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Effect of cooking on the antioxidant properties of coloured peppers

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ABSTRACT

Pepper (*Capsicum annum* L.) has long been recognized as an excellent source of antioxidants, being rich in ascorbic acid and other phytochemicals. This study was conducted to investigate the effect of different cooking methods on the antioxidant properties of coloured peppers. Six varieties of peppers were subjected to different cooking methods, such as microwave heating, stir-frying and boiling in water, for 5 min individually. The cooked and raw peppers were analyzed for radical-scavenging activity (RSA) and total polyphenol content (TP) using 1,1-diphenyl-2-picrylhydrazyl–high-pressure liquid chromatography (DPPH)–HPLC and Folin-Ciocalteu methods, respectively. The samples were also evaluated for ascorbic acid content (AsA) by HPLC. Total carotenoid content was determined spectrophotometrically. Results suggest that there is no significant ($P > 0.05$) difference in RSA, TP, AsA and total carotenoid contents between the cooked and raw peppers when processed for 5 min. However, the cooked peppers show marked differences ($P < 0.05$) in the RSA, TP and AsA when cooked for 5 min in boiling water with further reduction observed after boiling for 30 min. This may be due to the leaching of antioxidant compounds from the pepper into the cooking water during the prolonged exposure to water and heat. Therefore, it is vital to use less water and cooking time and also to consume the water used for boiling so as to obtain the optimum benefits of bioactive compounds present in peppers. It is concluded that microwave heating and stir-frying without using water are more suitable cooking methods for pepper, to ensure the maximum retention of antioxidant molecules.

1. Introduction

Pepper (*Capsicum annum* L.), is one of those vegetables which contain high levels of antioxidants. They are fast gaining popularity, not only for their attractive colours, characteristic taste and aroma, but also for their health-promoting properties. Peppers exhibit great genetic diversity in terms of colour, size, shape and chemical composition and therefore vary greatly in their antioxidant properties, vitamins and other phytochemicals. Fresh peppers have long been recognized as an excellent source of vitamin C. In addition, peppers are rich in polyphenols, particularly the flavonoids, quercetin and luteolin (Lee, Howard, & Villalon, 1995). Carotenoids are a class of natural pigments responsible for the diverse colours in fruits and vegetables and can be found in abundance in peppers. Carotenoids present in peppers are predominantly provitamin A (α- and β-carotene and β-cryptoxanthin) and xanthophylls, the oxygenated carotenoids (Howard, 2001). These fat-soluble compounds show potential action against certain cancers, prevent gastric ulcer, stimulate the immune system, prevent cardiovascular diseases and protect against age-related macular degeneration and cataracts (Krinsky & Johnson, 2005).

Peppers are commonly consumed raw in salads or blended into juice with other fruits and vegetables. In Asian cuisine, peppers are sometimes stir-fried or boiled with other vegetables. However, vegetables have always been considered to have lower nutritional value due to the loss of vitamin C content after cooking (Fennema, 1997). Reports on the effects of cooking on the antioxidant compounds in vegetables have been inconclusive. There are reports demonstrating an enhancement or no change in antioxidant activity of vegetables (Gahler, Otto, & Bohm, 2003; Turkmen, Sari, & Velioglu, 2005) while others have indicated a deterioration of activity after thermal treatment (Ismail, Marjan, & Foong, 2004; Zhang & Hamauzu, 2004).

There have been many studies conducted on fresh peppers, mainly to quantify the antioxidant activity of the various cultivars of peppers and the influence of maturity on their bioactive compounds (Howard, Talcott, Brenes, & Villalon, 2000; Lee et al., 1995; Marin, Ferreres, Tomas-Barberan, & Gil, 2004;

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Minguez-Mosquera & Hornero-Mendez, 1993; Simmone, Simmone, Eitenmiller, Mills, & Green, 1997). However, very little information is available on the effect of cooking on the antioxidant activities of peppers. Therefore, the purpose of this study was to investigate the effect of different cooking treatments on the antioxidant properties of coloured peppers.

2. Materials and methods

2.1. Plant materials

Six varieties of coloured peppers (*Capsicum annum* L.), namely green pepper, red pepper, green paprika, red paprika, orange paprika and yellow paprika, were purchased from local supermarkets in Nara, Japan. The green and red peppers, also known as *piman* and *aka piman*, respectively, in Japan, are larger in size on their upper body than the lower body whereas, for the paprika varieties, the diameters are identical on the upper and lower parts of the body.

2.2. Reagents

DPPH·, tris(hydroxymethyl)aminomethane (Tris), L-ascorbic acid (L-AsA), 2,4-dinitrophenylhydrazine, 2,6-dichloroindophenol and Folin-Ciocalteu reagent were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Acetonitrile and methanol (HPLC grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was obtained from Aldrich Chemical Co. (Milwaukee, WI). The water used in this experiment was purified with Milli-Q-Labo equipment (Millipore Japan, Tokyo). All other reagents used were of analytical grade.

2.3. Preparation of samples

2.3.1. General

The peppers were cooked in the same way as when prepared for consumption. In brief, peppers were cleaned with tap water and dried with absorbent paper. Stem and seeds were removed, edible portions of peppers (2 cm × 2 cm) were collected and weighed (~100 g). The first portion was used raw while the other three portions were subjected to either microwave heating or stir-frying or boiling for 5 min. The fifth portion of the sample was subjected to boiling for 30 min to study the effect of prolonged boiling on the antioxidant activity. From each of the raw and cooked samples (100 g), two portions of about 10 g were used for ascorbic acid content (AsA) analysis and the remaining samples were lyophilized in liquid nitrogen and then freeze-dried for 48 h using a freeze-dryer (Taitec VD-400F, Japan). Samples were ground into fine powder using a food grinder (Iwatani, Millser IFM-300DG) and were kept at −80 °C prior to analysis.

2.3.2. Cooking treatment

2.3.2.1. Microwave heating. Samples were placed in a Pyrex bowl, covered with a plastic cap for microwave heating to prevent water loss, and then cooked in a domestic microwave oven (Koizumi KRD-0106, 500 W) for 5 min. Total weight of the samples after cooking was measured before AsA analysis and freeze-drying.

2.3.2.2. Stir-frying. A minimum amount of cooking oil (2–3 g) was placed in a non-stick frying pan (diameter of 20 cm) and heated at 'high' on a hot plate (Sanyo IC-AI, 1200W, 100V) for 1 min. The cut peppers were then placed in the pan and the heating was reduced to 'medium'. Peppers were continuously stirred for 5 min. Total weight of the samples after stir-frying was measured before AsA analysis and freeze-drying.

2.3.2.3. Boiling. The cut peppers were heated in 500 ml of boiling water in a covered pan for 5 and 30 min, respectively. After boiling, the cooked tissues were drained using a wire mesh strainer. The weight of the cooked tissues and volume of the cooking water were determined. The cooking water was directly used for AsA analysis after appropriate dilution. For the radical-scavenging activity (RSA) and total polyphenol content (TP) analysis, 2 ml of cooking water were frozen with liquid nitrogen in a test tube and then freeze-dried. The cooked tissues were subjected to the respective procedures described for microwave heating and stir-frying.

2.4. Extraction of samples for DPPH radical-scavenging activity (RSA) and total polyphenol content (TP)

Powdered samples (50 mg) were extracted with 2 ml of 90% methanol containing 0.5% acetic acid. The solution was vortexed for 5 min, followed by centrifugation at 3000 rpm for 10 min at 4 °C. The extraction was repeated three times. The extracts were combined and dried under a stream of nitrogen. The residues were dissolved in 2 ml of methanol and filtered through a 0.45 μm filter (Ekicrodisc 25 mm syringe filter, Nacalai Tesque, Inc.). When necessary, solution was appropriately diluted before being used for RSA and TP analysis. Freeze-dried cooking water was used directly and no extraction was involved prior to analysis.

2.5. Determination of DPPH radical-scavenging activity (RSA)

The DPPH·–HPLC method was carried out according to the procedures of Yamaguchi, Takamura, Matoba, and Terao (1998). An aliquot of the sample solution (200 μl) was mixed with 100 mM Tris HCl buffer (pH 7.4, 800 μl) followed by 1 ml of 500 μM DPPH· in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left to stand in the dark at room temperature for 20 min. A blank was determined without sample solution. Trolox solution (200 μl) in ethanol was used as control (final concentration of 50 μM) and was also assayed, along with the experimental samples. DPPH· was measured by reversed-phase HPLC. The HPLC consisted of a L-7420 pump (Hitachi Co., Tokyo, Japan), a Rheodyne injector fitted with a 20 μl loop (Rheodyne, Rohnert Park, Cal., USA) and a L-7420 UV–Vis detector (Hitachi Co., Tokyo, Japan) set. Analyses were performed using a TSKgel Octyl-80Ts column (4.6 × 150 mm, Tosoh, Tokyo, Japan) at ambient temperature. The mobile phase consisted of methanol:water (70:30, v/v) at a flow rate of 1 ml/min. DPPH· RSA was calculated from the difference in peak areas of the DPPH radical detected at 517 nm between a blank and a sample. The activity was expressed as μmol trolox equivalents/100 g of fresh weight.

2.6. Determination of total polyphenol content (TP)

Total polyphenol content was determined by the Folin-Ciocalteu method. The TP was assayed colorimetrically by the procedure of Singleton and Rossi (1965) with slight modification. In brief, an aliquot (200 μl) of sample solution was mixed with 7.5% sodium carbonate (800 μl) followed by 1 ml of phenol reagent solution. The mixture was then shaken vigorously. The absorbance was measured at 765 nm after 30 min at room temperature using a UV-2100 spectrophotometer (Shimadzu, Kyoto, Japan). A mixture of water and reagents was used as a blank. The TP was expressed as μmol gallic acid equivalents/100 g fresh weight.

2.7. Determination of ascorbic acid content (AsA)

Ascorbic acid content was determined by the method of Kishida, Nishimoto, and Kojo (1992). In brief, sample (~10 g) was homogenized using a homogenizer (Kinematica Polytron

Homogenizer PT-MR 2000) in 5% metaphosphoric acid or 5% metaphosphoric acid containing 1% stannous chloride. The homogenate was then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant obtained was filtered through a 0.45 μm filter (Ekicrodisc 25 mm syringe filter, Nacalai Tesque, Inc.). After an appropriate dilution, 100 μl of 5% metaphosphoric acid filtrate were oxidized with 0.2% 2,6-dichloroindophenol (50 μl), followed by 1% stannous chloride in 5% metaphosphoric acid and 2% (dinitrophenyl) hydrazine (120 μl) in 4.5 M H_2SO_4 in both the filtrates. The mixture was incubated in a water bath for 3 h at 37 °C. Ethyl acetate (1 ml) and water (1 ml) were then added to the reaction mixture. After vortexing and centrifugation (3000 rpm, 5-min), 300 μl of ethyl acetate layer was withdrawn and evaporated under a stream of nitrogen to dryness. The residue was dissolved in 200 μl of acetonitrile prior to HPLC analysis. A standard curve of ascorbic acid was conducted for each run. HPLC analysis was carried out on a Cosmosil 5C18-AR-II column (4.6 × 250 mm, 5 μm particle size, Nacalai Tesque Inc.) with a SPD-10AV UV–Vis detector (Shimadzu, Kyoto, Japan) set at 505 nm and a Rheodyne injector fitted with a 20 μl loop (Rheodyne, Rohnert Park, Cal., USA). The mobile phase consisted of acetonitrile: water (50:50, v/v) adjusted to pH 3.5 with 0.1% triethylamine and phosphoric acid. The flow rate was 1 ml/min. The AsA was calculated by subtracting the value of the sample mixed with 2,6-dichloroindophenol from the value of the sample without 2,6-dichloroindophenol. The data were expressed as mg/100 g of fresh weight. The following equation was used for the calculation of the contribution of AsA to RSA and expressed as μmol of trolox equivalents/100 g of fresh weight of pepper (Yamaguchi et al., 2001)

$$\text{RSA derived from AsA } (\mu\text{mol of trolox equivalents /100 g of fresh weight of pepper}) = \frac{\text{AsA content } (\text{mg}/100\text{ g of fresh weight of pepper}) \times 10^3}{176.13(53.0/47.6)}$$

where 176.13 is the molecular weight of AsA, 53.0 is the concentration for 50% RSA of trolox and 47.6 is the concentration for 50% RSA of AsA.

2.8. Determination of total carotenoid content

Total carotenoid content was determined by the method of Alasalvar, Al-Farsi, Quantick, Shahidi, and Wiktorowicz (2005) with slight modification. In brief, freeze-dried samples (300–500 mg) were extracted with 5 ml of acetone–water (9:1, v/v) and centrifuged at 3000 rpm for 10 min at 4 °C. The clear supernatant was withdrawn and extraction was repeated for another five or six times with 3 ml of acetone–water until no colour was extracted. Extracts obtained were pooled and measured against an acetone blank at 471 nm using a UV-2100 spectrophotometer (Shimadzu, Kyoto, Japan). Total carotenoid content was calculated according to the following equation:

$$\text{Total carotenoid content } (\%) = \frac{\text{Abs}_{\max} \times 25\text{ ml acetone} \times 100}{\text{sample weight}}$$

2.9. Statistical analysis

All data were presented as means ± standard deviation of at least two duplicate experiments. Differences between variables were tested for significance by using ANOVA. Differences between means were considered to be significantly different at $P < 0.05$ (SPSS for Windows 13.0).

3. Results and discussion

3.1. Effect of cooking methods on the radical-scavenging activity (RSA)

Fig. 1a summarizes the data for RSA levels in raw peppers and its changes after cooking. The RSA levels of raw peppers were in the range of 519–1190 μmol trolox equivalents/100 g fresh weight and are listed in descending order: orange paprika > red paprika > red pepper > yellow paprika > green paprika > green pepper.

Though RSA declined after cooking, differences were not significant ($P > 0.05$) between raw, microwave heating and stir-frying in all the samples (Fig. 1a). However, RSA was found to have reduced

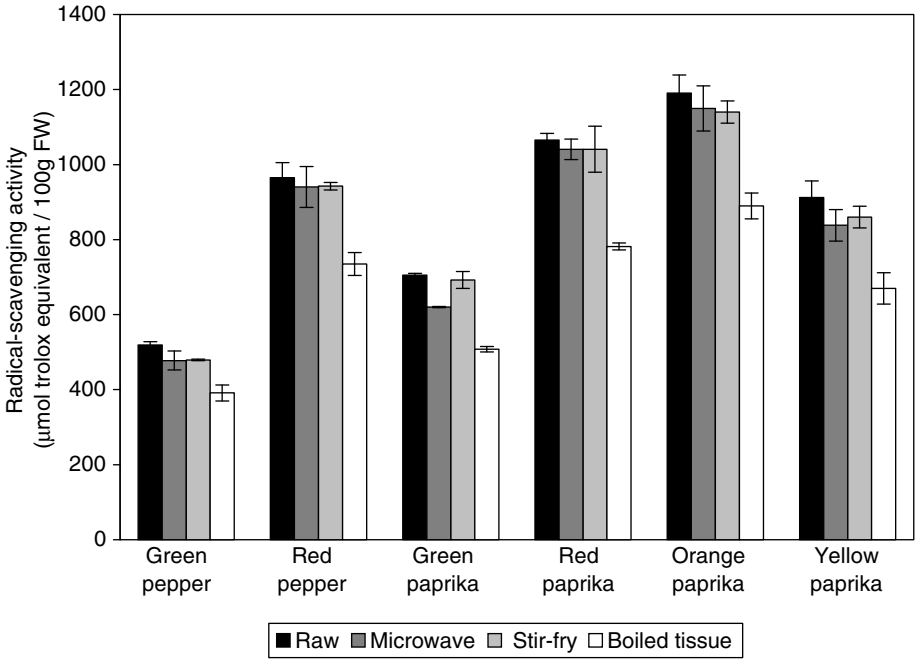

Fig. 1a. Change in radical-scavenging activity of coloured peppers after 5 min of cooking.

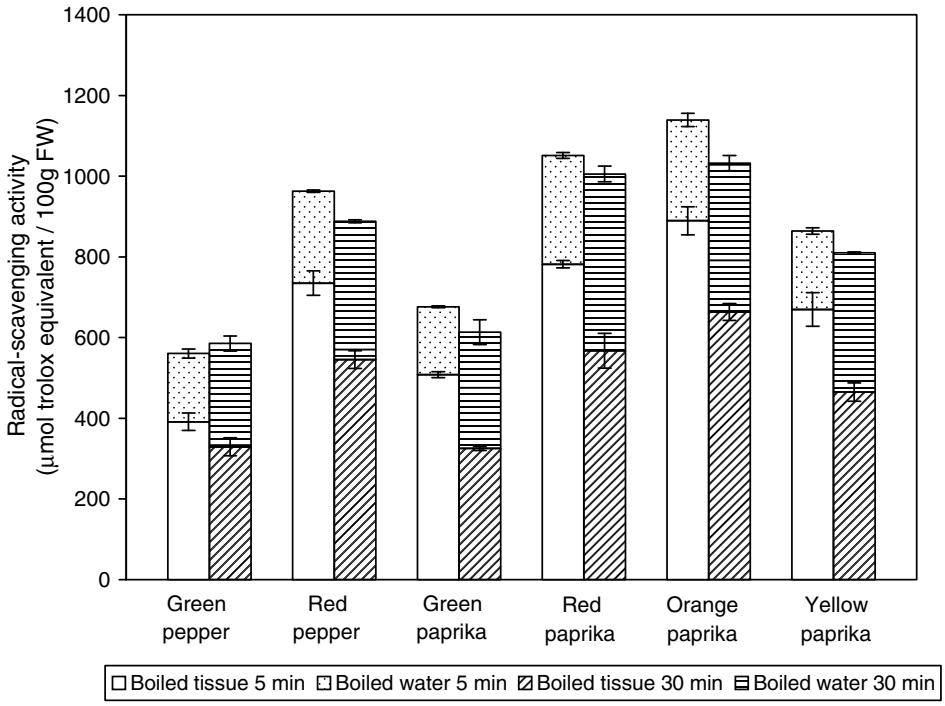

Fig. 1b. Change in radical-scavenging activity of coloured peppers after 5 and 30 min of boiling.

considerably ($P < 0.05$) in the cooked tissues, after boiling for 5 min, to below 77% of its initial level. Further reduction ($P < 0.05$) of the RSA to 64% was observed when the boiling time was prolonged to 30 min (Fig. 1b). Howard, Wong, Perry, and Klein (1999) noted that the loss of antioxidant activity in cooked tissue during blanching was due to the large surface area of the vegetables in contact with the water. On the other hand, increasing the boiling time from 5 to 30 min caused significant ($P < 0.05$) loss of antioxidants from the samples to cooking water in all of the tested samples (Fig. 1b). This is an indication that the activity could have leached into the boiling water. The sum of the RSA values in cooked tissues and cooking water after 5 min of boiling did not differ significantly ($P > 0.05$) from the activity in the initial raw pepper samples. As for boiling at 30 min, the sums of RSA were significantly ($P < 0.05$) reduced when compared with the initial uncooked tissues. Nonetheless, this was not the case with red pepper and red paprika when subjected to 30 min of boiling, as both showed resistance to deterioration in their RSA activity. It was also interesting to note that the total RSA values, in both the tissues and cooking water, increased in the green pepper during boiling for 5 and 30 min. This phenomenon was not observed in the other pepper varieties. Yamaguchi et al. (2001) attributed the increases of RSA in several vegetables after boiling to the suppression of oxidation by antioxidants due to thermal inactivation of oxidative enzymes. In addition, the destruction of cell walls and subcellular compartments of vegetables during boiling causes the release of potent radical-scavenging antioxidants. Gahler et al. (2003) reported an improvement in the antioxidant activity of tomatoes after heat treatment due to the increased release of phytochemicals, such as lycopene, from the matrix. Turkmen et al. (2005) reported that boiling, microwave cooking and steaming induced significant increases in total antioxidant activity of pepper, green beans, broccoli and spinach. Conversely, antioxidant levels were reported to decrease after aquathermal treatment of broccoli (Zhang & Hamauzu, 2004) and selected cruciferous vegetables (Sikora, Cieslik, Leszczynska, Filipiak-Florkiewicz, & Pisulewski, 2008). Puupponen-Pimia et al. (2003) reported that the DPPH· index of cauliflower decreased by 23% during blanching in water but increased by 9% in the case of cabbage.

3.2. Effect of cooking methods on the total polyphenol content (TP)

The total polyphenol content in the different varieties of peppers, before and after cooking, is shown in Fig. 2a. A positive correlation of $R = 0.929$ was observed between the TP and RSA in cooked peppers (data not shown), suggesting that the higher the TP, the higher is the RSA. The highest level of TP in the raw peppers was found in red pepper (594 μmol gallic acid equivalents/100 g FW). This was followed by red and orange paprikas, which were found to contain equally high levels at 458 and 444 μmol GAE/100 g FW, respectively, and its levels in yellow and green paprika and in green pepper were 367 μmol GAE/100 g FW, 279 μmol GAE/100 g FW and 259 μmol GAE /100 g FW, respectively.

Results show that, after cooking, TP was reduced in all the tested samples but the decline was not statistically significant ($P > 0.05$) between raw, microwave heating and stir-frying (Fig. 2a). This may be attributed to the inactivation of the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenols degradation (Yamaguchi et al., 2003). Similarly to RSA, TP content was significantly ($P < 0.05$) reduced in the cooked tissues of all the peppers after boiling for 5 min and was further reduced after boiling for 30 min (Fig. 2b). Although TP contents of the combined cooked tissues and cooking water were reduced after boiling was extended to 30 min, the decline was not appreciable ($P > 0.05$) in comparison to boiling for 5 min, demonstrating that the polyphenols were stable and did not lose their activity during 30 min of boiling. According to Sikora et al. (2008), the degree of polyphenols degradation depends very much on the processing time and the size of the vegetables. Ewald, Fjelkner-Modig, Johnsson, Sjoholm, and Akesson (1999) reported that boiling, microwaving, frying or further warm holding did not affect the levels of polyphenols, quercetin and kaempferol in onions, green beans and peas. On the other hand, losses of polyphenols upon boiling or blanching have been reported in selected cruciferous vegetables

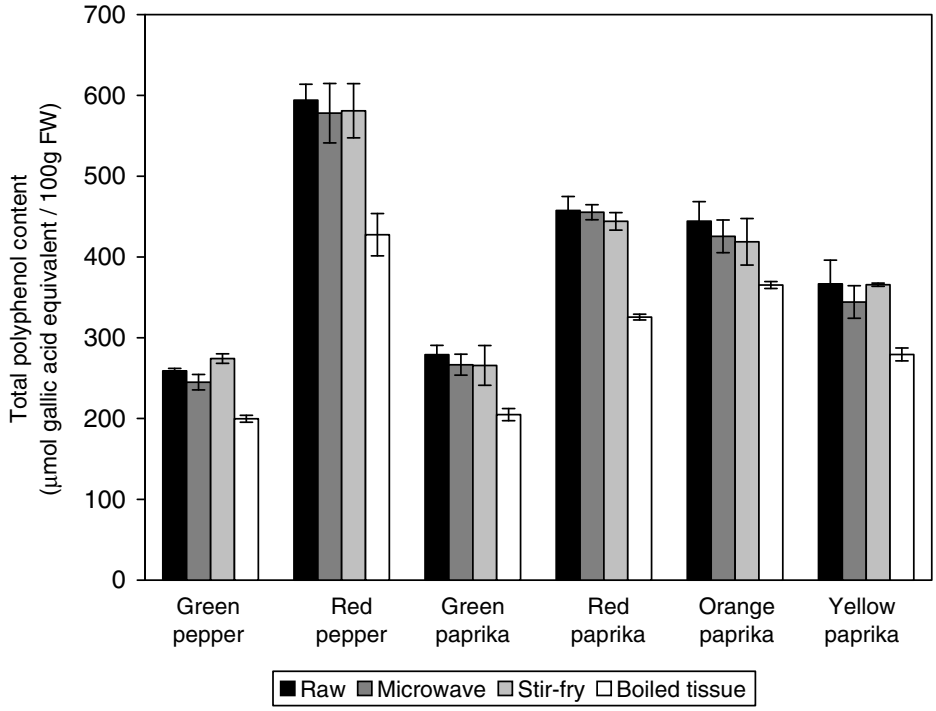

Fig. 2a. Change in total polyphenol content of coloured peppers after 5 min of cooking.

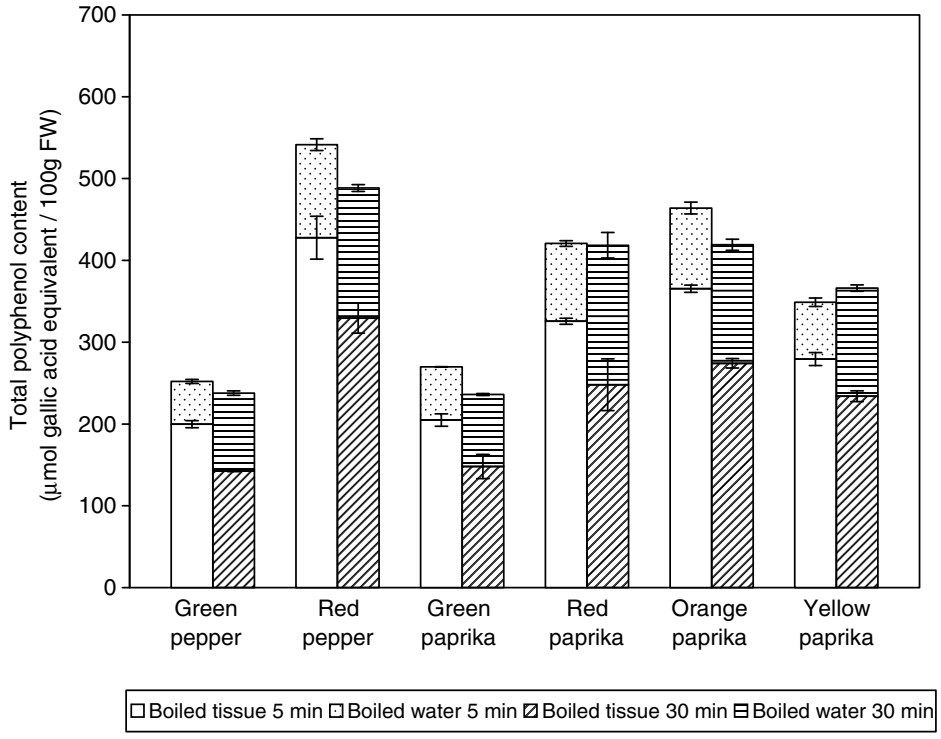

Fig. 2b. Change in total polyphenol content of coloured peppers after 5 and 30 min of boiling.

(Sikora et al., 2008), broccoli (Zhang & Hamauzu, 2004), kale, spinach, cabbage, swamp cabbage and shallots (Ismail et al., 2004), probably due to the dissolution of polyphenols into the cooking water. In contrast, Turkmen et al. (2005) concluded that cooking resulted in an increase in the phenolics content in vegetables.

3.3. Effect of cooking methods on the ascorbic acid content (AsA)

Fig. 3a shows the contents of ascorbic acid in different varieties of raw and cooked peppers. Only the AsA was evaluated in this analysis since dehydroascorbic acid did not show any radical-scav-

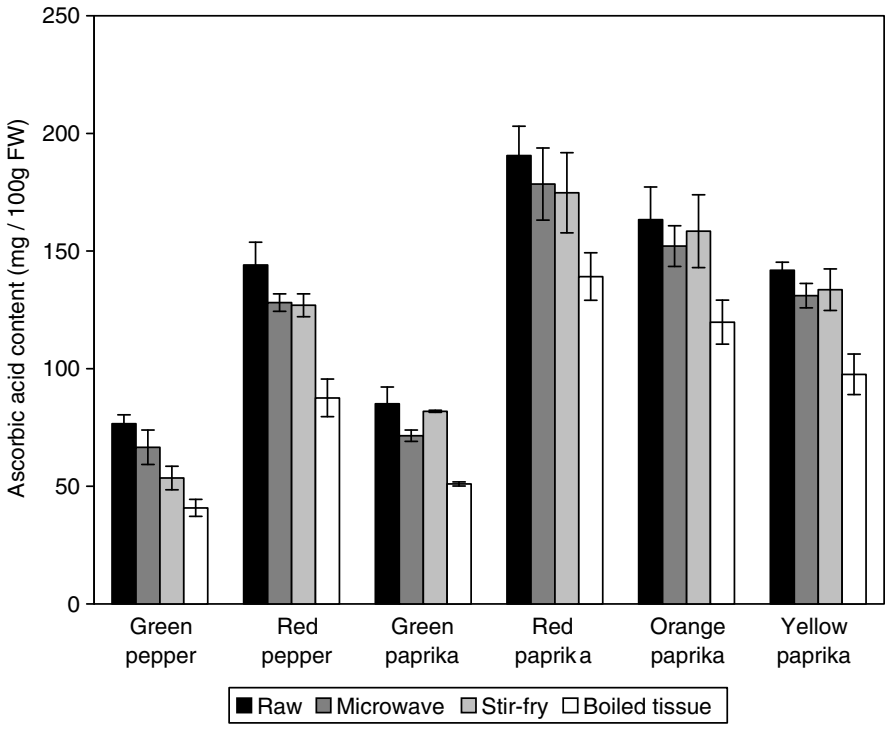

Fig. 3a. Change in ascorbic acid content of coloured peppers after 5 min of cooking.

enging activity against DPPH· or hydroxyl radicals (Takamura, Yamaguchi, Terao, & Matoba, 2001).

The AsA contents of the raw peppers used in this study were different from previous reports and this could be due to variations in cultivar, genetics, maturity, fertilization and environmental growing conditions (Lee et al., 1995; Howard, 2001; Simmone et al., 1997). Raw red paprika was found to have the highest level of AsA (191 mg/100 g of fresh weight), followed by orange paprika (163 mg/100 g FW), red pepper (144 mg/100 g FW), yellow paprika (142 mg/100 g FW) and green paprika (85.1 mg/100 g FW). The lowest level of AsA was found in green pepper (76.7 mg/100 g FW) (Fig. 3a). Almost all the pepper varieties are green at the unripe stage, turning to yellow, orange, red or purple when they reach maturity (Marin et al., 2004). Studies have also reported an increase in the level of AsA during pepper ripening (Howard et al., 2000; Marin et al., 2004; Simmone et al., 1997). This is in agreement with the present results, which show higher AsA in the red, orange, and yellow varieties than in the green cultivars. Mozafar (1994) reported that the higher levels of AsA found during ripening might be related to the light intensity and greater levels of glucose, the precursor of AsA. The calculated contribution of AsA to the RSA in raw peppers can be seen in Fig. 3b. AsA was the major component in red paprika, responsible for 91.2% of the RSA. A drastic degradation in the AsA could have rendered a low RSA in red paprika. Contributions of AsA to RSA in other cultivars ranged from 61.5% to 79.3%, demonstrating that phytochemicals other than AsA could have contributed more to its RSA than in red paprika.

All the peppers, regardless of the cooking treatments, showed a reduction in their AsA when cooked (Fig. 3a). Loss of AsA is expected, as thermal treatment is known to accelerate oxidation of ascorbic acid to dehydroascorbic acid, followed by the hydrolysis to 2,3-diketogulonic acid and eventually polymerization to other nutritionally inactive components (Gregory, 1996). However, reductions in the AsA were found to be not significantly ($P > 0.05$) different from the raw peppers after microwave heating or stir-frying, whereas green and red peppers showed significant ($P < 0.05$) reduction from the raw samples after stir-frying. Microwave cooking has been found to have only minimal effects on the AsA of carrot, green beans and broccoli (Howard et al., 1999). Yadav and Sehgal (1995) attributed the loss of AsA during stir-frying to high temperatures, long cooking time, enzymatic oxidation during the preparation and cooking processes and frequent stirring that expose the materials to atmospheric oxidation. AsA levels were also found to have declined markedly ($P < 0.05$) after boiling for 5 min in all the cooked tissues with further reduction observed after 30 min (Fig. 3c). Erdman and Klein (1982) noted that using minimal cooking water and cooking for shorter time periods resulted in better AsA retention. On the contrary, the AsA detected in the cooking water of all the samples increased with further boiling. Levels reached in the water were almost equal to those found in the cooked tissues at 30 min (Fig. 3c). Nonetheless, the sum of AsA in the boiling water and cooked tissues virtually equalled the initial AsA content in raw peppers for both boiling duration in red, orange and yellow paprika. Gil, Ferreres, and Tomas-Barberan (1999) found that only 40% of the initial vitamin C content was retained in the cooked tissues of spinach while the remaining 60% was recovered in the cooking water. Kalt (2005) and Somsub, Kongkachuichai, Sungpuag, and Charoensiri (2008) attributed the degradation of AsA during boiling or blanching to leaching of the AsA from the plant tissues into the cooking water, thus confirming our results that the AsA in the cooked tissues was not actually destroyed in total. This is not surprising since AsA is highly soluble in water. However, the same cannot be concluded for green and red peppers, which showed significant ($P < 0.05$) losses in the sum of AsA after boiling for 5 and 30 min. This could be attributed to the fact that green and red peppers have a thinner pericarp layer, and consequently a higher surface to mass ratio than do the thicker paprika varieties. The thinner cell membranes would be more permeable to heat, resulting in rapid leaching of AsA into the water as the cells die. In addition, the penetration of heat throughout the boiling medium is easier because the heat transfer

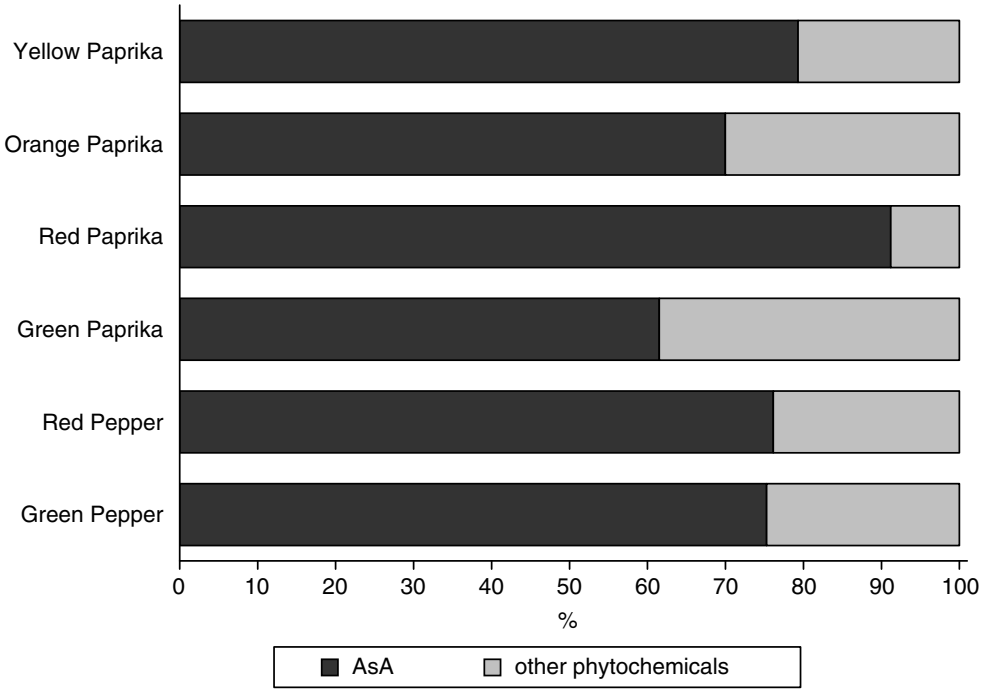

Fig. 3b. Percentage contribution of ascorbic acid content to the radical-scavenging activity of raw coloured peppers.

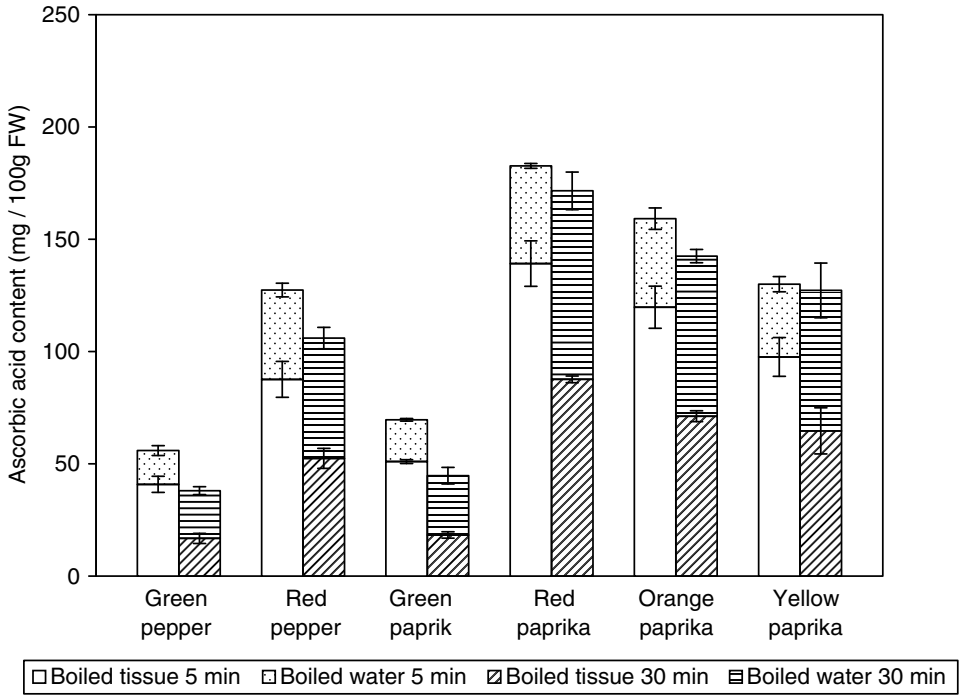

Fig. 3c. Change in ascorbic acid content of coloured peppers after 5 and 30 min of boiling.

coefficient is higher in aqueous solutions; hence destruction of the heat-sensitive nutrients is expected to be greater (Selman, 1994). A 40% loss of AsA has been reported during water blanching of green bell peppers (Matthews & Hall, 1978). Masrizal, Giraud, and Driskell (1997) reported significantly higher vitamin C retention values in vegetables (bean sprouts, green beans, nappa cabbage and spinach) prepared by microwave cooking and stir-frying than in those subjected to boiling. Loss of AsA after cooking has also been observed in other vegetables, such as peas, carrots, spinach, potatoes and several brassicas (Puupponen-Pimia et al., 2003), broccoli (Zhang & Hamauzu, 2004), fenugreek (Yadav & Sehgal, 1995) and some selected Thai vegetables (Somsub et al., 2008). Among the commonly practised cooking methods, boiling has always been consistently recorded to contribute to the highest loss in vitamin C content in vegetables (Masrizal et al., 1997; Somsub et al., 2008; Zhang & Hamauzu, 2004).

Table 1
Effects of cooking on the total carotenoids content of coloured pepper

Pepper variety	Cooking treatment Total carotenoid content[A]								
	Raw	Microwave Heating		Stir-frying		Boiling 5-min		Boiling 30-min	
	mg/100 g FW	mg/100 g FW	% Retention	mg/100 g FW	% Retention	mg/100 g FW	% Retention	mg/100 g FW	% Retention
Green pepper	2.42 ± 0.03^{a}	1.99 ± 0.11^{b}	82.3	2.44 ± 0.07^{a}	101	1.97 ± 0.07^{b}	81.4	1.79 ± 0.16^{b}	73.8
Red pepper	5.34 ± 0.57^{ab}	4.80 ± 0.10^{ab}	89.8	5.86 ± 0.57^{b}	110	4.25 ± 0.12^{a}	79.6	4.18 ± 0.07^{a}	78.3
Green paprika	1.56 ± 0.01^{a}	1.28 ± 0.07^{ab}	82.0	1.41 ± 0.08^{ab}	90.8	1.38 ± 0.24^{ab}	88.4	1.00 ± 0.01^{b}	64.4
Red paprika	4.50 ± 0.19^{ab}	4.34 ± 0.09^{ab}	96.4	5.09 ± 0.53^{b}	113	3.77 ± 0.13^{a}	83.7	3.56 ± 0.03^{a}	79.0
Orange paprika	6.49 ± 0.39^{a}	6.28 ± 0.47^{ab}	96.8	6.25 ± 0.18^{ab}	96.3	5.07 ± 0.21^{bc}	78.2	3.83 ± 0.17^{c}	59.1
Yellow paprika	1.81 ± 0.03^{a}	1.16 ± 0.34^{a}	64.0	1.16 ± 0.20^{a}	64.3	1.17 ± 0.22^{a}	64.8	1.29 ± 0.44^{a}	71.7

Mean values in a row with different letters are significantly different at $P < 0.05$.
[A] Data are expressed as means ± SD of at least two duplicate experiments.

3.4. Effects of cooking methods on the total carotenoid content

The concentrations and compositions of carotenoids are responsible for the diverse and attractive colours observed in peppers (Howard, 2001). The red pigments in red bell pepper and red paprika are mainly capsanthin and capsorubin (Matsufuji, Nakamura, Chino, & Takeda, 1998). Chlorophyll contributes to the green colour (Marin et al., 2004) whereas α- and β-carotene, zeaxanthin, lutein and β-cryptoxanthin are responsible for the yellow–orange colour in peppers (Howard, 2001). Paprika juice, rich in the oxygenated carotenoid capsanthin, has been implicated in the prevention of colon cancer (Narisawa et al., 2000). Table 1 summarizes the total carotenoid contents of raw and heat-treated peppers used in this study. Orange paprika showed the highest carotenoid content, followed by red pepper and red paprika while no variation ($P > 0.05$) was found in the carotenoid contents among green pepper, yellow paprika and green paprika. Although, in general, the results showed that the total carotenoid contents in peppers were reduced after cooking, the data were not significantly different from those of the control. This reduction was only significant ($P < 0.05$) during boiling for 5 min for the green pepper and orange paprika. The other cultivars experienced no significant ($P > 0.05$) change in the carotenoid level, regardless of the style of cooking. This result may be explained by the differences in the degree of susceptibility of peppers to heat treatment and also to varietal differences. However, for all the cultivars, after boiling for 5 min, total carotenoid content remained stable for up to 30 min in the cooked tissues. It has been reported that there is an enhanced bioavailability of carotenoids after heat treatment in carrots and spinach (Rock et al., 1998). Howard et al. (1999) and de Sa and Rodriguez-Amaya (2003) attributed the increase in carotenoids content in cooked vegetables to the ease of chemical extraction after cooking. On the other hand, studies also observed a decrease in the retention of carotenoids during cooking of green leafy vegetables (Chandrika, Svanberg, & Jansz, 2006; Zhang & Hamauzu, 2004). Kalt (2005) and Puupponen-Pimia et al. (2003), however, concluded that carotenoids in selected vegetables were not adversely affected by heat treatment.

4. Conclusion

In general, microwave heating and stir-frying did not affect the RSA, TP, AsA or total carotenoid contents while their levels were partly degraded during boiling. Loss of antioxidant activity was greater in the cooked tissues with prolonged boiling time. This finding indicated that cooking of peppers by microwave heating and stir-frying is better to ensure a higher retention of the bioactive components in peppers. When boiling is unavoidable, it is recommended to use less water and less cooking time, so as to retain the optimum benefits of the bioactive compounds present in peppers during cooking. It is also vital to consume the water used for boiling in addition to the peppers, as bioactive compounds will be present in the water as a result of leaching from the cooked tissues. Generally, red, orange and yellow paprika retained higher levels of antioxidant compounds during cooking than did green and red peppers.

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