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Radical-Scavenging Activity of Vegetables and the Effect of Cooking on Their Activity

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The radical-scavenging activity of vegetables was measured using the 1,1-diphenyl-2-picrylhydrazyl-HPLC method, and the effect of cooking on this activity was determined. The content of ascorbic acid having radical-scavenging activity was highest in burdock among the 18 fresh vegetables tested. In some vegetables, the activity increased in spite of the loss of ascorbic acid content after boiling, while in other vegetables, the activity had decreased after boiling. This decrease may be due to release of the activity from cooked tissue into the cooking water during boiling. Both activity and ascorbic acid content of the vegetables cooked in a microwave were generally higher than in those cooked by boiling.

Keywords: radical-scavenging activity, 1,1-diphenyl-2-picrylhydrazyl, ascorbic acid, vegetables, heating effect

Antioxidants in food have received considerable attention in recent years for their role in human health (Ames *et al*., 1993; Aruoma, 1994). Dietary vegetables contain a wide variety of free radical-scavenging antioxidants, for example, flavonoids, phenolic acids, and antioxidant vitamins (Huang *et al*., 1994; Shahidi & Naczk, 1995). There are many studies on the antioxidant activity and the mechanism of individual antioxidants. In addition, epidemiological studies have shown that the consumption of fruits and vegetables is associated with a reduced risk of life-style related diseases such as cancer and coronary heart disease (Steinmetz & Potter, 1991; Ames *et al*., 1993; Joshipura *et al*., 1999). Over 170 studies on epidemiological cancer have also been reviewed and consistently showed that there is a lower risk with increased intake of fruits and vegetables (Block, 1992). Therefore, the World Cancer Research Fund and the American Institute for Cancer Research (1997) recommended the eating of 400–800 g or five or more portions (servings) a day of a variety of vegetables and fruits for cancer prevention. In Japan, the recommended intake of vegetables is 350 g/day/person, while the average dietary intake of vegetables in 1998 was 292 g/day/person (Health Service Bureau, Community Health, Health Promotion and Nutrition Division, Office for Life-Style Related Diseases Control, Japan, 1998).

Vegetables are the main source of vitamin C in the diet, and are usually consumed after processing and cooking but also in their fresh state. There are several ways to cook vegetables such as boiling, steaming, baking, and frying. Boiling is the most conventional process, which heats food in water near 100°C. Vegetables are boiled for a few minutes to soften the plant tissues and remove bitterness. They are sometimes boiled for a longer time with seasonings. Recently, microwave heating, which utilizes the interaction of an electromagnetic field with chemical constituents of food, has been used for cooking. Microwave heating has well-known advantages over boiling, the conventional heating process, because foods are heated directly and rapidly without contact with hot surfaces (Young & Jolly, 1990). Many experiments have been carried out on the effect of processing and cooking on vitamin C content in vegetables (Erdman & Klein, 1982; Kiribuchi & Kawashima, 1987; Sako *et al*., 1996; Howard *et al*., 1999). It is generally believed that these steps result in significant loss of vitamin C. However, the effect of processing and cooking on the radical-scavenging activity of vegetables has not yet been fully approached. To determine the optimum cooking method which results in the highest retention of the radical-scavenging activity, and to obtain high quality food beneficial for the extension of human life, the effect of processing and cooking of vegetables on their radical-scavenging activity must be understood.

In this study, we investigated the radical-scavenging activity of commercial fresh vegetables using the 1,1-diphenyl-2-picrylhydrazyl (DPPH)-HPLC method. We further looked at the effect of cooking by boiling and by microwave heating on this activity. Ascorbic acid (ASA), the reduced form of vitamin C, is a common component of vegetables and has strong radical-scavenging activity. The ASA content of vegetables and its changes during cooking were determined, and the contribution of ASA on this activity is discussed here.

Material and Methods

Materials DPPH, L-ASA, tris(hydroxymethyl)aminomethane (Tris), 2,4-dinitrophenylhydrazine, and acetonitrile (HPLC grade) were obtained from Nacalai Tesque Inc. (Kyoto). Ethanol and methanol (HPLC grade) were obtained from Wako Pure Chemical Industries (Osaka), and 6-hydroxy-2,5,7,8-tet-

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ramethylchroman-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).

Eighteen vegetables, asparagus (*Asparagus officinalis* L.), broccoli (*Brassica oleracea* L. var. *italica* PLENCK), burdock (*Arctium lappa* L.), cabbage (*Brassica oleracea* L. var. *capitata* L.), carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), Chinese cabbage (*Brassica campestris* L.), Chinese chive (*Allium tubersum* ROTTLER), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), green pepper (*Capsicum annuum* L.), Japanese radish (*Raphanus sativus* L.), kidney beans (*Phaseolus vulgaris* L.), lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), pumpkin (*Cucurbita maxima* DUCHESNE), spinach (*Spinacia oleracea* L.), and tomato (*Lycopersicon esculentum* MILL.) were purchased from local markets in Nara, Japan.

Preparation of vegetable extracts The edible portions of fresh vegetables (10–20 g) were cut into small pieces and homogenized for 20–30 s using a homogenizer (Kinematica Polytron Homogenizer PT-MR 2000) in 30 ml of water, 5% metaphosphoric acid, or 5% metaphosphoric acid containing 1% stannous chloride. The resulting homogenate was centrifuged at 27,000×*g* for 20 min at 4°C, and the supernatant was filtered through a 0.45 μm filter (Cosmonice Filter W, 13 mm, Nacalai Tesque Inc.). After appropriate dilution, the filtrate of the "water extract" was used for the measurement of radical-scavenging activity, whereas the filtrates of "water extract", "5% metaphosphoric acid extract", and "5% metaphosphoric acid containing 1% stannous chloride extract" were used to measure ASA content. The solutions of 5% metaphosphoric acid with or without 1% stannous chloride were generally used for extraction of ASA in vegetables.

Cooking by boiling Edible portions of asparagus, burdock, green pepper, kidney beans, and spinach were cut into small pieces of 3 cm each. Carrot, pumpkin, and Japanese radish were cut into small cubes of 1.5 cm × 1.5 cm × 1.5 cm; cabbage and Chinese cabbage were cut into pieces of 3 cm × 3 cm. Tomato and eggplant were sliced to 1 cm, onion was vertically cut into eight pieces, and heads of broccoli were cut to ~5 cm floret diameter with a ~3 cm stalk below the floret. Then each sample of 80–150 g fresh weight was heated in 500 ml of boiling water for 5 min. The influence of boiling time on the radical-scavenging activity was examined in broccoli, eggplant, and Japanese radish by heating, respectively, in boiling water for 30 min.

After boiling, the total weight of cooked tissue was measured and an extract of the cooked vegetable was prepared following the same procedure as described in the preceding section for fresh vegetables. The volume of cooking water determined after boiling was used to measure radical-scavenging activity and ASA content after concentration. Before this experiment, a model solution of ASA was assayed to evaluate the effect of boiling: an ASA aqueous solution (500 μM) in a vial was heated in a water bath for 30 min, and then both radical-scavenging activity and ASA content of the solution were measured.

Cooking by microwave heating Vegetables were cut to the same dimensions as for the boiled samples. An amount of 80–150 g of each sample was put on a ceramic plate, wrapped in a plastic cap for microwave heating, and placed in a microwave oven for 1, 3, and 5 min. The microwave oven used was a Koizumi KRD-0106 with 500 W effective power. After heating, the total weight of cooked tissue was measured, and the extract of cooked vegetable was prepared following the same procedure as for fresh vegetables above.

Measurement of DPPH radical-scavenging activity Radical-scavenging activity was measured according to the DPPH-HPLC method of Yamaguchi *et al*. (1998) as follows. An aliquot of sample solution (200 μl) was mixed with 100 mM of Tris-HCl buffer (pH 7.4, 800 μl) and added to 1 ml of 500 μM DPPH in ethanol. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The reaction mixture was then subjected to HPLC analysis.

The HPLC analysis was carried out on a TSKgel Octyl-80Ts column (4.6× 150 mm, Tosoh, Tokyo) equipped with a Shimadzu LC-6A pump, a Rheodyne injector fitted with a 20 μl loop and a Shimadzu SPD-10AV UV-VIS detector set at 517 nm at ambient temperature. The mobile phase consisted of methanol/water (70:30, v/v), and the flow rate was 1 ml/min. Trolox was used as the control standard and 200 μl of Trolox solution (final concentration of 50 μM) in ethanol was assayed similarly during each run.

The DPPH radical-scavenging activity was evaluated by the difference in peak area decrease of the DPPH radical detected at 517 nm between a blank and a sample. The activity was expressed as μmol of Trolox equivalent per 100 g of each fresh vegetable.

Measurement of ASA content in vegetables ASA content in both fresh and cooked vegetables was determined by HPLC according to Kishida *et al*. (1992) as follows. Briefly, each vegetable extract (100 μl) was mixed with or without 0.2% 2,6-dichloroindophenol (50 μl); 1% stannous chloride in 5% metaphosphoric acid solution (50 μl) and 2% 2,4-dinitrophenylhydrazine (120 μl) in 4.5 M sulfuric acid was added to the solution. The mixture was incubated in a water bath for 3 h at 37°C, then, ethyl acetate (1 ml) and water (1 ml) were added. After shaking and centrifuging (1500×*g*, 4°C) for 5 min, 300 μl of the ethyl acetate layer was taken out and dried under nitrogen. The residue was dissolved in 200 μl of acetonitrile and applied to HPLC analysis.

HPLC analysis was carried out on a Cosmosil $5C_{18}$-AR-II column (4.6×250 mm, Nacalai Tesque Inc.) with a Shimadzu SPD-10AV UV-VIS detector set at 505 nm and a Rheodyne injector fitted with a 20 μl loop. The mobile phase was acetonitrile/water (50:50, v/v) adjusted at pH 3.5 with 0.1% triethylamine and phosphoric acid; the flow rate was 1 ml/min.

ASA content was calculated by subtracting the value of sample mixed with 2,6-dichloroindophenol from the value of sample without 2,6-dichloroindophenol. The data were expressed as mg per 100 g of each fresh vegetable. To calculate the contribution of ASA to the radical-scavenging activity, ASA content was expressed as μmol of Trolox equivalent per 100 g of each fresh vegetable. The equation for calculation is as follows:

Radical-scavenging activity derived from ASA (μmol of Trolox eq./100 g of each fresh vegetable)

=ASA content (mg/100 g of each fresh vegetable)/176.13 $\times 10^3$/(53.0/47.6)

= ASA content (mg/100 g of each fresh vegetable) ×5.1

where 176.13: the molecular weight of ASA, 53.0: the concen-

tration for 50% radical-scavenging activity of Trolox, and 47.6: the concentration for 50% radical-scavenging activity of ASA.

Results and Discussion

Radical-scavenging activity of fresh vegetables The radical-scavenging activity of fresh vegetables is shown in Table 1. Among the 18 vegetables, the highest activity was found in burdock (490.0 μmol Trolox eq./100 g) followed by broccoli (468.3), green pepper (453.0), and asparagus (448.1). However, carrot (31.9) and cucumber (47.0) showed poor activity. There have been some studies that evaluated the antioxidant activity of vegetables. Tsushida *et al.* (1994) measured the antioxidant activity using the β-carotene bleaching method coupled with the oxidation of linoleic acid, which showed the high activity of asparagus, sweet pepper, Chinese chive, and mini-tomato. Cao *et al.* (1996) reported that spinach, broccoli, onion, and eggplant have medium activity and cabbage and carrot have low activity on peroxyl radicals measured by oxygen-radical absorbance capacity assay. Vinson *et al.* (1998) also measured the antioxidant activity of vegetables against the inhibition of LDL oxidation, showing that asparagus, onion, and tomato had higher activity than kidney beans, broccoli, carrot, bell pepper, and spinach. A recent study using linoleic acid emulsion showed relatively high activity in eggplant and low activity in pumpkin, cabbage, Chinese cabbage, spinach, and carrot (Azuma *et al.*, 1999). Thus, the order of antioxidant activity of vegetables is attributed to the difference of analytical method.

ASA content of fresh vegetables and its contribution to radical-scavenging activity ASA content of fresh vegetables is shown in Table 1. Since dehydroascorbic acid had no radical-scavenging activity on DPPH or hydroxyl radicals (Takamura *et al.*, 2001), we measured only the ASA of these vegetables. ASA content of broccoli was the highest (79.5 mg/100 g), followed by green pepper (74.4), and cauliflower (61.9).

The calculated contribution of ASA to the radical-scavenging activity ranged from 2% (burdock) to 87% (broccoli) in vegetables (Table 1). ASA was the main antioxidant component only in broccoli, cabbage, cauliflower, and green pepper. Therefore, the major portion of the radical-scavenging activity of other vegetables seems to be derived from other compounds, such as phenolic compounds and chlorophyll. The correlation of phenolic content and antioxidant activity of the vegetable extracts has been reported (Tsushida *et al.*, 1994). Many phenolic compounds have been isolated from vegetables, and their antioxidant activity has been evaluated by several researchers (Tsushida *et al.*, 1994; Maruta *et al.*, 1995; Price *et al.*, 1998). Chlorogenic acid was found as the antioxidant in burdock (Maruta *et al.*, 1995) and eggplant (Winter & Herrmann, 1986), and was reported to have a strong radical-scavenging activity on DPPH radical (Kono *et al.*, 1998). Therefore, the higher activity of burdock and eggplant is probably derived from chlorogenic acid.

Many researchers have investigated flavonoids in various vegetables (Hertog *et al.*, 1992a, 1992b; Crozier *et al.*, 1997; Guo *et al.*, 1997). The major antioxidant components of onion and eggplant are 2 types of quercetin glycosides (Tsushida & Suzuki, 1995; Price *et al.*, 1997) and nasunin (Sakamura *et al.*, 1963), respectively. Broccoli contains quercetin glycosides, kaempferol glycosides and 4 types of hydroxycinnamic acid esters as the

Table 1. Radical-scavenging activity and ASA content of fresh vegetables.

Vegetables	Radical-scavenging activity (μmol Trolox eq./100 g)	ASA content (mg/100 g)	Contribution of ASA (%)[a]
Asparagus	448.1±29.3[b]	6.6±1.1	8
Broccoli	468.3±35.5	79.5±5.7	87
Burdock	490.0± 2.5	1.8±0.5	2
Cabbage	228.6±26.6	37.7±3.3	84
Carrot	31.9± 4.9	1.9±0.6	31
Cauliflower	386.7±13.4	61.9±1.9	82
Chinese cabbage	138.8±21.2	17.5±3.7	64
Chinese chive	388.6±41.7	18.2±1.6	24
Cucumber	47.0± 1.1	5.9±0.2	64
Eggplant	342.4±29.9	3.3±0.4	5
Green pepper	453.0± 7.7	74.4±3.1	84
Japanese radish	285.7±22.9	15.9±1.4	28
Kidney beans	223.1±21.5	7.7±2.7	18
Lettuce	104.5±17.6	4.5±1.6	22
Onion	138.2±20.5	4.2±0.8	15
Pumpkin, summer	273.0±28.8	40.5±6.8	76
Spinach, summer	374.3±26.7	17.4±2.3	24
Tomato	213.4±13.9	15.6±0.9	37

[a]The contribution of ASA to radical-scavenging activity of the vegetables was calculated as percentage.
[b]The values are the means±SD for three determinations.

Table 2. Radical-scavenging activity of vegetables after boiling.

Vegetables	Radical-scavenging activity					
	Cooked tissue		Cooking water		Total	
	μmol Trolox eq./100 g	%[a]	μmol Trolox eq./100 g	%	μmol Trolox eq./100 g	%
Asparagus	492.4± 24.3[b]	110	68.8±16.9	15	561.2± 38.7	125
Broccoli	390.7± 21.1	83	371.3±41.2	79	762.0± 68.6	163
Burdock	1076.1± 10.3	220	897.4±26.6	183	1973.5± 36.9	403
Cabbage	196.0± 6.5	86	110.9±19.6	49	307.0± 26.9	134
Carrot	35.3± 6.0	111	18.1± 4.3	57	53.4± 10.3	168
Chinese cabbage	74.9± 2.8	54	42.7± 7.5	31	117.6± 11.3	85
Eggplant	577.4±113.5	169	275.7±77.8	81	853.2±168.4	249
Green pepper	632.6± 11.4	140	157.4± 1.7	35	790.0± 17.6	174
Japanese radish	100.1± 5.9	35	60.0± 5.7	21	160.0± 14.6	56
Kidney beans	144.0± 16.2	65	16.2± 4.4	7	160.2± 25.9	72
Onion	60.9± 4.4	44	14.7± 2.7	11	75.6± 8.5	55
Pumpkin	260.5± 22.0	95	44.6±12.0	16	305.0± 8.3	112
Spinach	73.6± 11.3	20	284.4±25.4	76	358.0± 35.3	96
Tomato	151.4± 9.7	71	44.2± 9.2	21	195.7± 0.5	92

[a]Percentage to the value for fresh materials.
[b]The values are the means±SD for three determinations.

major antioxidant components (Plumb *et al*., 1997; Price *et al*., 1998). Tsushida *et al*. (1994) reported that the most significant compound in asparagus is rutin and that its contribution of antioxidant activity is approximately 75%. Flavonoids as well as ASA are universally distributed in vegetables, therefore, both seem to play important roles in the antioxidant activity of fresh vegetables.

Carrot had a very poor radical-scavenging activity (Table 1) in spite of its high content of β-carotene, one of the antioxidant vitamins. β-Carotene is an efficient singlet oxygen quencher but is not a hydrogen donor in the present DPPH-HPLC reaction system (Foote & Denny, 1968). On the other hand, Endo *et al*. (1985) suggested that the antioxidant effect of chlorophyll takes place through the same mechanism as phenolic antioxidants, that is, the antioxidant activity occurs by donation of a hydrogen atom. Dark green-colored vegetables may thus show higher activity than light-colored vegetables. Further study is needed to identify the individual antioxidants and their contribution to free radical-scavenging activity in vegetables.

Effect of boiling on radical-scavenging activity and on ASA content The radical-scavenging activity and ASA content of vegetables after heating in boiling water were measured. Table 2 shows the radical-scavenging activity and Table 3 shows the ASA content of both cooked tissue and cooking water. The activity in cooked tissue of asparagus, burdock, carrot, eggplant, and green pepper increased after boiling for 5 min in spite of the loss of ASA content: 1.1-, 2.2-, 1.1-, 1.7-, and 1.4-fold, respectively.

Maeda *et al*. (1992) stated that an increase in the activity of vegetables after boiling might be due to the thermal destruction of vegetable cell walls and subcellular compartments which liberates more components, and/or thermal chemical reaction which produces more potent radical-scavenging antioxidants. With respect to thermal change of flavonol, protocatechuic acid and some of the other degradation products have been detected during heating of quercetin and rutin under aqueous conditions (Makris & Rossiter, 2000). The degradation products of flavonol glycoside are expected to show more potent radical-scavenging activity. In addition, we presumed that the higher activity of cooked vegetables may be due to inactivation of oxidative enzymes by heating. Chlorogenic acid, an antioxidant contained in high amount in burdock and eggplant, is a substrate for enzymatic browning. The content of chlorogenic acid was reported to

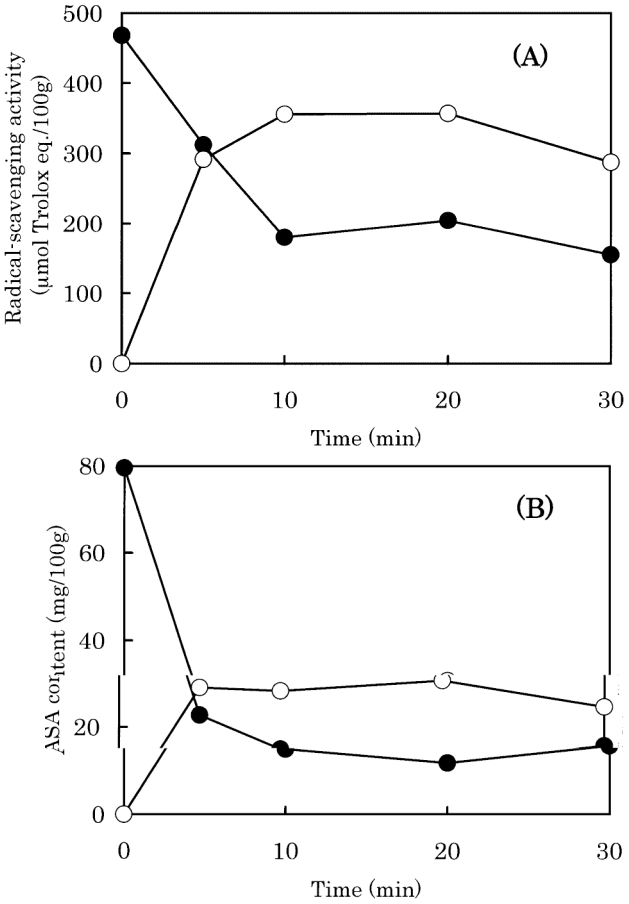

Fig. 1. Change in radical-scavenging activity and ASA content of broccoli during boiling. (A), radical-scavenging activity. (B), ASA content. ●, Cooked tissue; ○, Cooking water.

Table 3. ASA content of vegetables after boiling.

Vegetables	ASA content						Contribution of ASA in cooked tissue[b] (%)
	Cooked tissue		Cooking water		Total		
	mg/100 g	%[a]	mg/100 g	%	mg/100 g	%	
Asparagus	4.2±1.0[c]	63	0.5±0.1	7	4.7±1.1	70	4
Broccoli	38.4±3.3	48	27.8±3.5	35	65.6±3.7	83	50
Burdock	tr[d]	—	tr	—	tr	—	—
Cabbage	23.0±1.8	61	9.8±1.2	26	32.7±0.9	87	60
Carrot	0.6±0.1	33	0.5±0.2	24	1.1±0.3	57	9
Chinese cabbage	5.5±1.0	31	5.5±0.8	31	11.0±1.6	63	37
Eggplant	0.6±0.2	19	tr	—	tr	—	1
Green pepper	61.6±5.1	83	13.1±2.2	18	74.7±2.9	101	50
Japanese radish	8.1±1.1	51	4.0±0.2	25	12.1±1.8	76	41
Kidney beans	5.1±0.5	66	0.8±0.4	10	5.9±0.9	76	18
Onion	2.6±0.4	62	0.6±0.1	15	3.2±0.5	76	22
Pumpkin	16.7±1.5	41	4.8±0.9	12	21.5±0.8	53	33
Spinach	1.8±0.6	10	1.2±0.3	7	3.1±0.7	18	12
Tomato	9.3±1.2	59	1.9±0.3	12	11.2±1.4	72	31

[a]Percentage to the value for fresh materials.
[b]The contribution of ASA to radical-scavenging activity of the vegetables was calculated as percentage.
[c]The values are the means±SD for three determinations.
[d]tr, Trace amounts.

be decreased by polyphenol oxidase, an enzyme naturally present in vegetables and some other plants (Shahidi & Naczk, 1995). Ascorbate oxidase is also present in vegetables, and this enzyme is inactivated by heating. However, ASA content in the cooked tissues of all vegetables decreased after boiling.

In the case of broccoli, Chinese cabbage, Japanese radish, kidney beans, onion, spinach, and tomato, boiling caused a significant decrease in the radical-scavenging activity (20–83% of fresh materials) and ASA content (10–66% of fresh materials) of their cooked tissues. There are reports of the effect of boiling on the content of individual antioxidant components in vegetables (Crozier *et al*., 1997; Price *et al*., 1997, 1998; Chuda *et al*., 1998; Hirota *et al*., 1998; Gil *et al*., 1999). Of these, Price *et al*. (1997, 1998) reported that the content of flavonol glycosides in broccoli and onion retained in the cooked tissue was 14–28% and 81–88%, respectively. In the present study, both radical-scavenging activity and ASA were detected in the cooking water (Tables 2 and 3). For spinach and tomato, the total activity in cooked tissue and cooking water was nearly the same as that in fresh materials. Therefore, the antioxidant compounds of these vegetables were stable during heating, but were leached out into the cooking water during boiling. Erdman and Klein (1982) reported that when a large volume of water was used for boiling, a higher content of ASA was found in the cooking water, which emphasizes the importance of using a small volume of water in vegetable cookery.

The decrease in total activity of cooked tissue and cooking water was observed for the boiling of Chinese cabbage, Japanese radish, kidney beans, and onion. In the cases of broccoli, cabbage, and pumpkin, the activity of cooked tissue decreased slightly, but total activity increased from 112 to 163%.

Based on the above findings, three possibilities are suggested for the increase in radical-scavenging activity of vegetables after boiling: 1) liberation of a great amount of antioxidant components due to thermal destruction of cell walls and subcellular compartments; 2) production of stronger radical-scavenging antioxidants by thermal chemical reaction; 3) suppression of the oxidation of antioxidants by thermal inactivation of oxidative enzymes. However, it is not clear to what extent each possible factor contributes to the increase of activity.

Effect of boiling time on radical-scavenging activity and on ASA content A model solution of ASA was assayed to investigate the effect of boiling time on the radical-scavenging activity and ASA content (data not shown). Both activity and ASA content decreased with boiling time. The decrease of activity depended on the decrease of ASA content, reaching approxi-

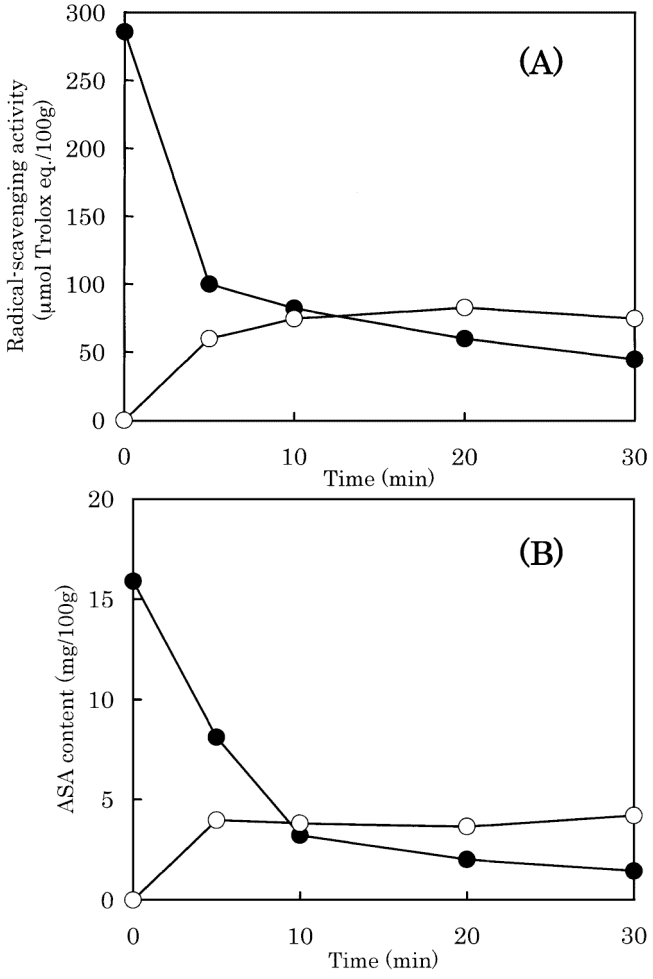

Fig. 2. Change in radical-scavenging activity and ASA content of Japanese radish during boiling. (A), radical-scavenging activity. (B), ASA content. ●, Cooked tissue; ○, Cooking water.

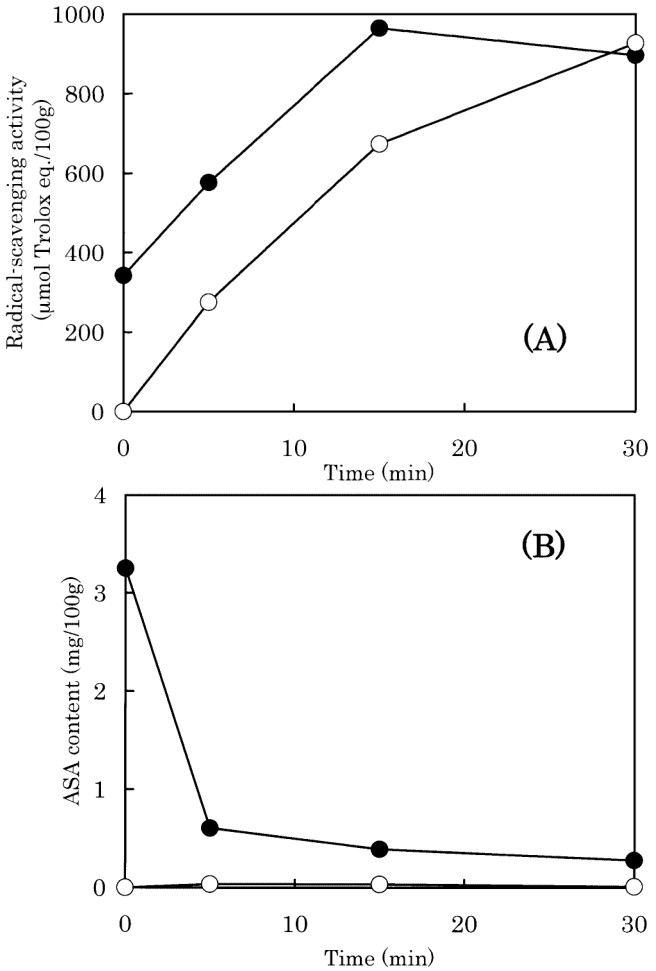

Fig. 3. Change in radical-scavenging activity and ASA content of eggplant during boiling. (A), radical-scavenging activity. (B), ASA content. ●, Cooked tissue; ○, Cooking water.

mately 60% of the initial activity after 30 min of boiling.

Three different types of vegetables, broccoli, eggplant, and Japanese radish were boiled in water for 30 min. Change in the radical-scavenging activity and ASA content of broccoli during boiling is shown in Fig. 1. Both activity and ASA content of cooked tissue decreased with boiling time while those in the cooking water increased. The activity in the cooking water was higher than that of cooked tissue after boiling for 10 min. Total activity of cooked tissue and cooking water was higher than that of fresh material.

In the case of Japanese radish, the activity and ASA content of cooked tissue also decreased with boiling time while those in the cooking water increased (Fig. 2). Total activity of cooked tissue and cooking water was only 30% of the fresh material. This is a common ingredient in traditional Japanese cooking (*Nimono*) and requires a long boiling time, so that the remaining activity and ASA of Japanese radish in *Nimono* could thus be estimated to be low.

Change in the radical-scavenging activity and ASA content of eggplant during boiling is shown in Fig. 3. The activity of cooked tissue and cooking water generally increased with boiling time, however, the ASA content in both the tissue and cooking water after the boiling was very low. After boiling for 30 min, total activity of cooked tissue and cooking water increased 4-fold, and the activity in the tissue was at the same level as that in the cooking water. Therefore, boiling of eggplant for a long time optimize its radical-scavenging activity.

Thus, these three vegetables showed different behavior of radical-scavenging activity and ASA content during boiling. This difference may be due to the differences in tissue hardness and polyphenol profile of each vegetable.

Effect of microwave heating on radical-scavenging activity and ASA content Changes in the radical-scavenging activity and ASA content of vegetables after microwave heating are shown in Table 4. The activity of asparagus, broccoli, burdock, cabbage, carrot, Chinese cabbage, eggplant, green pepper, and

Table 4. Radical-scavenging activity and ASA content of vegetables after microwave heating.

Vegetables	Time (min)	Radical-scavenging activity		ASA content		Contribution of ASA (%)[b]
		μmol Trolox eq./100 g	%[a]	mg/100 g	%	
Asparagus	1	546.0±13.8[c]	122	6.1±0.4	93	6
	3	553.6±56.5	124	3.7±0.2	55	3
	5	475.1±40.4	106	4.0±0.7	61	4
Broccoli	1	415.3±21.0	89	68.6±3.4	87	85
	3	625.8±12.7	134	60.1±3.3	76	49
	5	648.3±16.1	138	59.4±4.1	75	47
Burdock	1	334.6±21.7	68	0.7±0.0	nc[d]	1
	3	1703.3±159.4	348	0.1±0.1	nc	<1
	5	2072.5±147.1	423	0.7±0.0	nc	<1
Cabbage	1	169.4±48.9	74	27.1±3.5	72	81
	3	357.7±21.3	156	33.0±2.6	88	47
	5	383.5±17.7	168	28.6±3.0	76	38
Carrot	1	58.5±1.6	184	2.3±0.8	121	20
	3	51.9±3.4	163	1.4±0.6	72	14
	5	46.8±2.4	147	0.8±0.3	41	9
Chinese cabbage	1	158.6±10.2	114	15.9±1.9	91	51
	3	137.6±47.7	99	13.9±0.9	79	51
	5	181.6±22.1	131	12.6±0.4	72	35
Eggplant	1	185.5±17.8	54	1.1±0.1	nc	3
	3	1390.4±102.5	406	0.1±0.1	nc	<1
	5	1311.2±228.1	383	0.9±0.2	nc	<1
Green pepper	1	445.0±46.1	98	68.5±5.0	92	78
	3	777.8±32.9	172	68.1±8.3	92	45
	5	671.0±40.8	148	53.8±6.2	72	41
Japanese radish	1	192.2±14.0	67	12.0±0.6	75	32
	3	104.3±23.1	36	10.4±0.6	65	51
	5	100.5±11.0	35	8.1±1.2	51	41
Kidney beans	1	136.7±2.6	61	7.1±1.0	92	27
	3	138.2±15.2	62	6.5±0.3	85	24
	5	199.7±4.3	90	3.4±0.6	44	9
Onion	1	100.7±8.8	73	2.8±0.7	66	14
	3	72.6±6.4	53	2.1±0.8	51	15
	5	45.6±6.3	33	2.1±0.3	50	23
Pumpkin	1	127.9±17.6	47	20.3±2.8	50	81
	3	195.3±17.7	72	13.6±1.5	34	35
	5	254.0±16.3	93	18.7±1.5	46	37
Spinach	1	257.1±18.0	69	10.2±3.1	59	20
	3	246.7±33.6	66	10.4±2.7	60	21
	5	282.6±8.7	76	8.5±0.8	49	15
Tomato	1	221.5±11.0	104	13.6±2.0	87	31
	3	204.6±0.9	96	10.3±0.8	66	26
	5	245.5±19.4	115	12.9±1.0	83	27

[a] Percentage to the value for fresh materials.
[b] The contribution of ASA to radical-scavenging activity of the vegetables was calculated as percentage.
[c] The values are the means±SD for three determinations.
[d] nc, not calculated because of its low content.

tomato increased after heating for 5 min, with the highest increase being observed in burdock (4.2-fold). However, the activity of Japanese radish, kidney beans, onion, pumpkin, and spinach decreased after heating for 5 min. Nishibori and Namiki (1998) studied the effect of microwave heating of 22 vegetables on the superoxide anion radical-scavenging activity using a nitro blue tetrazolium method and reported that the activity of most vegetables decreased. Their result was inconsistent with ours. Vegetables including broccoli, carrot, green pepper, Chinese cabbage, cabbage, and tomato showed an increase of activity in our experiment. The discrepancy is probably due to the differences in cutting size of vegetables, power of the microwave oven, and/or method of radical-scavenging activity assay.

The effect of heating time on this activity is shown in Table 4. Increase was observed in asparagus and carrot, compared with the activity of fresh materials (Table 1), but the activity was decreased somewhat by long-time heating. On the other hand, the activity of Japanese radish and onion decreased with increase in heating time, and after heating for 5 min was about 35% that of fresh materials. Crozier *et al*. (1997) reported that microwave heating of onion resulted in 36% decrease of quercetin content caused by flavonoid breakdown during the heating. The main antioxidant component of onion is quercetin glycoside (Tsushida & Suzuki, 1995; Price *et al*., 1997), so the lower activity in the present experiment may be due to the decrease of quercetin content during heating. In the present study, there is no thermal data on the vegetables during cooking. However, the internal temperature of each vegetable is probably associated with inactivation of oxidative enzymes and destruction of cell walls which are responsible for radical-scavenging activity. Clarification of the correlation between this activity and thermal condition will therefore be helpful.

After microwave heating for 5 min, the ASA content was markedly decreased in all vegetables, and in carrot, kidney beans, onion, pumpkin, and spinach was more than 50% lower (Table 4). The contribution of ASA to the radical-scavenging activity was also lower after heating for this period except in Japanese radish and onion.

Finally, changes in both this activity and ASA content by boiling for 5 min were compared with those by microwave heating. From the results in Tables 2, 3, and 4, higher activity and ASA content were generally found in vegetables cooked by microwave oven. This difference can be explained by the present results that boiling of vegetables caused the release of both ASA and other antioxidants from cooked tissue into the cooking water, while microwave heating did not. Therefore, microwave heating of vegetables is a better cooking process to retain these active components in cooked tissue. When vegetables are cooked by boiling, using the cooking water in vegetable soup or stew is recommended.

In summary, as part of our studies on the importance of vegetables, we evaluated the radical-scavenging activity and ASA content and their changes with cooking. Vegetables in human diets contain a wide variety of free radical-scavenging antioxidants such as flavonoids and phenolic acids as well as ASA. Further work is in progress in our laboratory to elucidate the effect of cooking on vegetable polyphenol content.

Conclusions

The radical-scavenging activity of some vegetables was increased in spite of the loss of ASA content after cooking. The activity and ASA content of those cooked by microwave oven were generally higher than of those cooked by boiling, because microwave heating does not stimulate the release of either ASA or other antioxidants from cooked tissue into cooking water. Microwave heating of vegetables is thus the better of the two processes to retain these active components in cooked tissue. Use of the cooking water of boiled vegetables in vegetable soup or stew is recommended. These findings have identified the optimum method of cooking vegetables which results in the highest retention of their radical-scavenging activity and assures a higher quality food for the maintenance of human health.

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