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Effect of hydrothermal treatment on the antioxidant properties of broccoli (*Brassica oleracea* var. *botrytis italica*) florets

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Abstract

In the extracts of fresh raw and frozen broccoli, caffeic, ferulic, sinapinic acids and kaempferol were identified. Boiling reduced the amounts of caffeic acid and kaempferol in both samples. The concentration of polyphenols was 2.69 mg/g fresh mass and 0.96 mg/fresh mass in fresh raw broccoli and frozen raw broccoli, respectively. Boiling significantly decreased the amounts of phenolic compounds in fresh broccoli (1.58 mg/g of fresh mass). In the case of frozen broccoli, boiling increased the concentration of polyphenols by 38%. Fresh broccoli extract neutralized free radicals by 19.87%. Boiling significantly reduced its antiradical activity (to 15.06%). Samples of frozen broccoli had a 27.06% antiradical ability. Boiling did not change the antiradical activity in frozen broccoli case. Hydrothermal processing significantly influenced on the ability of the extracts to inhibit the decolorization of β-carotene emulsion. The extract of fresh broccoli had a higher activity when uncooked. Boiling seemed to increase this activity in the case of frozen broccoli samples. The results of the studies on the ability of broccoli extracts to inhibit linoleic acid autooxidation were ambiguous and depended on the method applied. No correlation was found between the content of phenolic compounds and the antioxidant activity of the extracts, regardless of the experimental variant and technique used.

Keywords: Broccoli; Phenolic compounds; Antioxidant activity; Boiling

1. Introduction

A diet rich in fruits and vegetables, which has been recommended for many years, provides secondary metabolites that contribute to the prevention of many civilization-related diseases such as: arteriosclerosis, insulin-dependent diabetes, cancer, ischemic heart disease and brain stroke. It has been determined that antioxidant activity, among other factors, is associated with anticancerogenic and antimutagenic properties. Therefore natural plant-derived antioxidants have became the object of vast interest. The extracts of many vegetables, fruits and cereals (Kähkönen et al., 1999; Velioglu, Mazza, Gao, & Oomach, 1998) as well as semi-products such as apple peels, grain husks, seeds and rinds of citrus fruits (Moure, Cruz, & Franco, 2001) demonstrated high antioxidant activity in model systems. Most of the antioxidant potential in vegetables, herb and spices is due to the redox properties of phenolic compounds which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Flavonoids and phenolic acids in particular have strong antioxidant properties. Antioxidant activity of polyphenols is exerted through different mechanisms. They act as reducers, have an ability to scavenge free radicals and chelate metal ions – cofactors of enzymes catalyzing oxidative reactions, inhibit oxidases, terminate radical chain reactions and stabilize free radicals (Rice-Evans, Miller, & Paganga, 1997; Weiqun-Wang, Marc, & Goodman, 1999). The content of phenolic compounds depends on plant species, cultivation technique and – in the case of food products – on the technological procedures used for raw material processing. Among vegetables, broccoli has been recognized as an important source of various biologically active compounds

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including polyphenols (mainly hydroxycinnamic acid esters, kaempferol and quercetin glucosides and traces of isorhamnetin) (Moreno, Carvajal, Lopez-Berenguer, & Garcia-Viguera, 2006). However, until quite recently there were no extensive studies on the effects of the hydrothermal food processing on the levels and antioxidant properties of broccoli polyphenols. Therefore this was the objective of this study.

2. Materials and methods

2.1. Plant materials and extracts preparation

Frozen broccoli of the "Hortex" brand and fresh broccoli available on the local market were used in the study. The raw material was boiled in 100 ml of distilled water for 5 (frozen broccoli) or 10 min (fresh broccoli). Phenolic compounds were extracted from both boiled and raw vegetables with the methanol: water (1:1 v/v) solvent system. Ten grams of broccoli sample and 50 ml of 50% (v/v) methanol were mixed and extracted twice using magnetic stirrer for 30 min. Then mixture was centrifuged and the supernatants were collected and used in the further analysis. The total content of phenolic compounds in the extracts was determined and their antioxidant activity was examined using techniques based on the different mechanisms of their action.

2.2. Chemicals and reagents

Folin-Ciocalteau reagent, *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), pyridine, chlorogenic acid, 1.1-diphenyl-2-picryl-hydrazyl (DPPH), β-carotene, linoleic acid, Tween 20, thiocyanate were purchased from Sigma-Aldrich. All others reagents were of analytical grade.

2.3. Identification of polyphenolic compounds by mass spectrometry

Polyphenols were extracted from 10 g of plant material with 100 ml of 50% (v/v) methanol. The solvent was evaporated and the remaining water phase was concentrated under reduced pressure. Such processed samples were transferred to columns packed with the stationary phase with chemically bounded octadecyl groups (C-18). Pure methanol was used to elute the column. The collected polyphenol fraction was methanolized for 2 h at 100 °C. The solvent was evaporated under nitrogen, and polyphenolic compounds were converted into trimethylsilyl derivatives (TMS) by the reaction of phenols with *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in the presence of trimethylchlorosilane (TMCS) in pyridine solution (Stobiecki, 1995). The TMS derivatives were prepared during 20 min in 100 μl of the pyridine: BSTFA (2:3) mixture at 90 °C, and analyzed with the Hewlett Packard GC/MS system which consisted of a gas chromatograph, model 5890/II, and a mass detector, model 5971A equipped with a Restek XTI-5 capillary column (length – 30 mm, diameter – 0.25 mm, film thickness – 0.25 μm). The carrier gas was helium at a low-rate 1 ml/min. the column temperature was programmed from 140 °C (held or 2 min) at 5 °C × min^{-1} to 300 °C, which was maintanted or 5 min. The injector temperature was 250 °C. Mass spectra were recorded in the range 50–650 amu.

2.4. Total phenols

Total phenols were estimated according to the Folin–Ciocalteau method (Singleton & Rossi, 1965). A 0.5 ml sample of the extract was mixed with 0.5 ml of H_2O, 2 ml of Folin reagent (1:5 H_2O) and after 3 min with 10 ml of 10% Na_2CO_3. After 30 min the absorbance of mixed samples was measured, at a wavelength of 720 nm, against the standard sample prepared with pure methanol. The concentrations of phenolic compounds were determined based on the standard curve for chlorogenic acid.

2.5. Determination of antioxidant activity

2.5.1. DPPH assay

The ability of the extracts to scavenge DPPH free radicals was determined by the method of Brand-Williams, Cuvelier, and Berset (1995). A 0.08 ml sample of methanol extract containing phenolic compounds was mixed with 3.92 ml of methanol solution of DPPH* (6×10^{-5} mol/L). Absorbance was measured at a wavelength of 515 nm at 0 time and every 15 min during one hour. Pure methanol was used as a control sample. Antioxidant activity was expressed as a percentage of inhibition calculated according to the formula

$$\%\text{inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \times 100$$

where $A_{C(0)}$ – absorbance of the control sample at 0 time, $A_{A(t)}$ – absorbance of the experimental sample measured every 15 min during 1 h.

The percentage of the remaining DPPH free radicals was also calculated based on the standard curve.

2.5.2. β-Carotene bleaching assay

The antioxidant activity of broccoli extracts was assayed based on the β-carotene bleaching method reported by Lee, Howard, and Villalón (1995). To prepare β-carotene emulsion, 5 mg of β-carotene was dissolved in 50 ml of chloroform, and 6 ml of the chloroform solution of β-carotene was added to a flask containing 120 μl of 60% linoleic acid and 1200 μl of Tween 40. Chloroform was evaporated at 40 °C under reduced pressure, and β-carotene emulsion was treated with 200 ml of 30% hydrogen peroxide. Six milliliter samples of oxidated emulsion were transferred into tubes and mixed with 80 μl samples of the examined extract. The tubes were immediately placed in the thermostat and incubated at 50 °C. The oxidation of β-carotene emulsion was measured spectrophotometrically

at 470 nm. Absorbance was measured at 0 time and at 15 min intervals during 1 h. In the control sample the extract was replaced by 80 μl of pure methanol. Antioxidant activity was expressed in two ways. First, the level of decolorization (R) of β-carotene was calculated according to the formula

$$R = \ln(A_{t=0}/A_{t=t}) \times 1/t$$

where $A_{t=0}$ – emulsion absorbance (470 nm) at 0 time, $A_{t=t}$ – absorbance (470 nm) after time t; t – incubation time in minutes.

Antioxidant activity (ANT) was expressed as a percentage of inhibition of β-carotene decolorization in comparison to the control, according to the equation

$$\%\mathrm{ANT} = 100 \times (R_k - R_s)/R_k$$

where R_k – degree of decolorization of the control sample, R_s – degree of decolorization of the experimental sample.

2.5.3. Determination of inhibition of linoleic acid autooxidation

Determination of inhibition of linoleic acid autooxidation was determined according Lingnert, Vallentinn, and Eriksson (1979) method. A 100 ml solution of linoleic acid (0.5 ml of 60% linoleic acid) and Tween 20 (0.5 ml) in 0.1 M phosphate buffer, pH 6.5, was prepared. The linoleic acid emulsion (2 ml) was mixed with 0.2 ml of the experimental extract or with 0.2 ml of distilled water (control sample). All samples were incubated at 37 °C for 24 h. A 0.2 ml aliquot of each sample was added to 8 ml 45% (v/v) methanol. Absorbance was measured immediately at a wavelength of 234 nm against 60% methanol. Antioxidant activity was determined according to the formula

$$\mathrm{AAC} = (\Delta A_{234\mathrm{nm(C)}} - \Delta A_{234\mathrm{nm}})/\Delta A_{234\mathrm{nm(C)}}$$

where $\Delta A_{234\,\mathrm{nm}}$ – increase in the absorbance of the experimental sample, $\Delta A_{234\,\mathrm{nm(C)}}$ – corresponding increase in the absorbance of the control sample.

2.5.4. Determination of antioxidant activity by the thiocyanate method

Determination of antioxidant activity was assayed based on the thiocyanate method according to Masuda and Jitoe (1994). A linoleic acid solution in 96% ethanol was prepared by adding 90% ethanol to 4.65 ml of 60% linoleic acid to reach the volume of 200 ml. A 1 ml aliquot of this solution was mixed with 1 ml of 0.05 M phosphate buffer, pH 7.0, 0.2 ml of the examined extract and 0.3 ml distilled water, to obtain an incubation mixture. In the control sample, the extract was replaced by 0.2 ml of distilled water. All samples were incubated at 40 °C in darkness. After incubation, 0.1 ml of the incubation mixture was mixed with 9.7 ml of 75% methanol, 0.1 ml of 30% of ammonium thiocyanate and 0.1 ml of 0.02 M $FeCl_3$ in 3.5% HCl. Absorbance was measured at a wavelength of 500 nm against 75% methanol at 0 time and after 24-h incubation. Antioxidant activity was calculated according to the formula

$$\mathrm{AAC} = (\Delta A_{500\mathrm{nm(C)}} - \Delta A_{500\mathrm{nm}})/\Delta A_{500\mathrm{nm(C)}}$$

where: $\Delta A_{500\,\mathrm{nm}}$ – increase in the absorbance of the experimental sample, $\Delta A_{500\,\mathrm{nm(C)}}$ – corresponding increase in the absorbance of the control sample.

2.6. Statistical analysis

All determination was performed in triplicate. The obtained data were subjected to statistical analysis using Statistica 5.0. The evaluations were analyzed for one-factor variance analysis. Statistical differences between the treatment groups were estimated by Tukey's test. All statistical tests were carried out at significance level of $\alpha = 0.05$. The linear correlation coefficients to determine the relationship between the concentrations of polyphenols and antioxidant activity and between different antioxidant activities were also evaluated.

3. Results and discussion

Broccoli is a significant source of biologically active dietary components such as hydroxycinnamic acids and flavonoids. In this reason the aim of my work was to evaluate the influence of hydrothermal treatment on their level and activity.

3.1. Identification of phenolic compounds by GC–MS

The following acids were detected in the extract of fresh raw broccoli, isolated with 50% (v/v) methanol: caffeic, sinapinic, ferulic and kaempferol. Caffeic acid was not found and only trace quantities of kaempferol were detected in the extracts of boiled broccoli (Fig. 1).

The extract of frozen raw broccoli, isolated with 50% methanol, contained the following acids: ferulic, caffeic, sinapinic and kaempferol (Fig. 2). In the boiled broccoli samples, kaempferol was not detected and only trace amounts of caffeic acid were present.

Analysis of decoctions of broccoli samples showed that alls compounds leached into the cooking water. Decoction of fresh broccoli contained the following acids: ferulic, caffeic, sinapinic and kaempferol. In the sample of frozen broccoli decoction kaempferol, ferulic and sinapinic acids were identified but only trace amounts of caffeic acid were present.

Herrmann (1989) found that broccoli contains chlorogenic acid (4-caffeoylquinic and 3-caffeoylquinic), 3-*p*-coumaroylquinic and glucose esters of acids: caffeic, ferulic and sinapinic. Additionally, Beveridge, Loubert, and Harrison (2000) identified *p*-coumaric and benzoic acids in broccoli cell wall material. Our results confirmed the presence of sinapinic, ferulic, caffeic and *p*-coumaric acids.

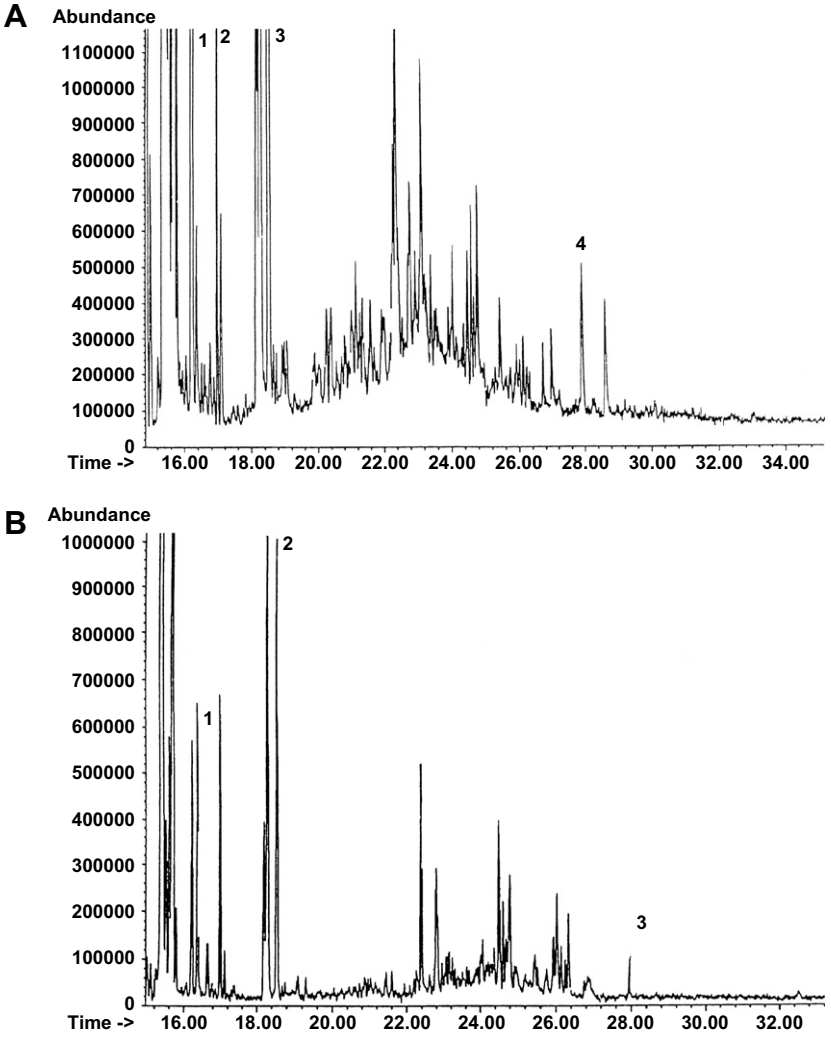

Fig. 1. Chromatograms of the total ionic current of fresh broccoli extracts. (A) before boiling (1 – ferulic acid, 2 – caffeic acid, 3 – synapic acid, 4 – kaempferol) and (B) after boiling (1 – ferulic acid, 2 – synapic acid, 3 – kaempferol).

Vegetables of the family *Brassicaceae* contain chlorogenic, *p*-coumaroylquinic and feruloylquinic acids. These compounds are present in the leaves of kale, cabbage and Brussels sprouts in the amount from 6–120 mg/kg to 104 mg/kg (Clifford, 1999). Broccoli contains approximately 60 mg/kg of chlorogenic acid and about 20 mg/kg of glycosides. Current studies have revealed that the head of some broccoli cultivars contains a mixture of different feruoyl and synapoyl esters with gentobiose in an amount exceeding 300 mg/kg. Broccoli heads and leaves of the cruciferous vegetables are a good source of sinapinic acid glycosides – broccoli heads contain over 10 mg of complexed sinapinic acid per 100 g (Clifford, 1999). The following acids: sinapinic, ferulic and *p*-coumaric, present in the form of esters incorporated into the cell wall, were also identified in radish (*Raphanus sativus* L.) cotyledons and hypocotyls (Chen, Gitz, & McClure, 1998). Carrot cell wall material was found to contain the following acids: 4-hydrobenzoic, vanillic, ferulic, coumaric and diferulic (Beveridge et al., 2000).

3.2. Determination of the total content of phenolic compounds

Kaur and Kapoor (2002) analyzed the total content of phenolic compounds (in catechol equivalents) in 33 of the most commonly eaten vegetables in India. Based on the findings, they divided these vegetables into three groups, i.e. vegetables with a high (>200 mg catechol/100 g), average (100–200 mg catechol/100 g) and low (<100 mg catechol/100 g) concentration of phenolic compounds. According to these authors, broccoli contains 87.5 mg of phenolic compounds in 100 g of fresh mass. In our studies, a similar amount was detected in frozen raw broccoli (Table 1). According to Vinson, Hao, Su, and Zubik (1998), broccoli contains 10.53 mg of phenolic compounds in 1 kg of fresh mass. The content of phenolic compounds in other cruciferous vegetables varies from 62.0 mg/100 g and 68.8 and for mustard and Brussels sprouts, respectively, to 92.5 and 127.0 mg/100 g for white cabbage and turnip, respectively (Kaur & Kapoor, 2002).

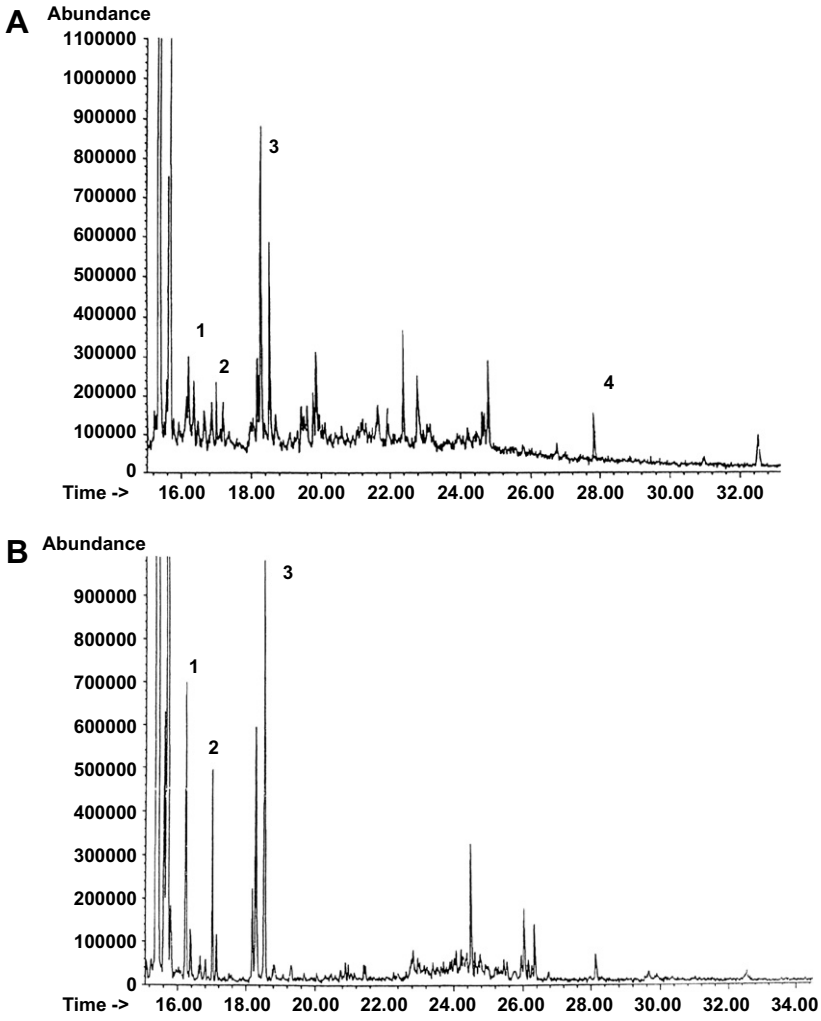

Fig. 2. Chromatograms of the total ionic current of frozen broccoli extracts. (A) before boiling (1 – ferulic acid, 2 – caffeic acid, 3 – synapic acid, 4 – kaempferol) and (B) after boiling (1 – ferulic acid, 2 – caffeic acid, 3 – synapic acid).

Table 1
Total content of phenolic compounds in broccoli extracts

Sample of broccoli	Phenolic compounds content
Raw fresh (mg/g f m)	2.70 ± 0.02
Boiled fresh (mg/g f m)	1.60 ± 0.02
Decoction of fresh broccoli (mg/ml)	0.160 ± 0.001
Raw frozen (mg/g f m)	1.00 ± 0.07
Boiled frozen (mg/g f m)	2.55 ± 0.03
Decoction of frozen broccoli (mg/ml)	0.11 ± 0.01

The results of this study did not find unequivocally that boiling affects on the total content of phenolic compounds in broccoli. This kind of treatment increased the content of phenolic compounds of frozen broccoli. Hydrothermal processing of frozen broccoli increased the amounts of phenolic compounds from 0.964 to 2.497 mg/g of fresh mass (Table 1). Similarly, Villavicencio, Mancini-Filho, Delincée, and Greiner (2000) found that soaked and cooked beans had a higher content of phenolic compounds than uncooked beans. This finding was explained based on the difference in extraction efficiency. High temperature could modify the structure of compounds building the cell walls, or release phenolics from insoluble complexes, therefore making them more accessible for extraction. In the case of fresh broccoli, boiling decreased the amount of phenolic compounds from 2.70 to 1.60 mg/g of fresh mass. Because the structure of fresh broccoli cell walls was not damaged prior to boiling, a smaller amount of phenolic compound was released (see Fig. 3).

3.3. Antioxidant assay

3.3.1. DPPH assay

Studies of the antioxidant activity of different extracts from various parts of broccoli revealed that the methanol extracts of polyphenols had the highest activity (determined against DPPH in comparison to acetone and water extracts (Guo, Lee, Chiang, Lin, & Chang, 2001). These

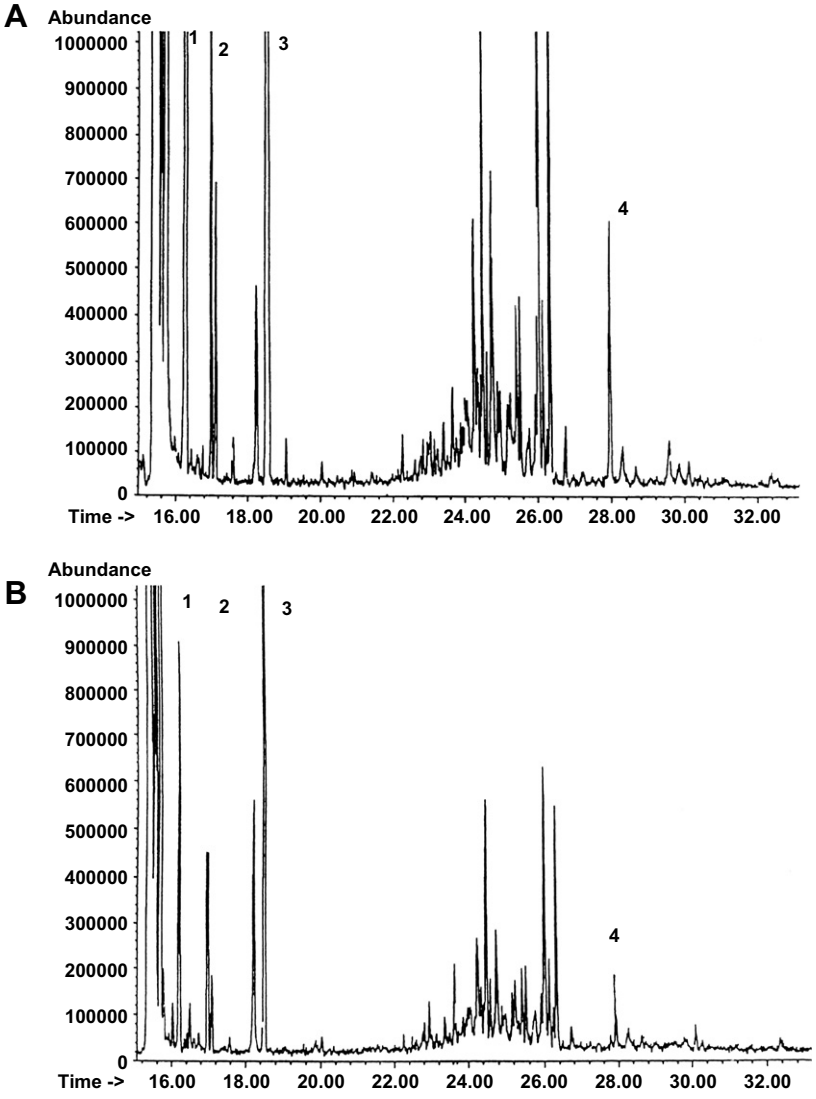

Fig. 3. Chromatograms of the total ionic current of decoction of fresh (A) and frozen (B) broccoli (1 – ferulic acid, 2 – caffeic acid, 3 – synapic acid, 4 – kempferol).

authors also proved that polyphenols from broccoli heads and stems had stronger antioxidant activity than those from broccoli leaves. The methanol extract of broccoli heads and stems (4 mg/ml) had antioxidant activity above 94%, similar to that of tocopherol and BHA (Guo et al., 2001). The ability to neutralize DPPH free radicals by phenolic compounds isolated from fresh broccoli did not exceed 20% and did not depend on incubation time. Boiling decreased the antiradical activity, which did not exceed 15.06%. Also in this case the time of incubation did not have an effect on antiradical activity. Relatively high antiradical capacity was found in the case of fresh broccoli decoction. They are significantly higher than activity of the samples obtained from boiled broccoli. Probably the compounds responsible for antiradical activity passed to decoction during cooking (Fig. 4).

The ability of phenolic compounds extracted from frozen broccoli to neutralize DPPH free radicals (expressed as a percentage of inhibition) did not exceed 30% and was lower than that described by others for freeze–dried broccoli heads stored at 4 °C (Guo et al., 2001). Boiling frozen broccoli did not significantly change their antiradical activity. However, incubation time had a significant effect on their antiradical ability. Activity of frozen broccoli samples decreased after 60 min of incubation. The significant differences were observed in the case of the raw and boiled broccoli samples while the decoction of frozen broccoli showed a very low antiradical activity (Fig. 5).

3.3.2. β-Carotene bleaching assay

The results of antioxidant activity measured against β-carotene revealed that phenolic compounds extracted from fresh raw broccoli had the highest activity, which changed over time. It is interesting that the highest activity measured with this method was found in the case of decoction. This activity was significantly higher than the activity of fresh broccoli samples. Probably the antioxidative com-

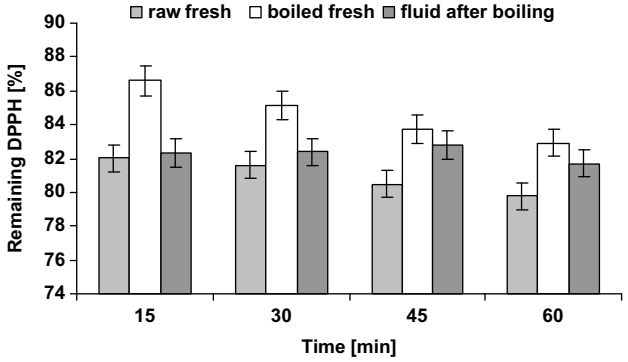

Fig. 4. The ability of extracts from fresh broccoli, before and after boiling, to neutralize DPPH free radicals.

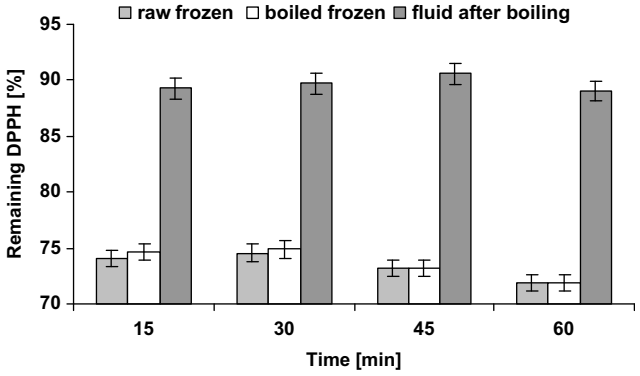

Fig. 5. The ability of extracts from frozen broccoli, before and after boiling, to neutralize DPPH free radicals.

pounds released during cooking nearly totally diffunded to decoction (Fig. 6).

In the case of frozen broccoli the significantly higher activity was found in the samples of cooked vegetable. Also in this case the activity of decoction was initially very high, but the differences were decreasing during incubation (Fig. 7).

The experiment performed by Kähkönen et al. (1999) on extracts from 92 plants (including 22 vegetables) revealed that phenolic compounds found in broccoli had the highest antioxidant effect measured against β-carotene. Kaur and

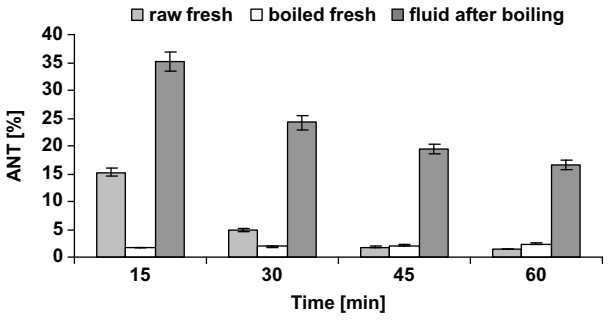

Fig. 6. The ability of extracts from fresh broccoli, before and after boiling, to inhibit the oxidation of β-carotene emulsion.

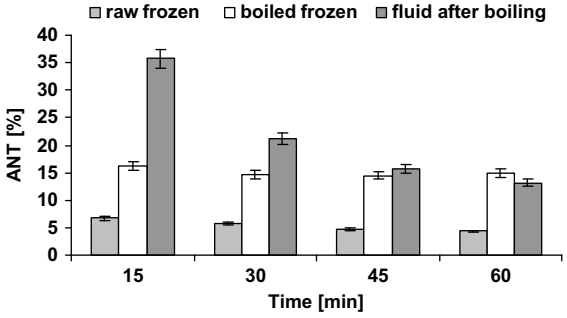

Fig. 7. The ability of extracts from frozen broccoli, before and after boiling, to inhibit the oxidation of β-carotene emulsion.

Kapoor (2002) analyzed the antioxidant activity of extracts from 34 vegetables and also concluded that broccoli belongs to the group of vegetables with a high antioxidant activity (>70%). These authors included Brussels sprouts, tomatoes, curcuma, beets and carrots into the same group. As a result of their study on the antioxidant properties of phenolic compounds, Vinson et al. (1998) concluded that this activity depends more on which vegetable the polyphenols were extracted from rather than on which solvent system was used for extraction. The authors extracted polyphenols at 90 °C from 23 sources of raw materials, using two different solvent systems: 50% methanol or 50% methanol with hydrochloric acid. They did not detect significant differences for broccoli, cauliflower and corn, but for potato, tomato, different cultivars of beans, cucurbit and onion, different values of antioxidant activity were measured (Vinson et al., 1998).

3.3.3. Determination of inhibition of linoleic acid autooxidation

One of the indicators of antioxidant activity is an ability to inhibit self-oxygenation of unsaturated fatty acids. In this study, two methods of antioxidant activity detection were compared – the Lingnert's method and the thiocyanate method. The results were ambiguous and depended upon which method was used. The analysis of fresh broccoli with the thiocyanate method did not reveal any differences, while the results obtained with the Lingnert's method showed that raw broccoli had higher activity than cooked vegetables. Independently on the method used, the lowest activity was found in the case of decoction (Fig. 8). Basing on the results obtained by the Lingnet's method no significant differences between the activities of samples obtained from frozen broccoli were found. The results obtained using thiocyanate method shown that cooked broccoli sample had the lower activity in comparison to raw broccoli and decoction samples. It is noteworthy that these activities were not significantly different (Fig. 9).

An important antioxidant compound present in broccoli is ascorbic acid, however the extraction procedures used in my research (solvent, pH, time) were not optimally for it isolation. Also in papers of Lin and Chang (2005) and

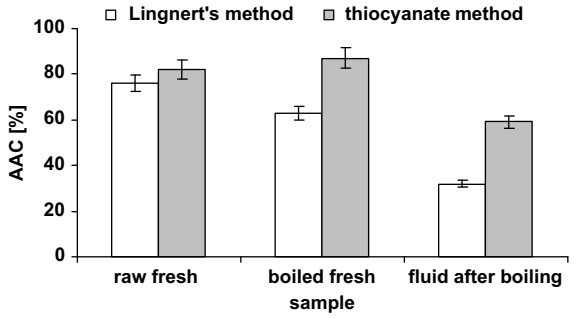

Fig. 8. Antioxidant activity of fresh broccoli extracts, before and after boiling.

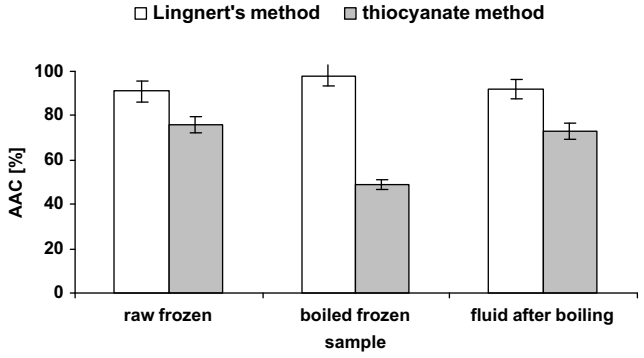

Fig. 9. Antioxidant activity of frozen broccoli extracts, before and after boiling.

Sun, Powers, and Tang (2007) concerning antioxidant properties of broccoli this effect on total antioxidant activity are neglected. Also the hydrothermal treatment caused losses in ascorbic acid content. The results obtained for Zhang and Hamauzu (2004) shows that the content of vitamin C declined dramatically during cooking. Lisiewska and Kmiecik (1996) showed that greatest losses in ascorbic acid content occurred during blanching while Hunter and Fletcher (2002) shows that cooking by boiling of peas caused only a small loss of water and lipid-soluble antioxidant activities. A result obtained in this work shows that ascorbic acid not influence on antioxidant activity of water-methanolic extracts of broccoli.

Numerous studies have been conducted on the relationship between the antioxidant activity and content of phenolic compounds in plant extracts. Very often the results of these studies are contradictive. Some researchers have found a correlation between the concentration of polyphenols and antioxidant activity (Nowak & Gawlik-Dziki, 2007; Velioglu et al.; 1998; Vinson et al., 1998), while others have reported to the contrary. Gazzani, Papetti, Massolini, and Daglia (1998) and Kähkönen et al. (1999) did not find a positive correlation between the content of phenolic compounds and antioxidant activity of plant extracts. According to them, phenolic compounds react differentially with Folin–Ciocalteau reagent therefore the antioxidant activity of the extract should not be determined based on the total content of phenolic compounds. In this study a positive correlation was found only between the content of phenolic compounds and antioxidant activity measured as the ability to inhibition of β-carotene emulsion decolorization, the correlation coefficient $r = 0.81$.

In this study the correlation between different antioxidant activities were found. Strong negative correlation between the DPPH radical scavenging activity and ability to inhibition of β-carotene emulsion decolorization were found in the case of raw fresh and raw frozen broccoli extracts ($r = -0.90$ and -0.99, respectively), however positive correlation in the case of cooked vegetables were found ($r = 0.78$ for fresh and $r = 0.74$ for frozen broccoli). These results indicate the complexity of the changes occurring during hydrothermal treatment. The GC–MS analysis show that extracts of hydrothermal broccoli and the residue after boiling are a wide mixture of phenolic compounds not identified in this study. It is possible that these constituents may interact to produce synergistic or antagonistic antioxidant effects with each other and with other compounds. Very little is known about such effects.

To measure the antioxidant potential of examined raw materials, it is not only necessary to use many different methods to determine antioxidant activity, but also to take into consideration the content and composition of phenolic compounds in the extracts. Many studies have been conducted to find a correlation between the structure of phenolic compounds and their antioxidant activity (Cuvelier, Rihard, & Berset, 1992; Rice-Evans, Miller, & Paganga, 1996). However, due to diverse methods used for the determination of antioxidant activity, a correlation has not been elucidated. It is also necessary to learn more about the bioavailability of phenolic compounds from plant foods, and to determine how technological processing affects the content and structure of these compounds.

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