

The Effect of Cooking Conditions on Hydrophilic Antioxidants in Brussels Sprouts

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ABSTRACT

Knowledge on the stability and antioxidant activity of natural vegetable antioxidants affected by cooking conditions can help in planning diets for enhanced antioxidant intake. This study evaluates the effect of boiling, microwaving and steaming on antioxidant activity and content of both vitamin C and phenolic compounds present in Brussels sprouts (cvs. 'Ajax' and 'Filemon'). Steaming cooking is recommended to minimise the loss of hydrophilic antioxidants, while conventional cooking significantly reduces the content of vitamin C (up to 53%) and phenolics (up to 64%) in cooked Brussels sprouts. The losses of dietary antioxidants tested caused a decrease of the free radical scavenging activity towards ABTS^{•+} and DPPH[•] radicals for all the samples cooked.

Keywords: ABTS, *Brassica*, cooking methods, DPPH, phenolic compounds, vitamin C

Abbreviations: ABTS, 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonic acid); DPPH, (1,1-diphenyl-2-picrylhydrazyl); FRAP, Ferric Reducing Antioxidant Power; TEAC, Trolox Equivalent Antioxidant Capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

INTRODUCTION

A diet rich in *Brassica* vegetables (Cruciferous family) is thought to be associated with reduced risk of chronic diseases like cardiovascular disease and cancer (Cohen *et al.* 2000; Chu *et al.* 2002; Uhl *et al.* 2004; Boivin *et al.* 2009). The beneficial dietary properties of these vegetables have been attributed to their dietary antioxidants and also to the products of the glucosinolates hydrolysis. These antioxidants include water-soluble vitamin C and phenolic compounds, as well as lipid-soluble tocopherols and carotenoids. Among *Brassica* vegetables Brussels sprouts (*Brassica oleracea* var. *gemmifera*) are an especially rich source of vitamin C. They also contain moderate levels of lipid-soluble antioxidants (Podsędek 2007). According to numerous reports, the amount of total phenolics in Brussels sprouts ranged from 37 to 331 mg/100 g fresh weight (Table 1). Brussels sprouts have been also reported to have high antioxidant activity (Cao *et al.* 1996; Honer and Cervellati 2002; Boivin *et al.* 2009). Composition and activity of Brussels sprouts antioxidants are usually determined on raw material; however, from a nutritional point of view it should be considered that before consumption this vegetable is cooked in different ways. Variation in cooking conditions can affect both the sensory quality and chemical composition of vegetable, as well as antioxidants content. Some studies have indicated that in *Brassica* vegetables subjected to

cooking the amounts of both vitamin C and phenolics were reduced by 4-80% (Czarniecka-Skubina 2002; Zhang and Hamauzu 2004; Lopez-Berenguer *et al.* 2007; Podsędek 2007; Sikora *et al.* 2008; Wachtel-Galor *et al.* 2008). The losses of dietary antioxidants depend on the cooking conditions like type of cooking (conventional, steaming, microwaving, etc.), cooking time and amount of water.

The objective of the present work was to study the influence of several cooking methods on the content of hydrophilic antioxidants in Brussels sprouts, such as vitamin C and phenolic compounds and their scavenging capacity towards free radicals, as well as estimation of the conditions that maximise retention of these dietary antioxidants.

MATERIALS AND METHODS

Chemicals

Chlorogenic acid, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), dithiothreitol, potassium persulphate and meta-phosphoric acid were purchased from Sigma-Aldrich (Steinheim, Germany) and ascorbic acid was obtained from Sigma-Aldrich (St. Louis, USA). HPLC grade methanol, acetonitrile and formic acid were purchased from J.T. Baker (Germany). All other chemicals were reagent grade products purchased from POCh (Gliwice, Poland). Ultra pure water was produced in the laboratory using a SimplicityTM Water Purification System (Millipore, Marlborough, MA, USA).

Plant materials

Brussels sprouts samples (cvs. 'Ajax' and 'Filemon'), field-grown in commercial gardens near Łódź (central region of Poland) were harvested in November. External damaged leaves were removed, and edible parts of Brussels sprouts were cooked in one of three different ways. Each of the cooking procedures was repeated in triplicate. Raw Brussels sprouts were used as a reference sample.

Table 1 Literature data for total polyphenols content (mg/100 g) in Brussels sprouts

Variety	Extraction solvent	Content	References
Zenk	80% ethanol	37.7 ^a	Singh <i>et al.</i> 2007
nd	70% methanol	91.0	Cieślak <i>et al.</i> 2006
nd	70% acetone	257.1 ^a	Brat <i>et al.</i> 2006
Maczuga	70% methanol	331.0 ^b	Sikora <i>et al.</i> 2008

^a content is expressed as gallic acid

^b content is expressed as chlorogenic acid

nd – indefinite

Cooking

The cooking conditions were selected according to literature data (Czarniecka-Skubina 2002; Viña *et al.* 2007; Sikora *et al.* 2008) and traditional polish style of cooking. For conventional cooking, fresh Brussels sprouts samples (500 g) were cooked in 500 or 1000 ml of boiling water for 10 or 20 min. Then, excessive water was dripped off and the cooked vegetable was homogenized in a blender (Braun GmbH–Germany). For microwaving, 300 ml (room temperature) of water were added to a 300 g portion of Brussels sprouts in a glass beaker and microwaved (600 W) for 10 min in a conventional microwave oven (LG Group–Korea). The vegetable was then drained and homogenized. For steaming, Brussels sprouts (500 g) were placed on tray in a pot, and then covered with a lid and steamed over boiling water for 20 min. The cooked Brussels sprouts were then homogenized. Next, one part of the homogenized samples was used for determination of dry matter and vitamin C contents. The second part was lyophilized and ground into a powder and stored at -20°C for further analysis (phenolic compounds content and their scavenging activity).

Dry matter determination

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

Vitamin C extraction and analysis

The extraction method used was a modification of that described by Howard *et al.* (1999). Raw and cooked Brussels sprouts samples (10 g) were extracted with a 1% solution of meta-phosphoric acid (25 ml) for 15 min at room temperature and centrifuged at $2500 \times g$ for 10 min. The residue was re-extracted with 10 ml of the extracting solution and centrifuged. The combined supernatants were diluted to 50 ml with 1% meta-phosphoric acid. One ml of this solution and 1 ml of dithiothreitol (5%) were mixed and kept in the dark for 18 h at room temperature. Then, after dilution with meta-phosphoric acid the samples were analyzed using a Knauer high performance liquid chromatograph equipped with a Eurospher-100 C-18 column (25 cm \times 4.6 mm; 5 μm) fitted with the same guard column. The HPLC method was adapted from Gliszczynska-Swiglo and Tyrakowska (2003). For analysis of vitamin C the following gradient of methanol (solvent A) and 5 mM KH_2PO_4 , pH 2.6 (solvent B) was used: linear increase of solvent A from 5 to 22% in 6 min and then return to the initial conditions within the next 9 min with a flow rate of 1 ml/min with a UV-Vis detector set at 245 nm. Ascorbic acid was identified by comparison with its genuine sample.

Phenolics extraction and determination

Lyophilized raw or cooked vegetables (2 g) were extracted twice with 50 ml of a 70% solution of methanol for 15 min at room temperature (Vallejo *et al.* 2003). The mixture was then centrifuged at $2500 \times g$ for 15 min, and the resulting supernatant was evaporated under reduced pressure ($T < 40^{\circ}\text{C}$). The aqueous extracts were diluted to 20 ml with water, 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 and Pompei 1971). Briefly, 10 ml of water, 0.1–0.6 ml of the 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 of incubation in the dark, the absorbance was measured at 700 nm. The total phenolics amount was expressed as gallic acid equivalent in mg/100 g of fresh weight.

Phenolic profiles were determined using a HPLC Knauer system equipped with UV-Vis detector and a Eurospher-100 C-18 column. The binary mobile phase according to Tsao and Yang (2003) consisted of 6% acetic acid in 2 mM sodium acetate (solvent A) and acetonitrile (solvent B). The flow rate was 1 ml/min and a 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 two groups. The hydroxybenzoic acid deriva-

tives were quantified at 280 nm and expressed as gallic acid equivalents, and hydroxycinnamic acid derivatives at 320 nm as chlorogenic acid equivalents.

ABTS radical cation scavenging activity

ABTS^{•+} scavenging activity was determined according to the procedure described by Re *et al.* (1999). 2,2'-Azinobis(3-ethyl-benzothiazoline-6-sulphonic acid) radical cation (ABTS^{•+}) was produced by reacting 7 mM ABTS water solution with 2.45 mM potassium persulphate (final concentration) followed by an incubation of the mixture in the dark for 12–16 h at room temperature. Then, water phenolic extract (20 μl) was mixed with 1 ml of diluted ABTS^{•+} solution and after 6 min incubation at 30°C this mixture was measured at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard and the antioxidant capacity was expressed as μmol of Trolox per 1 g of the vegetable – fresh or cooked (TEAC - Trolox Equivalent Antioxidant Capacity).

DPPH radical scavenging activity

DPPH[•] scavenging activity was determined using a method of Kim *et al.* (2002). Water phenolic extract (0.1 ml) was mixed with 2.9 ml of 100 μM DPPH[•] 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. Trolox was used as a standard and the antioxidant capacity was expressed as μmol of Trolox per 1 g of the vegetable – fresh or cooked (TEAC - Trolox Equivalent Antioxidant Capacity).

Statistical analysis

The analysis of variance was performed on data for differences between the cooking methods using the ANOVA (Statistica Ver. 6.0, USA), followed by the Tukey's posthoc test with significance level $p < 0.05$.

RESULTS AND DISCUSSION

Due to the beneficial effects of vegetable antioxidants, there is an increasing interest in evaluation of their changes during postharvest treatments, technology processing (e.g. blanching, freezing, canning), and domestic cooking. Brussels sprouts can be cooked in various ways, but most common are boiling, steaming and microwaving. Our previous studies have demonstrated that water-soluble vitamin C and phenolic compounds are the main dietary antioxidants in Brussels sprouts. The total content of lipid-soluble carotenoids and α -tocopherol was more than one hundred-fold lower than that of hydrophilic antioxidants. Moreover, the contribution of lipophilic antioxidants to the total antioxidant capacity of Brussels sprouts towards ABTS radical was below 1% (Podszędek *et al.* 2006).

The effect of the cooking method on vitamin C content

The vitamin C content in two varieties of Brussels sprouts ('Ajax' and 'Filemon') cooked under different conditions is shown in **Table 2** and **Fig. 1**. Vitamin C concentration significantly ($p < 0.05$) decreased after all cooking treatments. The losses of vitamin C content in cooked Brussels sprouts were caused by its thermal degradation and leaching into the cooking water. The highest vitamin C retention was found for steamed Brussels sprouts (the losses from 9 to 14%). In the case of conventional cooking, the amount of water strongly influenced the content of vitamin C. When the water to vegetable ratio was 2:1 (v/w) and Brussels sprouts were cooked for 20 min, 52.7% ('Filemon') and 65.0% ('Ajax') of vitamin C was retained (**Fig. 1**). Reduction of the water volume by half (1:1 v/w) resulted in slightly higher level of vitamin C (59.8% for the 'Filemon' variety and 68.3% for the 'Ajax'). On the other hand, shortening of the cooking time from 20 to 10 min affected the

Table 2 Effect of various cooking methods on the antioxidants content in two cultivars of Brussels sprouts.

Cooking method	Cooking time (min)	Vegetable: water (g/ml)	'Ajax'			'Filemon'		
			Dry matter (%)	Vitamin C ^a (mg/100 g)	Phenolics ^b (mg/100 g)	Dry matter (%)	Vitamin C ^a (mg/100 g)	Phenolics ^b (mg/100 g)
Raw cabbage	---	---	18.10 ± 0.04 a	127.77 ± 7.82 a	140.13 ± 5.67 a	20.64 ± 0.25 a	129.27 ± 2.96 a	133.46 ± 6.43 a
In boiling water	20	1:2	16.17 ± 0.53 c	81.41 ± 1.45 b	97.67 ± 6.72 b	14.24 ± 0.39 c	64.07 ± 1.22 b	80.26 ± 5.81 b
	20	1:1	16.98 ± 0.08 b	85.69 ± 2.76 bc	110.60 ± 8.49 bc	15.52 ± 0.26 b	73.56 ± 1.73 c	92.29 ± 3.65 bc
In steam	10	1:1	17.31 ± 0.08 b	94.01 ± 1.36 d	114.85 ± 2.93 c	15.60 ± 0.24 b	80.15 ± 3.52 d	100.64 ± 2.52 c
	20	---	19.60 ± 0.08 d	118.04 ± 1.29 e	142.73 ± 5.69 a	17.28 ± 0.38 d	107.56 ± 1.64 f	133.35 ± 9.74 a
In microwave	10	1:1	17.49 ± 0.18 ab	89.60 ± 3.43 cd	119.59 ± 1.65 c	15.36 ± 0.13 b	84.18 ± 1.70 e	98.18 ± 6.69 c

Mean ± SD, n≥3; Treatments with the same letters are not significantly different (p < 0.05; Tukey's test)

^a vitamin C values are expressed as ascorbic acid equivalents

^b phenolics values are expressed as gallic acid equivalents

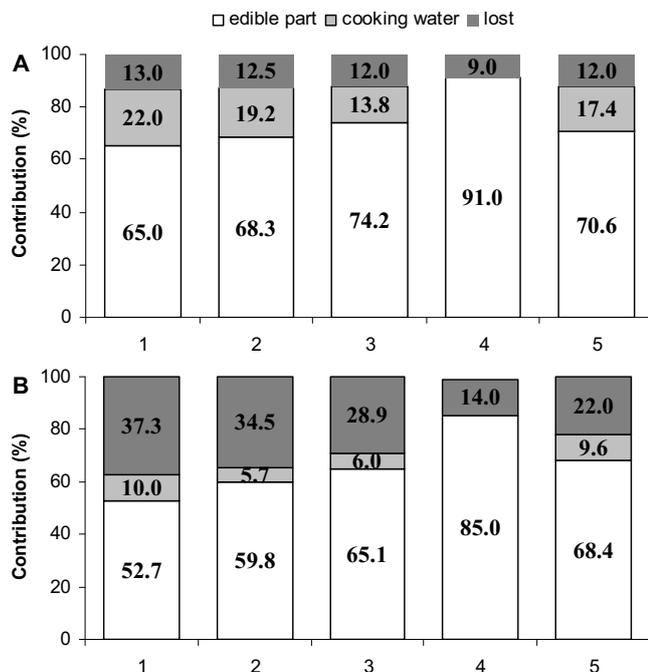


Fig. 1 Distribution of vitamin C in edible part, cooking water and lost observed for two varieties (A = cv. 'Ajax', B = cv. 'Filemon') of Brussels sprouts cooked in different conditions. 1 - conventional cooking for 20 min, ratio vegetable: water = 1: 2 (w/v); 2 - conventional cooking for 20 min, ratio vegetable: water = 1: 1 (w/v); 3 - conventional cooking for 10 min, ratio vegetable: water = 1: 1 (w/v); 4 - steaming for 20 min; 5 - microwaving for 10 min.

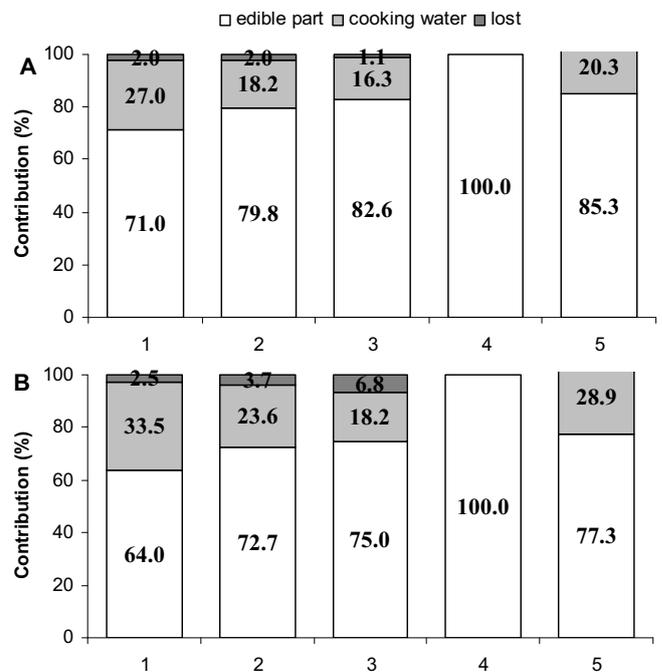


Fig. 2 Distribution of total phenolics in edible part, cooking water and lost observed for two varieties (A = cv. 'Ajax', B = cv. 'Filemon') of Brussels sprouts cooked in different conditions. 1 - conventional cooking for 20 min, ratio vegetable: water = 1: 2 (w/v); 2 - conventional cooking for 20 min, ratio vegetable: water = 1: 1 (w/v); 3 - conventional cooking for 10 min, ratio vegetable: water = 1: 1 (w/v); 4 - steaming for 20 min; 5 - microwaving for 10 min.

retention of vitamin C by 5.3 and 5.9% in the 'Filemon' and 'Ajax' variety, respectively. The losses of vitamin C during microwaving were comparable with the results obtained for the best conventional cooking method with water to vegetable ratio 1:1 (v/w), and the cooking time 10 min. Regarding the Brussels sprouts varieties tested, retention of vitamin C in the cooked vegetable was better for the 'Ajax' variety. Our results are consistent with those reported by Czarniecka-Skubina (2002). The conventional cooking in pot starting with boiling water (water to vegetable ratio was 2:1 (v/w)) for 26-27 min, caused losses of vitamin C from 35.9 to 40.4% depending on the vegetable variety. On the contrary, this author has observed higher retention of vitamin C during the cooking in microwave oven (96.3%) in comparison to our results (68.4-70.6%). According to Sikora *et al.* (2008) the amount of vitamin C in Brussels sprouts prepared by the boiling for 12-15 min decreased by 70%. Viña *et al.* (2007) have studied the effect of blanching methods on vitamin C content in Brussels sprouts. The authors found that the blanching in water for 1 and 3 min did not cause changes in ascorbic acid content, but blanching for 4 min caused 24% decrease in amount of this vitamin. Conversely, microwave heating for 5 min, followed by blanching in boiling water for 2 min gave a slight increase in the ascorbic acid content.

The effect of the cooking method on phenolics content

The total phenolics content in Brussels sprouts cooked under different conditions was determined by Folin-Ciocalteu assay (Table 2) while the contents of total hydroxybenzoic and hydroxycinnamic acids were measured by HPLC method (Table 3). The level of total phenolics in uncooked Brussels sprouts was 133.46 mg/100 g for 'Filemon' and 140.13 mg/100 g for 'Ajax'. These values were higher than the contents (37.7 mg/100 g) obtained by Singh *et al.* (2007) and by Cieřlik *et al.* (2006) – 91 mg/100 g, but about 2.5-fold lower than the amount estimated by Sikora *et al.* (2008) – Table 1. These differences are probably caused by the use of different Brussels sprouts varieties.

After cooking of Brussels sprouts in boiling water and in microwave, the total phenolics content was significantly (p < 0.05) reduced (Table 2). Unlike vitamin C, the losses of phenolic compounds content were caused mainly by their leaching into the cooking water (Fig. 2). The phenolics level in the both varieties tested decreased in the following order: fresh > 20-min steamed > 10-min microwaved > 10-min cooked in boiling water > 20-min cooked in boiling water. The higher losses (about 10%) were observed for 'Ajax'. As for vitamin C, the lower volume of cooking

Table 3 Hydroxybenzoic acids and hydroxycinnamic acids contents^a (mg/100g) in raw and cooked Brussels sprouts.

Cooking method	Cooking time (min)	Cabbage: water (g/ml)	'Ajax'		'Filemon'	
			Hydroxybenzoic acids ^b	Hydroxycinnamic acids ^c	Hydroxybenzoic acids ^b	Hydroxycinnamic acids ^c
Raw cabbage	---	---	35.05 a	44.50 a	40.07 a	49.51 a
In boiling water	20	1:2	26.36 b	25.65 b	22.77 c	19.56 c
	20	1:1	26.26 b	26.65 b	22.63 c	21.63 c
	10	1:1	35.78 a	32.58 b	28.47 b	28.71 b
In steam	20	---	37.26 a	34.72 b	40.42 a	30.88 b
In microwave	10	1:1	30.18 b	26.04 b	20.39 c	20.77 c

^a Data expressed as means of two samples; Treatments with the same letters are not significantly different ($p < 0.05$; Tukey's test)

^b content based upon gallic acid as a standard

^c content based upon chlorogenic acid as a standard

water and shortening of the cooking time led to better retention of the phenolic compounds in the cooked vegetable. Brussels sprouts cooked in steam for 20 min retained 100% of the total phenolics present in fresh vegetable. Our results obtained for conventional cooking were similar to those previously reported by Sikora *et al.* (2008). Brussels sprouts boiled for 12-15 min lost 40% of total polyphenols. Wachtel-Galor *et al.* (2008) have examined the effect of boiling, steaming and microwaving of broccoli, cauliflower, cabbage and Chinese cabbage on total phenolics content. Boiling and microwaving had strong effect on cabbages and broccoli with a decrease of more than 60% in total phenolics, whereas, for cauliflower, a loss of 39% was noticed after microwaving, 4% after boiling and an increase by 45% was observed after steaming.

The level of phenolic acids in the Brussels sprouts tested before and after cooking is shown in **Table 3**. Hydroxycinnamic acids content was higher in raw vegetable than that of hydroxybenzoic acids, but hydroxybenzoic acids generally dominated in the cooked Brussels sprouts. After all cooking procedures, the hydroxycinnamic acids content was significantly ($p < 0.05$) reduced. In the case of hydroxybenzoic acids, steaming did not affect significantly their initial content of samples. The lowest reduction of both phenolic acids groups tested was observed in the case of conventional cooking for 10 min in boiling water in a 1:1 (v/w) ratio to weight of vegetable. To the best of our knowledge, there is no information on the quantitative and qualitative phenolic profiles in Brussels sprouts. Heimler *et al.* (2006) presented some data regarding to the content of phenolic acids (0.35 mg/g dry weight) and flavonols (1.12 mg/g dry weight) in Brussels sprouts, but according to the authors the attribution of peaks was difficult because of a very poor quality of HPLC profile obtained. In our study, we have not detected peaks with maximum absorbance at 360 nm at which flavonols should be quantified (data not shown).

The effect of the cooking method on free radicals scavenging activity

ABTS and DPPH are commonly used to assess antioxidant activity *in vitro*. Reduction of the radicals used by a hydrogen-donating antioxidant is monitored through a decrease in optical density at 734 and 517 nm, respectively. The antioxidant activity, which reflects the ability of methanolic extract to scavenge the ABTS^{•+} radical monocation and DPPH[•] radical, is presented in **Figs. 3, 4**. The results clearly showed that scavenging activity of Brussels sprouts towards the both synthetic, stable radicals tested significantly ($p < 0.05$) declined during cooking and showed similar trends for two varieties tested (**Figs. 3, 4**). Moreover, the tendency observed for TEAC values is in good correlation with the values of vitamin C and phenolic compounds losses (**Figs. 1, 2**). The largest changes (losses 35.2-36.5%) of the TEAC values were observed in the samples after conventional cooking for 20 min with double volume of the water, and the smallest (losses 4.7-12.2%), in the case of steaming. Previous studies have indicated the decrease of the antioxidant activity during the cooking of other *Brassica* vegetables such as broccoli, Swamp cabbage and kale (Ismail *et al.*

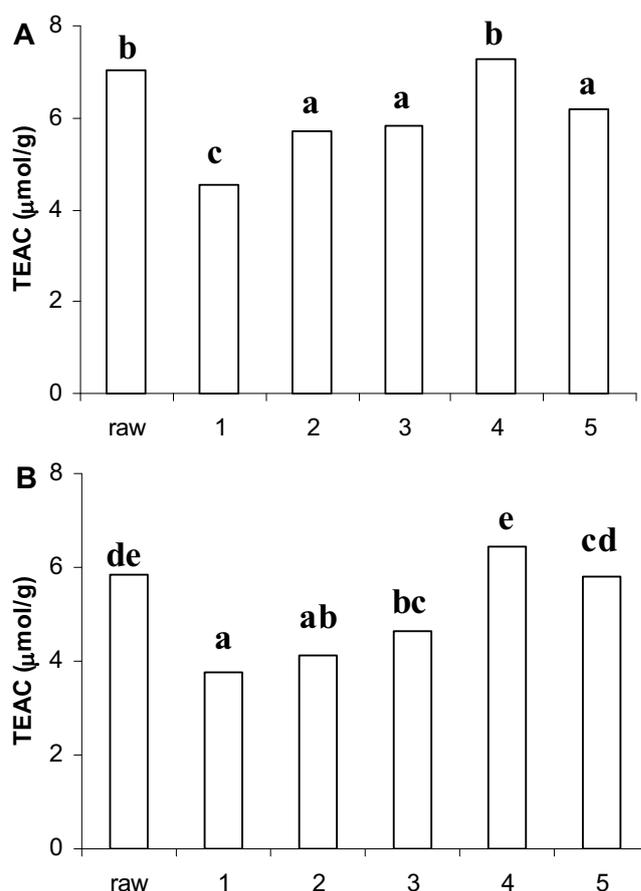


Fig. 3 Effect of different cooking methods on ABTS^{•+} radical scavenging capacity of two varieties (A = cv. 'Ajax', B = cv. 'Filemon') of Brussels sprouts methanolic extract. 1- conventional cooking for 20 min, ratio vegetable: water = 1: 2 (w/v); 2 - conventional cooking for 20 min, ratio vegetable: water = 1: 1 (w/v); 3 - conventional cooking for 10 min, ratio vegetable: water = 1: 1 (w/v); 4 - steaming for 20 min; 5 - microwaving for 10 min. Data are means \pm SD (n=3). Columns with the same letters are not significantly different ($p < 0.05$, Tukey's test).

2004; Lin and Chang 2005). Zhang and Hamauzu (2004) reported 65% decrease of the scavenging activity of broccoli towards DPPH radical during conventional and microwave cooking. However, other studies indicated that boiling, steaming, microwaving and blanching increased free radical scavenging activity of *Brassica* vegetables. Viña *et al.* (2007) noticed that blanching for 1-4 min gave 10-60% increases in the scavenging activity of Brussels sprouts as determined by the DPPH method. In the same studies the authors observed a 2-fold increase of the antioxidant activity in case of microwave heating for 5 min, followed by blanching in boiling water for 2 min. Wachtel-Galor *et al.* (2008) found that steaming of cauliflower, broccoli, cabbage and choy-sum led to an increase in the FRAP (ferric reducing antioxidant power) values from 13% to 3-fold. In their study the FRAP values increased also for cauliflower

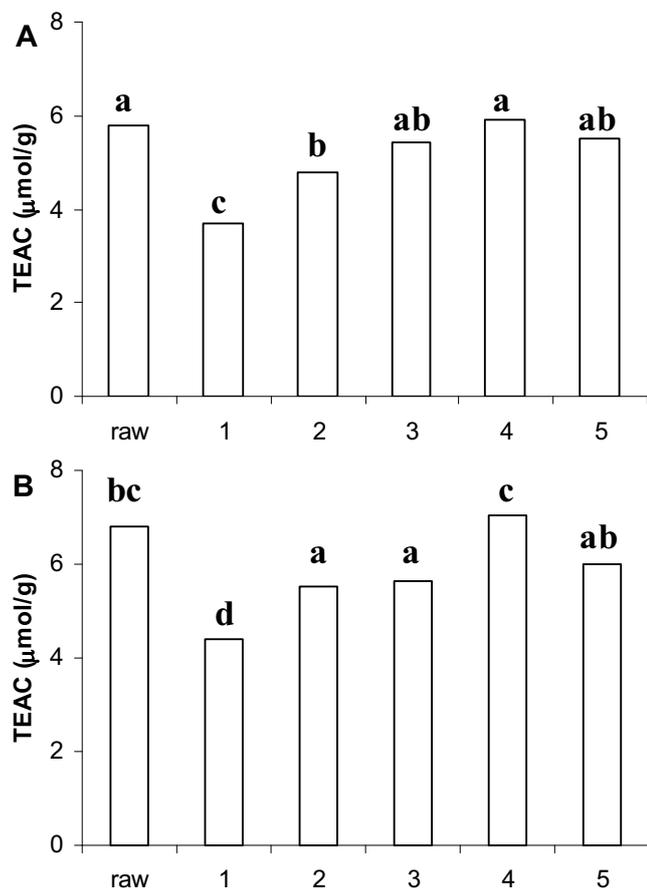


Fig. 4 Effect of different cooking methods on DPPH^{*} radical scavenging capacity of two varieties (A = cv. 'Ajax', B = cv. 'Filemon') of Brussels sprouts methanolic extract. 1- conventional cooking for 20 min, ratio vegetable:water=1:2 (w/v); 2 - conventional cooking for 20 min, ratio vegetable: water = 1: 1 (w/v); 3 - conventional cooking for 10 min, ratio vegetable: water = 1: 1 (w/v); 4 - steaming for 20 min; 5 - microwaving for 10 min. Data are means \pm SD (n=3). Columns with the same letters are not significantly different (p < 0.05, Tukey's test).

and broccoli boiled and microwaved for 5 and 10 min.

CONCLUSIONS

Our studies have shown that losses of vitamin C and phenolic compounds in Brussels sprouts during cooking vary depending on the cooking treatment and, to a lesser extent, on the Brussels sprouts variety. The results indicate that microwave cooking and boiling cause losses of hydrophilic antioxidants in Brussels sprouts and their free radical scavenging activity. Under these conditions vitamin C is destroyed, while phenolic compounds are largely leached into the cooking water. In order to minimise the losses of vitamin C and phenolic compounds during cooking, Brussels sprouts should be cooked in the steam.

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