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Antioxidant Activity and Phenolic Acid Content of Selected Vegetable Broths

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Abstract

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The antioxidant activity and content of phenolic substances in vegetable broths were determined. Green beans, beetroots, courgettes, onions, parsley, carrots, cabbages, celery, broccoli, spinach, cauliflowers, and tomatoes were subjected to boiling. Fresh vegetables and vegetable broths were analysed for ascorbic acid content, total phenolic content, ORAC and TEAC values. Phenolic acids were quantified using HPLC. The ascorbic acid content of vegetables ranged from 5–109 mg/100 ml, while no ascorbic acids could be detected in vegetable broths. Total phenolic content was between 17–1729 mg GAE/l for all samples. ORAC and TEAC values of vegetable broths were between 0–3 μmol TE/ml and 0–2 μmol TE/ml, respectively. Gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic acid were detected in both fresh vegetables and vegetable broths. The highest phenolic acid content was observed in water in which beetroots were boiled. It was found that the vegetable broths of beetroots, celery stalks, cabbages, parsley and broccoli harboured remarkable antioxidant activity.

Keywords: boiling; ORAC; phenolic acids; TEAC; antioxidant capacity; vegetable; cooking

Vegetables are an important part of a healthy diet, and the principal source of natural antioxidants such as vitamin C, α-tocopherol, and phenolic compounds (Ames *et al.* 1993). These antioxidants have been linked to the protection against diseases provided by fruits and vegetables (Wen *et al.* 2010; Harasym & Oledzki 2014).

Boiling is one of the most common methods used to prepare vegetables before consumption. The boiling process can lead to changes in the physical and chemical properties of vegetables; for example, several studies have shown that boiling is able to improve the palatability and bioavailability of naturally occurring nutrients in vegetables (Turkmen *et al.* 2005; Wen *et al.* 2010). When vegetables are subjected to boiling, their phenolic composition and antioxidant activities vary depending on the bioactive structures of the vegetables, the cooking method and length, the bioavailability of phenolics, temperature and resistance of the structure to heat (Jimenez-Monreal *et al.* 2009).

Although the levels of many food antioxidants are significantly diminished by the thermal process, the overall antioxidant properties of foods can be maintained or improved by various applications (Nicoli *et al.* 1997). In fruits and vegetables, phytochemicals with antioxidant properties are bound to the plant cell membranes or exist as free compounds. Antioxidants can be liberated due to the thermal destruction of cell walls and subcellular compartments leading to stronger radical-scavenging activity, which implies higher bio-accessibility. Boiling leads to the lixiviation phenomenon in which nutrients leach out of

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the vegetables into the cooking water (Amin *et al.* 2004; Jimenez-Monreal *et al.* 2009).

Even though a number of studies have been published regarding the effects of cooking on antioxidant and nutritional properties of vegetables (Amin *et al*. 2004; Amin & Lee 2005; Turkmen *et al.* 2005; Miglio *et al.* 2008; Xu & Chang 2008; Xu & Chang 2009; Jimenez-Monreal *et al.* 2009; Wen *et al*. 2010; Patras *et al.* 2011; Leong & Oey 2012), there is very little known about the retention of nutrients in water after the boiling of vegetables. The purpose of this study, therefore, was to determine the total antioxidant activity and phenolic acid content of selected vegetable broths.

MATERIAL AND METHODS

Sample preparation and experimental design. Green beans (*Phaseolus vulgaris*), courgettes (*Cucurbita pepo* L.), parsley (*Petroselinum crispum*), carrots (*Daucus carota* L.), tomatoes (*Lycopersicon esculentum* Mill. L.), celery (*Apium graveolens* L), broccoli (*Brassica oleracea* var. *italica*), onions (*Allium cepa* L.), beetroots (*Beta vulgaris* L. subsp. *conditiva*), spinach (*Spinacia oleracea*), white cabbages (*Brassica oleracea* L. var. *capitata* f. *alba*), and cauliflowers (*Brassica oleracea* L. var. *botrytis*) were purchased from a local fresh market at their mature stage. After washing with tap water, inedible parts were removed. Fresh vegetable samples were obtained using a juicer (HR1821/10; Philips, The Netherlands). For vegetable broth (VB) extraction, beetroots, courgettes, onions, carrots, cabbages, root celery, and tomatoes were sliced into 5-mm thick slices. Parsley, spinach, stalk celery, cabbages, broccoli, and cauliflowers were broken into pieces. Pre-treated vegetables were boiled in 10× volumes of water (w/v) until they become soft enough to easily poke with a fork. Each boiling experiment was carried out in duplicate on three separate occasions. Fifty ml of fresh vegetable and VB samples were transferred to Falcon® test tubes in an ice bath. Samples were then stored in a deep freezer (–40°C). The data are expressed as mean ± standard error. The percentage of leaching into VB was assessed in relation to fresh vegetables.

Ascorbic acid content. Ascorbic acid content was determined using a 2,6-dichlorophenol (DIP) titrimetric method (AOAC 1990). Five ml of homogenised sample was diluted with 25 ml of 6% oxalic acid solution and titrated with the 2,6-dichlorophenolindophenol dye until the appearance and persistence of pink colour.

Total phenolic content. Total phenolic content (TPC) of samples was determined according to the Folin-Ciocalteu method (Singleton & Rossi 1965; Singleton *et al.* 1999). Absorbance was measured at 760 nm. The concentration of total phenolic compounds in samples was determined as gallic acid equivalents (GAE) using an equation obtained from a gallic acid (Acros, USA) standard curve. The results are expressed as milligrams of gallic acid equivalents (GAE) per litre.

Oxygen radical absorbance capacity (ORAC) assay. Oxygen radical absorbance capacity (ORAC) was kinetically measured using a Biotek Synergy™ HT Multi-Detection Microplate Reader (Winooski, USA). Aliquot (25 µl) of the diluted samples and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Acros, USA) calibration solutions were added to wells of a 96-well bottom reading microplate (BD Falcon, USA). After the addition of 150 µM fluorescein (Acros, USA) stock solution to each well the microplate was incubated at 37°C for 30 minutes. Then, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH; Acros, USA) solution was added to start the reaction. The microplate reader was programmed to record the fluorescence reading with an excitation-emission wavelength of 485–520 nm using Gen 5™ software (Budak & Guzel-Seydim 2010). Results were expressed as µmol TE (Trolox Equivalents) per ml.

Trolox equivalent antioxidant capacity (TEAC) assay. 2,2'-Azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS+; Acros, USA) radical cation inhibition against Trolox was spectrophotometrically measured at 734 nm (Shimadzu Scientific Instruments Inc., Japan) (Re *et al.* 1999). TEAC values of samples was calculated from the Trolox standard curve and expressed as Trolox equivalents per millilitre of sample.

HPLC determination of phenolic acids. Phenolic acids were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu SCL-10A; Scientific Instruments Inc., Japan). Detection and quantification were carried out with a diode array detector at a wavelength of 198 nm. Separation of gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic acid (Sigma Chemical Co., Belgium) was conducted at 30°C on a C18 column (Gemini C18, 150 × 3 mm, 5 µm, 110A; Phenomenex, USA). Elution was performed at a flow rate

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Table 1. Correlation matrix of antioxidant capacity values

		ORAC	TEAC	TPC
FV	ORAC	1	0.280	0.297
	TEAC	0.280	1	0.820**
	TPC	0.297	0.820**	1
VB	ORAC	1	0.646*	0.399
	TEAC	0.646*	1	0.565*
	TPC	0.399	0.565*	1

*P = 0.005; ** P = 0.001

of 0.8 ml/min, using as mobile phase a mixture of 15% acetonitrile in water (for the first 3 min) and 18% acetonitrile in water (for next 3 min). pH values of mobile phases containing 50 mM *o*-phosphoric acid were adjusted to 4.5 with 1 N NaOH. Identification of phenolic acids was based on a comparison of retention times with standards. Quantification was carried out by comparison against an external standard method.

Data analysis. Results were analysed using SPSS (version 16). The data were expressed as mean ± standard error. The independent *t*-test was used to assess differences between results for fresh vegetables and VB. P values of < 0.05 were considered to be statistically significant. The relationship between TPC, ORAC and TEAC results was evaluated by calculating the Pearson correlation coefficient.

RESULTS AND DISCUSSION

The ascorbic acid content of vegetable samples ranged from 5 ± 0.3 to 109 ± 5 mg/100 ml (Figure 1). Parsley had the highest ascorbic acid content while beetroot had the lowest. Ascorbic acid was not detected

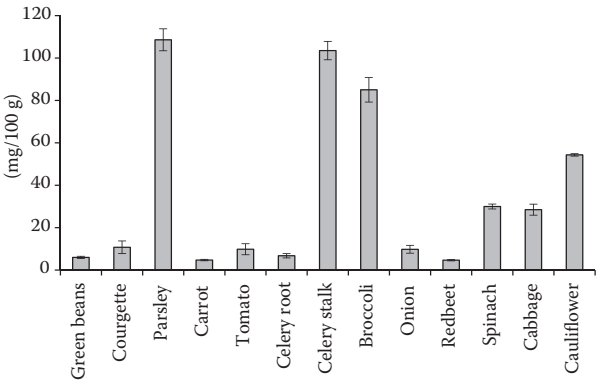

Figure 1. Ascorbic acid content in fresh vegetables (mg/100 ml)

in VBs. Ascorbic acid is a water-soluble and thermally labile vitamin, heat treatment has been shown to result in ascorbic acid loss in vegetables in various studies (Dewanto *et al.* 2002; Volden *et al.* 2009).

Total phenolic content (TPC) of vegetable juices and vegetable broths are presented in Figure 2. TPC values ranged between 306 ± 58 and 1728 ± 60 mg GAE/l and 17 ± 7 and 269 ± 16 mg GAE/l for vegetables and for vegetable broths, respectively. There were significant differences between the TPC values of fresh and VB samples (P < 0.05). A significant correlation was determined between the TPC and TEAC values of vegetables (P < 0.01; Table 1).

Broccoli, beetroots and celery stalks contained the highest levels of total phenolics among the studied vegetables. Song *et al.* (2010) reported that beetroots, broccoli and red peppers had the highest TPC values and the highest cellular antioxidant activities. Broccoli is a source of flavonols and hydroxyl cinnamoyl derivatives (Podsedek 2007). Mrkic *et al*. (2006) reported that the total polyphenol and free polyphenol (mostly corresponding to quercetin, kaempferol, and

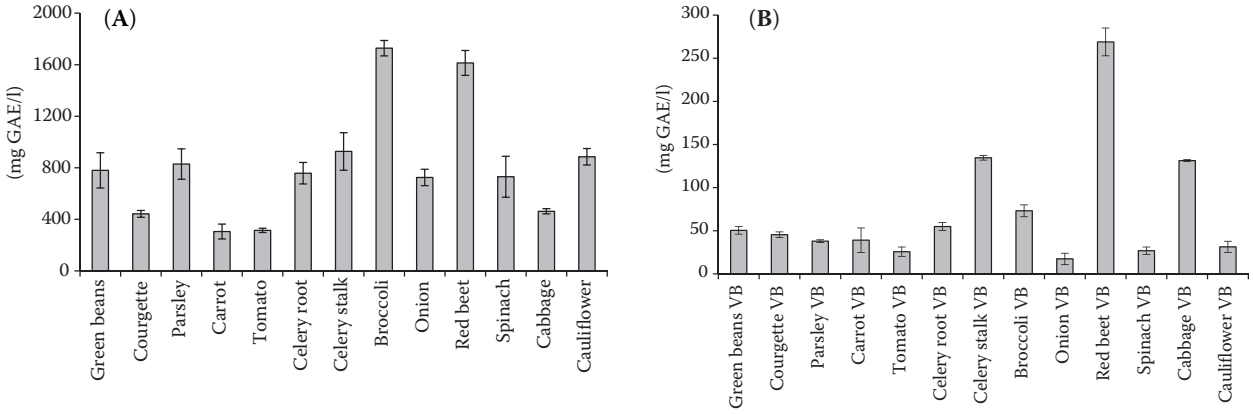

Figure 2. Total phenolic content (mg GAE/l) in (**A**) fresh vegetables and (**B**) vegetable broths

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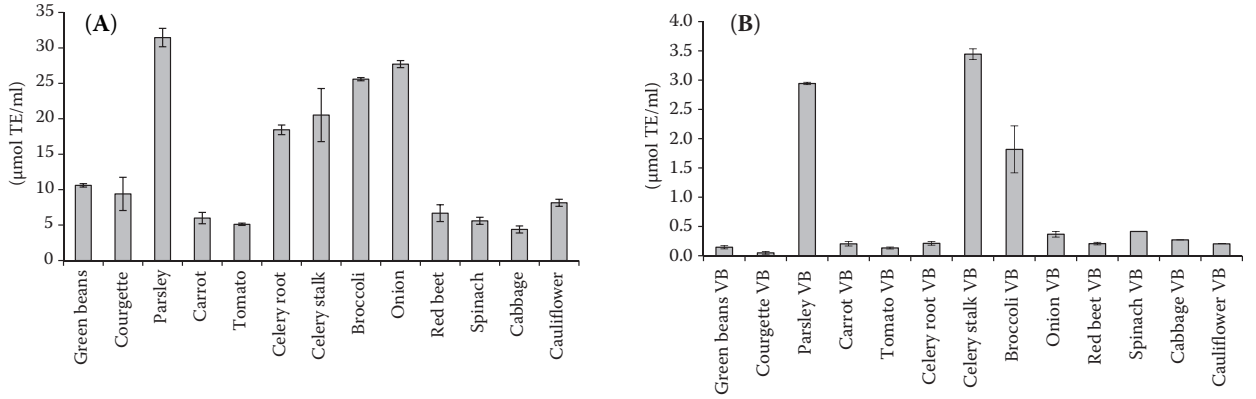

Figure 3. ORAC values (µmol Trolox equivalents-TE/ml) of (**A**) fresh vegetables and (**B**) vegetable broth

myricetin) contents of fresh broccoli were around 10.27 and 4.16 mg GAE/g dry weight, respectively.

TPC values were highest in beetroot VB with 17% of leaching. In agreement with this observation, Vinson *et al*. (1998) found that beetroots had the highest dry weight concentration of total phenols followed by red onions, broccoli, and kidney beans. It has been reported that beetroot extracts have relatively strong antioxidant activity in comparison to those of other vegetables, due to high concentrations of betalains, water-soluble nitrogen-containing pigments with antioxidant activity (Kujala *et al.* 2002). Betalains are more resistant to temperature changes than other natural pigments (Ravichandran *et al.* 2013). Previous studies showed that betalains considerably contributed to TPC (Kujala *et al.* 2002; Esquivel *et al.* 2007).

ORAC results are given in Figure 3. ORAC ranged between 4 ± 1 and 32 ± 1 µmol/ml, 0.1 ± 0.01 and 3.44 ± 0.09 µmol TE/ml for FV and VB samples, respectively. There were significant differences between the ORAC of fresh and VB samples ($P < 0.05$). The highest ORAC was detected in parsley (32 ± 1 µmol TE/ml), followed by onions, broccoli, celery stalks and beetroots. Celery stalk VB had the highest ORAC (3.44 ± 0.09 µmol TE/ml) (16.78% relative to fresh samples) followed by parsley and broccoli BW samples.

TEAC values were between 2.62 ± 1 and 16.69 ± 1 µmol TE/ml, 0.05 ± 0.01 and 1.98 ± 0.22 µmol TE/ml for FV and VB samples, respectively (Figure 4). Fresh vegetables exhibited significantly higher TEAC values than vegetable broths ($P < 0.05$). Broccoli had the highest TEAC values (16.69 µmol TE/ml), similar to the TPC results. Fresh vegetables exhibited higher TEAC values than vegetable broths. Celery stalk vegetable broths had the highest TEAC values (17.52%), similar to ORAC. A significant correlation was observed between TEAC and ORAC values for VBs ($P < 0.05$; Table 1).

The antioxidant properties of VB samples were found to be similar to those fresh vegetables with the highest antioxidant properties. Ciz *et al*. (2010) reported ORAC and total polyphenol that values to be higher in extracts of celery and parsley leaves than in any other vegetable. ORAC values were the highest in VBs of celery stalk and parsley.

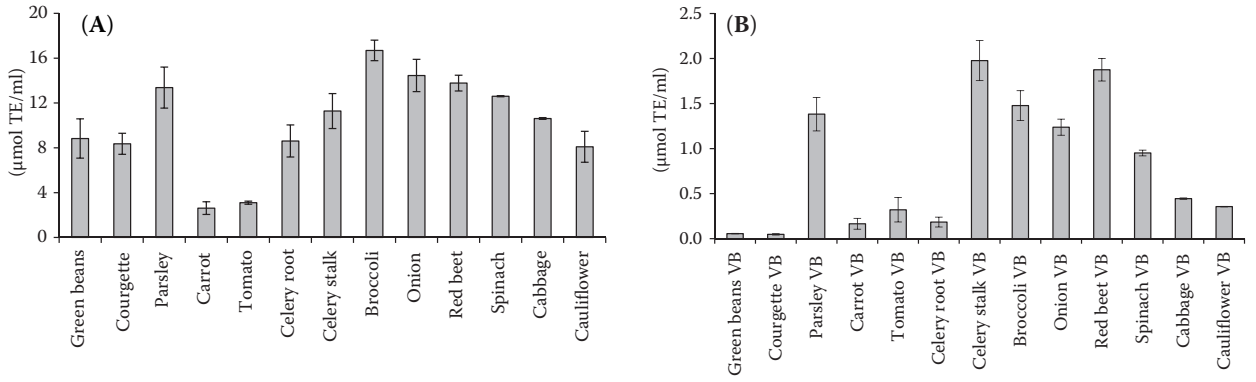

Figure 4. TEAC values (µmol Trolox equivalents-TE/ml) of (**A**) fresh vegetables and (**B**) vegetable broths

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Table 2. Phenolic acid contents of fresh vegetables (FV) and vegetable broths (VB) (mg/l)

	Gallic acid		Chlorogenic acid		Caffeic acid		*p*-Coumaric acid		Ferulic acid	
	FV	VB	FV	VB	FV	VB	FV	VB	FV	VB
Green beans	33 ± 5	–	–	–	–	–	24 ± 3	–	–	–
Courgette	–	–	–	–	30 ± 2	–	–	–	13 ± 1	–
Parsley	57 ± 7	1 ± 0.02	–	–	–	–	–	–	34 ± 5	2 ± 0.1
Carrot	–	–	64 ± 4	–	43 ± 3	2 ± 0.2	–	–	–	–
Tomato	–	–	–	–	10 ± 2	0.5 ± 0.02	–	–	–	–
Celery root	–	–	–	–	39 ± 3	1 ± 0.03	15 ± 1	–	–	–
Celery stalk	–	–	–	–	86 ± 4	3 ± 0.3	82 ± 7	2 ± 0.07	–	–
Broccoli	–	–	63 ± 5	–	–	–	24 ± 1	1 ± 0.01	82 ± 6	2 ± 1
Onion	11 ± 1	–	–	–	–	–	–	–	59 ± 3	–
Redbeet	–	–	25 ± 3	–	–	–	–	–	57 ± 4	5 ± 1
Spinach	–	–	–	–	–	–	70 ± 4	–	–	–
Cabbage	–	–	–	–	35 ± 2	2 ± 0.2	39 ± 2	–	27 ± 3	1 ± 0.01
Cauliflower	–	–	–	–	–	–	10 ± 2	–	72 ± 3	–

Cho *et al*. (2007) reported that green leafy vegetables including celery and parsley showed significantly higher ORAC values than fruits, light-coloured vegetables and red-orange coloured vegetables. Murcia *et al.* (2009) reported that the TEAC of broccoli was 11.5 μmol TE/ml. Amin *et al*. (2006) showed that boiling may result in a significant loss of the antioxidant components of leafy vegetables, which may be due to a leaching of water-soluble phenolic compounds into vegetable broth.

Not all phenolic acids identified in fresh vegetables were detected in the corresponding VBs (Table 2). The highest content of total phenolic acids was found in beetroot VB (5.67%). Gallic acid was identified in green beans, parsley and onions, and it leached into parsley VB at a rate of only 1%. Fresh broccoli, beetroots and carrots were found to contain chlorogenic acid; however, this compound was not detected in any of the VB samples. Caffeic acid was not detected in courgette VB, although it was detected in fresh courgettes. *p*-coumaric acid leached into celery stalks (2.31%) and broccoli (4.85%) VBs. Ferulic acid was the major phenolic acid in VB samples and it was found in parsley (5.75%), broccoli (2.76%), beetroot (8.20%), and cabbage (3.02%) VBs.

CONCLUSIONS

Our results show that fresh broccoli, onions, celery stalks, parsley and beetroots generally exhibited higher TPC, ORAC, and TEAC values than other examined vegetables. Similarly, the VBs of these vegetables were characterised by higher antioxidant activities. Parsley broth and celery stalk broth had the highest ORAC and TEAC values because of the leaching of antioxidative compounds into the water used for boiling. A few of the vegetable broths were found to contain either higher or moderate amount of phenolic acids. Ferulic acid was the major phenolic acid in fresh and VB samples. The quantity of antioxidant compounds leached during boiling varies depending on the characteristics of the food. High TPC values and antioxidant activities were observed not only in some vegetables, but also in some of the broths tested. Thus, according to our results, the wastewater generated during vegetable handling operations should be evaluated as a potential source of phytochemicals. Further, it may be recommended to use retained broth that contains important phytochemicals in alternative ways for nutraceutical production/food consumption.

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