemon	40.13 ± 3.11	49.58 ± 2.31	0	0	89.71
White cabbage	Almanag	5.91 ± 0.21	12.59 ± 0.56	0.45 ± 0.06	0	18.95
	Tukana	6.13 ± 0.18	7.74 ± 0.28	0.14 ± 0.01	0	14.01
	Vestri	10.09 ± 0.67	3.01 ± 0.11	0.03 ± 0.01	0	13.13
Savoy cabbage	Langedijker	13.56 ± 0.89	19.32 ± 1.61	1.38 ± 0.11	0	34.26
	60F/100	18.70 ± 1.12	21.36 ± 1.45	0.88 ± 0.06	0	40.94

[a]Content based upon gallic acid as standard.
[b]Content based upon chlorogenic acid as standard.
[c]Content based upon rutin as standard.
[d]Content based upon cyanidin 3-glucoside as standard.

generates superoxide $O_2^{\bullet-}$ radicals. Antioxidant activity of food phenolics extract depends strongly not only on total phenolic content but also on phenolic composition. Many studies suggested relationship between molecular structure of phenolics and free radical scavenging activity (Aaby *et al*., 2004; Taubert *et al*., 2003; Re *et al*., 1999) or inhibition of lipid peroxidation (Arora *et al*., 1998; Vinson *et al*., 1995). For example, according to Taubert *et al*. (2003) phenolics with pyrogallol or catechol moieties were revealed as the most rapid superoxide scavengers.

A number of studies on the quantitative phenolic profiles in Brassica vegetables concern mainly broccoli, which are a source of flavonol and hydroxycinnamoyl derivatives (Vallejo *et al*., 2003; Price *et al*., 1997,1998). White cabbage leaves contain a mixture of more than 20 phenolic compounds including glucosides of kaempferol and quercetin with/without further acylation with hydroxycinnamic acids (Nielsen *et al*., 1998). On the contrary, red cabbage contains 23 different anthocyanins, which were cyanidin derivatives highly conjugated with sugars (glucose and xylose) and acylated groups (caffeoyl, *p*-coumarolyl, feruloyl, *p*-hydroxybenzoyl, sinapoyl and oxaloyl) (Wu & Prior, 2005). The determination of phenolic profile in Brassica vegetables is difficult, because a majority of reference compounds are not commercially available. In order to carry out the quantitative analysis of phenolics in vegetable extracts, the hydrolysis procedure, especially acid hydrolysis, was used very often (Bahorun *et al*., 2004; Franke *et al*., 2004). In our study, phenolic profiles were determined using HPLC method based on the maximum absorption wavelength of different groups of phenolics, i.e. anthocyanins (520 nm), flavonols (360 nm), hydroxycinnamic acids (320 nm) and hydroxybenzoic acids (280 nm; Figs. 1 and 2). Table 4 shows the contents of phenolic compounds in the water phenolic extracts studied. Anthocyanin levels in red cabbage were as follows: 40.53 mg per 100 g FW for Koda and 76.16 mg per 100 g for Kissendrup. The average anthocyanin content determined spectrometrically in red cabbage cultivated in Italy was 125 mg per 100 g FW (Piccaglia *et al*., 2002). Mazza & Miniati (1993) reported the wide range of anthocyanin contents for red cabbage (25–495 mg per 100 g FW). Hydroxycinnamic acids predominated in other tested vegetables, except white cabbage Vestri, which contains 3.4-fold more hydroxybenzoic acids. Flavonols were found only in white and savoy cabbages, with concentration ranging from 0.03 to 1.38 mg per 100 g of FW. According to Chu *et al*. (2000), the flavonol contents (after acidic hydrolysis) were 0.15 and 0.11 mg per 100 g in purple and white cabbage, respectively. However, Bahorun *et al*., (2004) found higher levels of flavonols in hydrolysed white cabbage extract (5.9 mg per 100 g).

Conclusions

Among *B. oleracea* vegetables, red cabbage seems to be a very good source of dietary antioxidants possessing high antioxidant activity. This vegetable outstrips white cabbage, so far the most popular Brassica vegetable in Poland, in terms of phenolics (sixfold) and of vitamin C (2.5-fold). The results indicated that total phenolics are the major contributor to the free radical scavenging activity of red cabbage. In addition, the results suggest that anthocyanins can be considered the main source of antioxidant activity.

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