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The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables

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Abstract

Effects of microwave and conventional cooking methods were studied on total phenolics and antioxidant activity of pepper, squash, green beans, peas, leek, broccoli and spinach. Total phenolics content of fresh vegetables ranged from 183.2 to 1344.7 mg/100 g (as gallic acid equivalent) on dry weight basis. Total antioxidant activity ranged from 12.2% to 78.2%. With the exception of spinach, cooking affected total phenolics content significantly ($p < 0.05$). The effect of various cooking methods on total phenolics was significant ($p < 0.05$) only for pepper, peas and broccoli. After cooking, total antioxidant activity increased or remained unchanged depending on the type of vegetable but not type of cooking.

Keywords: Green vegetables; Phenolics; Antioxidant activity; DPPH

1. Introduction

Dietary antioxidants protect against free radicals such as reactive oxygen species in the human body (Nilsson, Stegmark, & Akesson, 2004). Free radicals are known to be a major contributor to degenerative diseases of aging (Atoui, Mansouri, Boskou, & Kefalas, 2005). Fruit and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other phenolic compounds. (Minussi et al., 2003; Zhang & Hamauzu, 2004).

Due to the detection of many bioactive compounds in food with possible antioxidant activity, there has been increased interest in the relationship between antioxidant and disease risks (Nilsson et al., 2004). Epidemiological studies have shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases such as cancer, cardiovascular disease, cataract and macular degeneration (Cheung, Cheung, & Ooi, 2003; Heim, Tagliaferro, & Bobilya, 2002; Hunter & Fletcher, 2002; Lopaczynski & Zeisel, 2001; Zhang & Hamauzu, 2004).

Most of the vegetables are cooked by boiling in water or microwaving before consumed. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of vegetables (Rehman, Islam, & Shah, 2003; Zhang & Hamauzu, 2004). Sahlin, Savage, and Lister (2004) showed that boiling and baking had a small effect on the ascorbic acid, total phenolic, lycopene and antioxidant activity of the tomatoes while frying significantly reduced the ascorbic, total phenolic and lycopene contents of tomatoes. Zhang and Hamauzu (2004) pointed out that cooking affected the antioxidant components and antioxidant activity of broccoli. Ismail, Marjan, and Foong (2004) found that thermal treatment decreased the total phenolic content in all vegetables such

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as kale, spinach, cabbage, swamp cabbage and shallots and antioxidant activity in some of them.

Green beans, peas, pepper, squash, broccoli, leek and spinach are common vegetables consumed as cooked. However, very little information is available in the literature regarding the antioxidant activity and total phenolics of these vegetables. Therefore, the present study was undertaken to investigate the effects of different cooking methods on antioxidant activity and total phenolics of the vegetables.

2. Materials and methods

2.1. Plant materials

Fresh broccoli, spinach, squash, leek, pepper, peas, green beans were purchased from several local markets in Ankara – Turkey and used as research material. The vegetables (2.5 kg each) were randomly sampled from the shelf.

2.2. Preparation of vegetable samples

Vegetables were washed with tap water after removing manually inedible parts with a sharp knife. Vegetables dried on paper towel and were cut into almost equal small pieces or slices, mixed well and 1200 g was taken and divided into four portions (300 g for each application). One portion was retained raw, others were cooked in three different methods in triplicate, as given below. Cooking conditions were determined, with a preliminary experiment for each vegetable.

2.2.1. Boiling

Vegetable (100 g) was added to 150 ml of water that had just reached the boil in a stainless steel pan and cooked for 5 min. The samples were drained off and cooled rapidly on plenty of ice.

2.2.2. Microwave cooking

Vegetable (100 g) was placed in a glass dish and 6 ml (12 ml for green beans) of distilled water was added. Dish were covered with cooking bag, having several holes, and cooked in a commercial – 1000 W microwave oven. Cooking took 1 min for squash, spinach, peas and 1.5 min for leek, broccoli, pepper and green beans. Samples were drained off and cooled rapidly on ice.

2.2.3. Steaming

Vegetable was placed on tray in a steam cooker (Tefal, Clipso-clipsoval, Model 4101, Groupe SEB-France) covered with lid and steamed over boiling water for 7.5 min under atmospheric pressure. The samples rapidly cooled on ice.

2.3. Analytical methods

Raw and processed vegetables were homogenised in a blender (Moulinex – France) for 2 min. Homogenised samples were kept at −20 °C until analyses.

2.3.1. Dry matter determination

Due to various water content of vegetables, all calculations were made according to dry matter basis.

For determination of the dry matter content, 3–4 g of raw or cooked homogenised sample (as triplicate) was dried in a convection oven at 70 °C for at least 2 days until reaching constant weight.

2.3.2. Determination of total phenolic content

The amount of total phenolic was determined using Folin–Ciocalteu reagent, as described by Singleton and Rossi (1965). About 1g raw and cooked homogenised samples were extracted with 80% aqueous methanol (4.5 ml) on a mechanical shaker for 2 h. The mixture was centrifuged at 10,000 rpm for 15 min and the supernatant decanted into polypropylene tubes. The pellets were extracted under identical conditions. Supernatants were combined and filtered through Whatman No.1 filter paper. The clear extracts were analysed both for determination of phenolic content and antioxidant activity. Results were expressed as milligram gallic acid equivalents (GAE)/100 g dry weight.

2.3.3. Determination of total antioxidant activity

Antioxidant activity was determined by the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method of Zhang and Hamauzu (2004) with some modifications. Vegetable content of the methanol extracts of fresh or cooked vegetables were adjusted to 6 mg/ml (on dry basis), which was chosen as an appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. An aliquot of 1.5 ml of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 ml of vegetable extract, at 6 mg/ml. Instead of methanolic extract of vegetables, pure methanol was used as control. The reaction mixture was vortex-mixed and let to stand at room temperature in the dark for 60 min before the decrease in absorbance at 517 nm was measured. Pure methanol was used to calibrate the spectrophotometer. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$AA(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100.$$

2.3.4. Statistical analysis

All data were recorded as means ± SE and analysed by SPSS for Windows (ver. 10.1.). One-way analysis of variance (ANOVA) and Duncan comparisons were carried out to test any significant differences between raw and cooked vegetables.

2.3.5. Chemicals

DPPH and Folin–Ciacalteu reagents were from Sigma (MO, USA) and Merck (Darmstadt, Germany), respectively. Other chemicals used were all analytical grade and from Merck.

3. Results and discussion

3.1. The effect of cooking methods on total phenolic content of vegetables

The total phenolic content of vegetables is shown in Table 1. The fresh vegetables contained 183.28–1344.77 mg GAE/100 g dm total phenolics and the rankings were pepper > spinach > broccoli > squash > green beans > leek > peas. After cooking procedures, the total phenolics content of squash, peas and leek was significantly ($p < 0.05$) reduced and reductions were the same in all cooking methods. Conversely, of pepper, broccoli and green beans total phenolic content was significantly ($p < 0.05$) increased to various extents, depending on the type of cooking method. Although, a little increase in total phenolics of spinach was also observed in cooking by all methods, this was not significant ($p < 0.05$).

Data on total phenolics in cooked green vegetables are very limited. Zhang and Hamauzu (2004) reported that raw broccoli floret contained 34.5 mg/100 g FW of total phenolics and the florets cooked for 5 min by boiling or microwave cooking retained 28.1% and 28.4% of total phenolics, respectively. However, in the present study it was found that raw broccoli contained 1204.29 mg/100 g dm of total phenolics content and cooking by both microwave and boiling did not cause any deleterious effect. The difference may have been due to the differences in the extraction and cooking methods. In a study carried out by Ismail et al. (2004) spinach was found to have the highest phenolic content, followed by swamp cabbage, kale, shallots and cabbage. Blanching for 1 min in boiling water reduced (12–26%) total phenolics in these vegetables. Sahlin et al. (2004) found that cooking by boiling, baking and frying resulted in a significant reduction ($p < 0.01$) in the total phenolic, ascorbic acid and lycopene contents in tomatoes. This study indicated that cooking caused loss of phenolics in squash, peas and leek, which is consistent with the previous findings (Fig. 1). This could be due to phenolics breakdown during cooking (Crozier, Lean, McDonald, & Black, 1997). On the other hand, cooking was found to give rise to an increase in phenolics in green beans, pepper and broccoli (Fig. 1). It was reported that heat treatment increased the level of free flavonols (Stewart et al., 2000).

3.2. The effect of cooking methods on total antioxidant activity of vegetables

Antioxidant activity of fresh vegetables as determined by the DPPH radical scavenging method decreased in the order: broccoli > peppe > spinach > green beans > peas > squash > leek (Table 2).

Among all these test vegetables broccoli showed highest scavenging activity with a inhibition of 78.17% whereas leek had lowest activity with 12.20%. Total antioxidant activity of pepper, green beans, broccoli and spinach significantly ($p < 0.05$) increased during cooking procedures compared to the values for the fresh ones.The increase was the same during cooking by all boiling, steaming and microwaving. However, antioxidant activity of squash, peas and leek remained the same as for fresh ones in each type of cooking.

The earlier study by Zhang and Hamauzu (2004) showed that there was no significant differences in the contents of antioxidant components and antioxidant activity between conventional and microwave cooking. Chu, Chang, and Hsu (2000) reported that scavenging

Table 1
Effect of different cooking methods on total phenolic content and retention factors of vegetables

Vegetables	Total phenolics (mg GAE/100 g DM)[a]						
	Fresh	Boiling		Steaming		Microwaving	
		Amount	%[b]	Amount	%	Amount	%
Pepper	1344.8 ± 26.25 a	1538.4 ± 78.92 bc	114	1371.0 ± 34.76 ab	102	1696.1 ± 53.91 c	126
Squash	833.0 ± 37.79 b	497.3 ± 9.72 a	60	581.7 ± 27.02 a	70	555.6 ± 26.75 a	67
Green Beans	355.3 ± 16.59 a	405.2 ± 14.95 b	114	463.3 ± 4.53 c	130	457.4 ± 9.30 c	129
Peas	183.3 ± 10.23 b	139.8 ± 2.06 a	76	160.6 ± 3.1 a	88	151.9 ± 7.10 a	83
Leek	300.8 ± 4.20 c	193.9 ± 10.87 a	64	254.4 ± 10.7 b	85	245.5 ± 6.41 b	82
Broccoli	1204.3 ± 12.6 a	1129.2 ± 20.30 a	94	1415.5 ± 52.6 b	118	1510.4 ± 17.65 b	125
Spinach	1274.8 ± 94.09 a	1291.8 ± 89.27 a	101	1315.3 ± 14.4 a	103	1390.7 ± 41.40 a	109

[a] Data are expressed as means ± SE of triplicate experiments (on dry basis) mean values in a row with different letters are significantly different at $p < 0.05$.
[b] Fresh = 100.

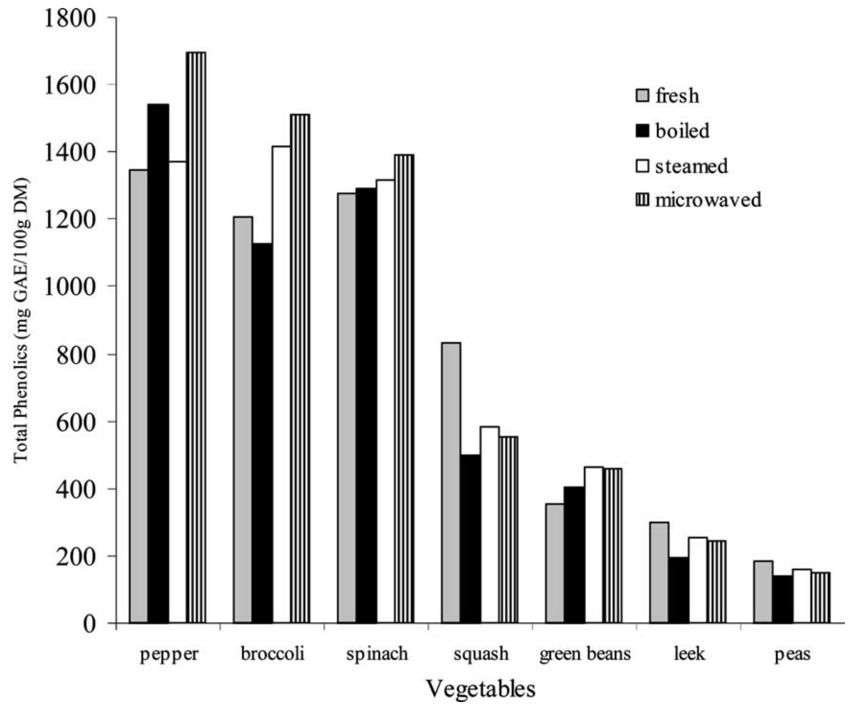

Fig. 1. The effects of cooking methods on phenolic content.

Table 2
Effect of different cooking methods on antioxidant activity[a] and retention factors of vegetables

Vegetables	Fresh[b]	Boiling		Steaming		Microwaving	
	Inhibition	Inhibition	%[c]	Inhibition	%	Inhibition	%
Pepper	68.5 ± 2.97 a	94.4 ± 0.11 b	138	94.5 ± 0.11 b	138	94.3 ± 0.28 b	138
Squash	15.8 ± 1.68 a	19.7 ± 4.25 a	125	26.0 ± 3.61 a	164	16.8 ± 1.03 a	106
Green beans	43.8 ± 2.66 a	70.8 ± 2.41 b	162	81.0 ± 3.53 c	185	82.2 ± 2.41 c	188
Peas	21.3 ± 0.57 a	17.9 ± 0.54 a	84	20.3 ± 2.12 a	95	19.1 ± 1.37 a	90
Leek	12.2 ± 1.39 ab	9.8 ± 0.70 a	80	14.8 ± 1.98 b	121	14.0 ± 0.43 ab	115
Broccoli	78.2 ± 2.53 a	90.6 ± 0.54 b	116	91.3 ± 0.18 b	117	91.2 ± 0.32 b	117
Spinach	67.4 ± 7.82 a	87.1 ± 0.40 b	129	85.5 ± 0.17 b	127	85.8 ± 0.22 b	127

[a] The extracts for antioxidant activity were tested at concentration of 6 mg/ml on dry basis.
[b] Data are expressed as means ± SE of triplicate experiments. Mean values in a row with different letters are significantly different at $p < 0.05$.
[c] Fresh = 100.

activities towards DPPH of green leaves of potatoes blanched for 2 min at 100 °C remained the same as for fresh ones. According to Hunter and Fletcher (2002) cooking by boiling and microwaving caused no significant losses of water and lipid soluble antioxidant activities evaluated by FRAP in frozen peas while an increase in frozen spinach. Peas had a lower total antioxidant activity than spinach (Ismail et al., 2004), which is in agreement with the results shown in the present report. Similarly, frozen peas was found to have a low total antioxidant activity (Nilsson et al., 2004). Zhang and Hamauzu (2004), reported that raw broccoli florets had total antioxidant activity measured by DPPH with 60.5% but after cooking for 5 min by boiling and microwaving the florets retained 35% and 34.7% of total antioxidant activity, respectively. However, our results showed that total antioxidant activity of raw broccoli florets was 78.17% more than reported by Zhang and Hamauzu (2004). After boiling for 5 min and microwaving for 1.5 min antioxidant activity increased by 15.90% and 16.68%, respectively. It was reported that the antioxidant activity of the vegetables as increased by boiling. This suggests that the pro-oxidant activity was due to peroxidases which were inactivated at high temperatures (Gazzani, Papetti, Massolini, & Daglia, 1998). Another research indicated that processing caused no change to antioxidant potential of fruit and vegetables or enhanced it due to improvement of antioxidant properties of naturally occurring compounds or formation of novel compounds such as Maillard reaction products having antioxidant activity (Manzocco, Calligaris, Masrrocola, Nicoli, & Lerici, 2001; Nicoli,

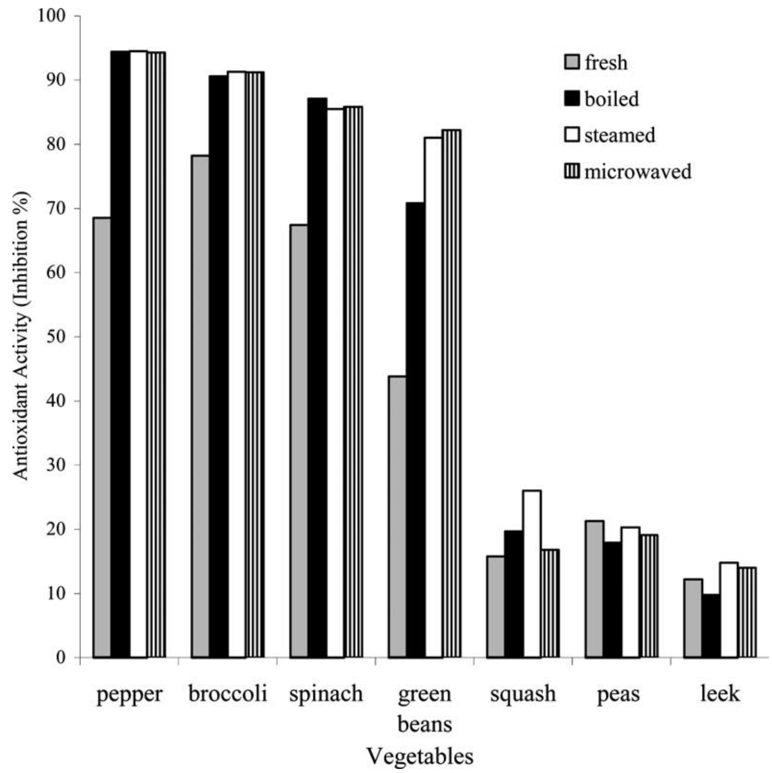

Fig. 2. The effects of cooking methods on antioxidant activity.

Anese, & Parpinel, 1999). In a study carried out by Manzocco, Anese, and Nicoli (1998), pasteurisation of tea extracts was found to cause an increase in antioxidant activity of teas, which was attributed to the formation of compounds having antioxidant activity during heat treatment. Ismail et al. (2004) reported that antioxidant activities of the 1 min boiled vegetables were similar to the fresh ones. Giovanelli, Zanoni, Lavelli, and Nani (2002) found that antioxidant activity of tomato pulp and halves was not significantly different from that of fresh samples. This study showed that cooking by different methods enhanced antioxidant activity in broccoli, spinach, green beans and pepper and caused no change to antioxidant activity in squash, peas and leek (Fig. 2).

4. Conclusions

Among green vegetables tested, pepper had the highest amount of phenolics and broccoli had the strongest antioxidant activity. This study indicated that vegetables except squash are rich in total phenolics showed strong antioxidant activity at the same time. Cooking had no deleterious effect on total antioxidant activity and total phenolics content of vegetables with the exception of some losses of phenolics in only squash, peas and leek. Moreover, moderate heat treatment might have been considered a useful tool in improving health properties of some vegetables.

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