

## Heat Processing of Edible Plants Grown in Korea Has Differential Effects on Their Antioxidant Capacity in Bovine Brain Homogenate

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### Abstract

Oxidant radicals are implicated as a causal factor in the pathogenesis of neurobiological disorders and neurodegenerative diseases. The objective of this study was to investigate the antioxidant activity of edible plants against oxidative stress in bovine brain tissue. Fifty five kinds of edible plants grown in Korea were dried either by freeze-drying or hot-air drying (70°C), and evaluated for their antioxidant activity by measuring TBARS (thiobarbituric acid-reactive substances) in brain homogenates subjected to Fe<sup>+2</sup>-mediated lipid peroxidation with or without the addition of botanical extracts. Heat-drying decreased the antioxidant activity of most plant extracts by 10~81%, compared with freeze-drying. However, *Arunucus americanus*, *Ligularia stenocephala*, *Artemisia princeps* var. *orientalis*, *Petasites japonicus* and *Aster scaber* showed very strong antioxidant activities regardless of processing, with or without heat treatment. The IC<sub>50</sub> values of the methanol extracts from these edible plants were in the range of 0.093~0.379 mg/mL, which was lower than that of ascorbic acid (0.79 mg/mL). Thermal processing of some edible plants enhanced their antioxidant activity.

**Key words:** edible plant, brain tissue, antioxidant activity, TBARS

### INTRODUCTION

Oxidative damage of brain tissue had been proposed to be a primary factor in the aging process as well as the etiology of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (1-4). As the elderly population increases, the prevalence of these age-related diseases is also likely to increase (5). Extensive evidence exists suggesting that lipid peroxidation is an important mechanism of neurodegeneration (6). The brain membrane is rich in polyunsaturated fatty acids, which are susceptible to oxidant radicals, while having a relative paucity of protective mechanisms. Polyunsaturated fatty acids or fatty acyl side chains in biological membranes can be peroxidized in the presence of oxidative enzymes, or in their absence by exposure to reactive oxygen species and transition metal ions which can initiate free radical chain reactions (7). The free radical reactions can result in lipid peroxidation which can be deleterious for membrane permeability and integrity, and can produce compounds that are toxic to humans. Numerous investigators have studied the antioxidant properties of plant extracts obtained from vegetables, fruits and spices (8-15). The effects of processing on the antioxidant activities of some vegetables (16), potatoes (10), tomatoes and coffee (17-19), and sweet corn (20) have been reported. However,

very few studies have investigated the antioxidant protection of vegetables against lipid peroxidation in brain tissue. Moreover, no information on the effects of heat treatment on the antioxidant capacity of edible wild vegetables is available, although most wild vegetables are typically consumed after heat treatment.

The objective of this study was to evaluate the antioxidant activities of edible plants grown in Korea, and to evaluate the effect of heat treatment on the antioxidant activities.

### MATERIALS AND METHODS

#### Materials

Sodium hydroxide, (NaOH) (Aldrich Chemical Company, ACS Reagent); ferric sulfate, [Fe(III)<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>], and thiobarbituric acid, (TBA) and 1,1,3,3,-tetraethoxypropane (Sigma Chemical Co, 98% pure) were used. Methanol, hexane and deionized water were used to prepare solutions.

#### Edible plants samples

Wild vegetables, as listed in Table 1, were obtained in June of 2001 at the Wild Vegetable Experiment Station, Kangwon Agricultural Research and Extensions Services, Kangwon-Do, Korea, at an altitude of 600~700 m. Cultivated vegetables, as listed in Table 1, were purchased from April 2001 to September of 2001 from a local organic food

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**Table 1.** Antioxidant activities of edible plants processed with freeze-drying on the lipid peroxidation in brain homogenate

Common name	Scientific name	Part used	Dry weight (mg)	Total ex. weight (mg)	Extract rate (%)	Antioxidant activity (%)
Nungaesengma	<i>Aruncus americanus</i>	leaf	304.8	96.7	31.7	100.0
Kondalbi	<i>Ligularia stenocephala</i>	leaf	304.2	52.0	17.1	100.0
Mugwort	<i>Artemisia princeps</i> var. <i>orientalis</i>	leaf	300.0	83.0	27.7	100.0
Bud of aralia	<i>Aralia elata</i>	Stem & bud	300.5	55.2	18.4	100.0
Sedum, raw	<i>Sedum sarmentosum</i>	leaf	301.7	13.0	4.3	100.0
Burdock	<i>Articum lappa</i>	root	302.1	71.5	23.7	100.0
Water dropwort	<i>Oenanthe stolonifera</i>	leaf	302.0	67.2	22.3	100.0
Butterbur	<i>Petasites japonicus</i>	stem	303.4	94.0	31.0	95.1
Rough aster	<i>Aster scaber</i>	leaf	304.7	35.8	11.7	94.8
Onion	<i>Allium cepa</i>	skin	302.1	71.5	23.7	91.5
Butterbur	<i>Petasites japonicus</i>	leaf & stem (young)	300.5	22.8	7.6	90.4
Plantain	<i>Plantago asiatica</i>	leaf	302.0	30.6	10.1	89.1
Eggplant	<i>Solanum melongena</i>	fruit	300.0	119.4	39.8	87.8
Lollo Rossa	<i>Lactuca sativa</i>	leaf	304.1	41.8	13.7	81.5
Perilla leaf	<i>Perilla frutescens</i>	leaf	304.5	18.0	5.9	80.9
Sowthistle	<i>Ixeris dentata</i>	leaf	301.7	13.0	4.3	79.7
Bud of aralia	<i>Aralia continentalis</i>	stem & bud	300.0	60.6	20.2	79.3
Water dropwort	<i>Oenanthe stolonifera</i>	stem	300.8	120.9	40.2	72.6
Paprica	<i>Capsicum annuum</i>	fruit (green)	302.0	134.5	44.5	71.9
Chicon	<i>Cichorium intybus</i>	leaf	303.0	42.9	14.2	70.8
Coriander	<i>Coriandrum sativum</i>	leaf	300.0	62.8	20.9	67.2
Cherry	<i>Prunus serrulata</i> Lindley	fruit	300.0	7.3	2.4	67.1
Wild garlic	<i>Allium victorialis</i>	leaf	301.0	75.1	25.0	66.5
Wild plant	<i>Cirsium setidens</i>	leaf	301.4	39.8	13.2	61.9
Radish	<i>Raphanus sativus</i>	sprout	307.0	87.4	28.5	60.3
Chicory	<i>Cichorium intybus</i>	leaf	302.8	34.6	11.4	60.1
Green Batavia lettuce	<i>Lactusa sativus</i>	leaf (green)	308.2	40.9	13.3	55.5
Lotus root	<i>Nelumbo nucifera</i>	root	300.0	67.8	22.6	51.0
Potato	<i>Solanum tuberosum</i>	skin	300.0	23.8	7.9	48.0
Dang Gui	<i>Angelica uchiyamana</i>	leaf	301.6	49.3	16.3	45.4
Bausae	<i>Basella rubra</i>	leaf	303.4	67.4	22.2	43.6
Mustard	<i>Brassica junacea</i>	leaf (red)	301.4	48.7	16.2	40.5
Soybean	<i>Glycine max</i>	sprout	305.1	37.9	12.4	39.5
Cham Na Mul	<i>Pimpinella brachycarpa</i>	leaf	303.0	22.6	7.5	38.9
O Su ri	<i>Heracleum moellendorffi</i>	leaf	300.4	42.3	14.1	38.3
Kale	<i>Brassica oleracea</i>	leaf	304.0	37.4	12.3	36.8
Apricot	<i>Prunus armeniaca</i>	fruit	300.0	192.6	64.2	36.4
Mustard	<i>Brassica Juncea</i>	leaf (green)	300.6	29.9	9.9	34.4
Tomato	<i>Lycopersicon esculentum</i>	fruit	300.0	173.5	57.8	29.1
Parsley	<i>Petrocelinum sativum</i>	leaf	304.1	66.6	21.9	27.8
Garlic	<i>Allium sativum</i>	root	301.2	90.3	30.0	27.8
Onion	<i>Allium cepa</i>	root	302.1	53.8	17.8	27.7
Carrot	<i>Daucus carota</i>	root	303.7	89.0	29.3	27.2
Amaranth	<i>Amaranthus mangostanus</i>	leaf	303.0	26.0	8.6	25.5
Welsh onion	<i>Allium fistulosum</i>	root	302.2	33.7	11.2	14.4
Kidney beans	<i>Phaseolus vulgaris</i>	seed	300.0	24.1	8.0	10.3
Leaf Parsley	<i>Petroselinum crispum</i>	leaf	301.7	51.9	17.2	8.8
Bakyuncho	<i>Opuntia ficus-indica</i> var. <i>saboten</i>	fruit	309.6	117.9	38.1	4.6
Crown daisy	<i>Chrysanthemum coronarium</i>	leaf (young)	300.2	42.0	14.0	2.0
Pumpkin	<i>Cucurbita moschata</i>	leaf	307.1	103.8	33.8	2.0
Cham Na Mul	<i>Pimpinella calycina</i>	leaf	303.0	22.6	7.5	1.5
Sweet pepper	<i>Capsicum angulosum</i>	fruit (red)	302.9	160.4	53.0	0.0
Sweet pepper	<i>Capsicum annunm</i>	fruit (green)	305.4	137.7	45.1	0.0
Quarri pepper	<i>Capsicum annuum</i>	fruit (green)	301.9	27.1	9.0	0.0
Beets	<i>Beta vulgaris</i>	root	301.9	71.9	23.8	0.0

market in Taejon.

### Preparation of vegetable extract and solvent fractionation

Vegetables were washed, drained, weighed, and then dried. Some of each vegetable was processed by freeze-drying and hot air (70°C)-convection drying. Dried samples were ground into a powder to pass through a 200 mesh sieve. Powdered samples (1 g) were immersed in absolute methanol (25 mL) and stored in the dark (15°C) for 3 days after which the methanol fraction was collected. The extraction was repeated three times and solvents were removed by a rotary evaporator. Chlorophyll was removed by extracting the residue with hexane. This chlorophyll free residue was allowed to stand at room temperature under vacuum to obtain a solvent free powder which was stored at -24°C. After rotary evaporation, the residue was dissolved in methanol and the solvent fraction assayed for antioxidant activity. Solvent fractionations of the methanol extracts from edible plants were performed as shown in Fig. 1.

### Preparation of brain homogenate

Immediately after an ox was slaughtered, it was decapitated and its brain surgically removed. The brain was washed in cooled 0.15 M NaCl, kept on ice, and subsequently blotted on filter paper. The brain tissue was then homogenized for 2 min in a glass-teflon homogenizer with equal volumes of brain tissue and cold 10 mM phosphate buffer. The homogenates were used to analyse the thiobarbituric acid reacting substances (TBARS) content.

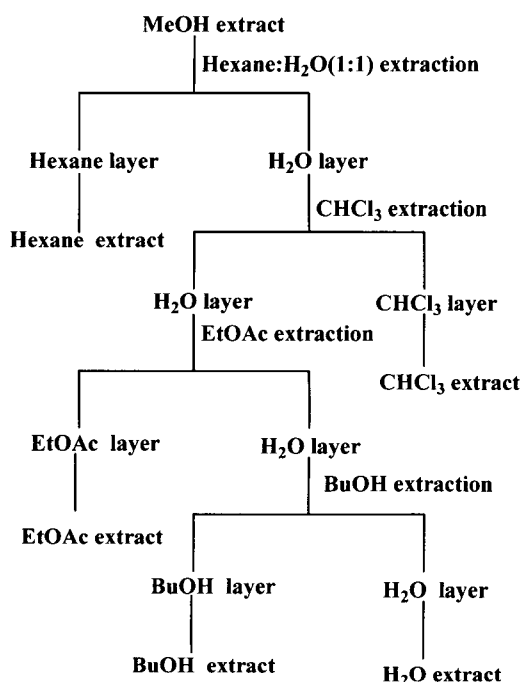


Fig. 1. Scheme of solvent fractionation.

### Biological lipid peroxidation assay

Fe<sup>+2</sup>-mediated lipid peroxidation in brain was induced with 0.2 mM Fe<sup>+2</sup> and 25 M ascorbic acid *in vitro* as described by Lee (21). The extent of lipid peroxidation was assayed as TBARS according to the method described by Bidlack and Tappel (22). Brain homogenates containing Fe<sup>+2</sup> and ascorbic acid, with or without plant extract, were placed in a shaking water bath at 37°C for 30 min; equal volumes of 15% trichloroacetic acid (TCA) and 0.75% thiobarbituric acid (TBA) were then added. The reaction mixtures were heated in boiling water for 15 min, kept in ice for 5 min, and then centrifuged for 10 min at 3000 rpm to separate corpuscolate particles. The absorbance of the supernatant was read in a spectrophotometer ( $\lambda = 533$  nm). Calibration was performed using a malondialdehyde standard prepared by hydrolysis of 1,1,3,3-tetraethoxypropane (23). Extract concentrations at which lipid peroxidation was inhibited by 50% inhibition (IC<sub>50</sub>) were derived by interpolation of a log concentration vs inhibition plot using eight concentrations of the extract, spanning the 50% inhibition point. All experiments were run in triplicate.

The antioxidant activity was expressed as the percentage decrease of TBARS relative to the control using the following equation.

$$\text{Antioxidant activity (\%)} = \left( 1 - \frac{A(\text{brain homogenate} + \text{ascorbate} + \text{Fe}^{2+} + \text{sample}) - A(\text{brain homogenate} + \text{sample})}{A(\text{brain homogenate} + \text{ascorbate} + \text{Fe}^{2+}) - A(\text{brain homogenate})} \right) \times 100$$

\*Sample: vegetable extracts.

A (brain homogenate): Absorbance from the incubation containing brain homogenate only.

A (brain homogenate + sample): Absorbance from the incubation containing brain homogenate and sample.

A (brain homogenate + ascorbate + Fe<sup>2+</sup>): Absorbance for the incubation containing brain homogenate, ascorbate and Fe<sup>2+</sup>.

A (brain homogenate + ascorbate + Fe<sup>2+</sup> + sample): Absorbance from the incubation containing brain homogenate, ascorbate, Fe<sup>2+</sup> and sample.

## RESULTS AND DISCUSSION

After freeze-drying, *Aruncus americanus*, *Ligularia ste-noccephala*, *Artemisia princeps* var. *orientalis*, *Aralia elata*, *Sedum sarmentosum*, *Arctium lappa*, *Oenanthe stolonifera* (leaf), *Petasites japonicus*, *Aster scaber*, *Allium cepa* (skin) and *Plantago asiatica* showed a very strong antioxidant activity (Table 1). In fact, in their presence, Fe<sup>+2</sup>-induced TBARS formation was almost completely suppressed (more than 90%) in brain homogenates. Freeze-dried *Solanum melongena*, *Lactuca sativa*, *Perilla frutescens*, *Ixeris*

**Table 2.** Antioxidant activities of edible plants processed with hot-air drying (70°C) on the lipid peroxidation in brain homogenate

Common name	Scientific name	Part used	Dry weight (mg)	Total ex. weight (mg)	Extract rate (%)	Antioxidant activity (%)
Butterbur	<i>Petasites japonicus</i>	leaf & stem (young)	304.5	37.6	12.3	100.0
Rough aster	<i>Aster scaber</i>	leaf	301.5	33.5	11.1	100.0
Mugwort	<i>Artemisia princeps</i> var. <i>orientalis</i>	leaf	317.2	39.2	12.4	100.0
Nungaesungma	<i>Arunucus americanus</i>	leaf	306.9	73.2	23.9	100.0
Coriander	<i>Coriandrum sativum</i>	leaf	302.2	1.1	0.4	100.0
Pumpkin	<i>Cucurbita moschata</i>	leaf	302.2	41.3	13.7	94.5
Plantain	<i>Plantago asiatica</i>	leaf	302.3	36.6	12.1	90.1
Kondalbi	<i>Ligularia stenocephala</i>	leaf	309.1	121.5	39.3	86.5
Bud of aralia	<i>Aralia continentalis</i>	Stem & bud	303.4	11.9	3.9	83.6
Lollo Rossa	<i>Lactuca sativa</i>	leaf	301.0	39.6	13.2	79.3
Potato, skin	<i>Solanum tuberosum</i>	skin	300.9	21.3	7.1	79.2
Cham Na Mul	<i>Pimpinella brachycarpa</i>	leaf	305.3	24.7	8.1	78.2
Bud of aralia	<i>Aralia elata</i>	Stem & bud	301.4	40.7	13.5	74.1
Burdock	<i>Artium lappa</i>	root	303.7	58.6	19.3	73.4
Butterbur, stem	<i>Petasites japonicus</i>	stem	306.5	105.5	34.4	73.0
Green Batavia lettuce	<i>Lactusa sativus</i>	leaf	300.4	34.0	11.3	63.6
Onion, skin	<i>Allium cepa</i>	skin	303.3	9.1	3.0	62.4
Sowthistle	<i>Ixeris dentata</i>	leaf	302.9	12.6	4.2	60.3
Tomato	<i>Lycopersicon esculentum</i>	fruit	304.8	129.8	42.6	54.4
Crown daisy	<i>Chrysanthemum coronarium</i>	leaf	302.3	81.1	26.8	53.7
Wild plant	<i>Cirsium setidens</i>	leaf	306.0	29.8	9.7	48.0
Sedum, raw	<i>Sedum sarmentosum</i>	leaf	303.3	128.9	42.5	47.1
Radish	<i>Raphanus sativus</i>	sprout	302.1	98.4	32.6	45.1
Wild garlic	<i>Allium victorialis</i>	leaf	307.7	66.0	21.4	44.1
Beets	<i>Beta vulgaris</i>	root	301.3	73.9	24.5	35.0
Leaf parsley	<i>Petroselinum crispum</i>	leaf	304.3	40.9	13.4	29.4
Quarri green pepper	<i>Capsicum annuum</i>	fruit	300.8	62.2	20.7	25.2
Water dropwort	<i>Oenanthe stolonifera</i>	leaf	300.4	40.3	13.4	24.2
Bakyuncho ficus-indica	<i>Opuntia ficus-indica</i> var. <i>saboten</i>	fruit	304.7	97.8	32.1	22.1
Dang Gui	<i>Angelica uchiyamana</i>	leaf	304.5	26.2	8.6	22.1
Sweet pepper	<i>Capsicum angulosum</i>	fruit (red)	302.0	116.7	38.6	20.4
O Su ri	<i>Heracleum moellendorffi</i>	leaf	310.3	166.4	53.6	20.4
Paprika	<i>Capsicum annuum</i>	fruit (red)	303.4	148.7	49.0	19.7
Kale	<i>Brassica oleracea</i>	leaf	303.7	42.7	14.1	17.4
Chicory	<i>Cichorium intybus</i>	leaf	301.2	30.8	10.2	16.0
Parsley	<i>Petrocelinum sativum</i>	leaf	300.3	39.5	13.2	15.8
Water dropwort	<i>Oenanthe stolonifera</i>	stem	300.8	143.8	47.8	15.6
Sweet pepper	<i>Capsicum annuum</i>	fruit (green)	302.8	97.1	32.1	14.9
Bausae	<i>Basella rubra</i>	leaf	302.5	31.7	10.5	13.1
Perilla leaf	<i>Perilla frutescens</i>	leaf	300.4	30.7	10.2	12.7
Cherry	<i>Prunus serrulata</i> Lindley	fruit	303.6	116.0	38.2	8.8
Onion	<i>Allium cepa</i>	root	301.7	136.5	45.2	7.8
Eggplant	<i>Solanum melongena</i>	fruit	302.0	104.0	34.4	6.0
Carrot	<i>Daucus carota</i>	root	301.1	93.4	31.0	5.7
Chicon	<i>Cichorium intybus</i>	leaf	300.1	58.2	19.4	3.8
Lotus	<i>Nelumbo nucifera</i>	root	300.2	18.0	6.0	2.5
Soybean	<i>Glycine max</i>	sprout	301.8	259.2	85.9	2.4
Garlic	<i>Allium sativum</i>	root	302.0	8.4	2.8	0.2
Welsh onion	<i>Allium fistulosum</i>	root	304.5	65.2	21.4	0.0
Mustard	<i>Brassica juncea</i>	leaf (green)	303.5	58.8	19.4	0.0
Mustard	<i>Brassica juncea</i>	leaf (red)	301.7	32.3	10.7	0.0
Apricot	<i>Prunus armeniaca</i>	fruit	301.4	131.6	43.7	0.0
Amaranth	<i>Amaranthus mangostanus</i>	leaf	301.3	30.3	10.1	0.0
Kidney beans	<i>Phaseolus vulgaris</i>	seed	303.5	126.9	41.8	0.0

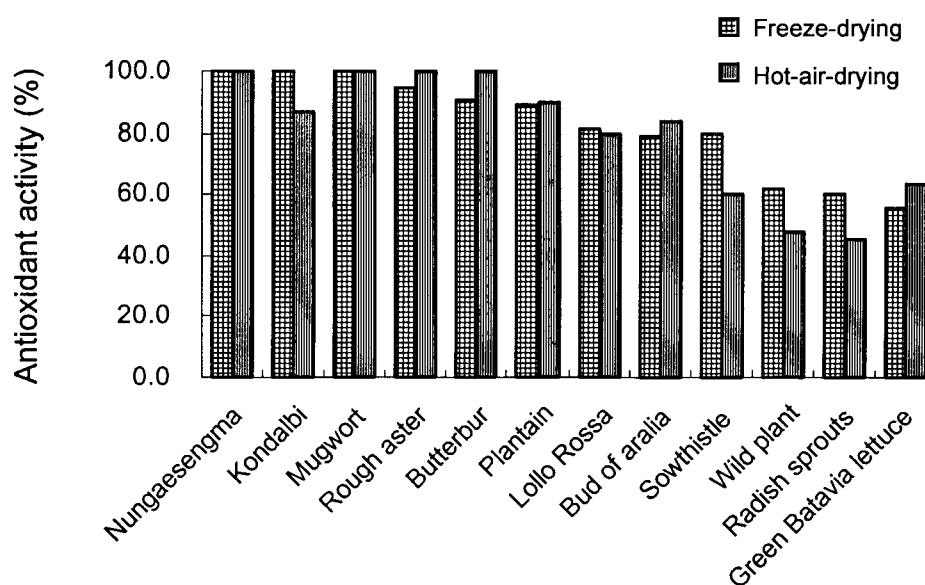
*dentata*, *Aralia continentalis*, *Oenanthe stolonifera* (stem), *Capsicum annum*, *Cichorium intybus*, *Lactuca, sativa*, *Perilla frutescens*, *Ixeris dentata*, *Aralia continentalis*, *Oenanthe stolonifera*, *Capsicum annum*, *Chicorium intybus*, *Coriandrum sativum*, *Prunus serrulata* Lindley, *Allium victorialis*, *Cirsium setidens*, *Raphanus sativum* (sprout), *Lactuca sativus* and *Nelumbo nucifera* all inhibited lipid peroxidation by more than 50%. *Capsicum annum*, *Beta vulgaris*, *Petroselinum crispum*, *Cucurbita moschata* (young leaf), *Pimpinella calycina*, *Chrysanthemum coronarium* and *Opuntia ficus-indica* var. *saboten* had very low antioxidant activity. When the vegetables showing strong antioxidant activity were treated with heat, most had decreased antioxidant activities in the brain tissues by 10~81% (Table 2), compared with freeze-drying. Heat drying resulted in a decrease of more than 50% in *Sedum sarmentosum*, *Oenanthe stolonifera* (stem), *Perilla frutescens*, *Allium cepa*, *Solanum melongena*, *Cichorium intybus*, and *sedum*, whereas the decrease was less than 20% of in *Ligularia stenocephala*, *Ixeris dentate*, *Raphanus sativus* (sprout), *Allium victorialis*, *Cirsium setidens*, *Heraclium mollendorffi*, *Artium lappa*, *Aralia elata*, *Petasites japonicus*, *Petrocelinum sativum*. However, *Aruncus americanus*, *Ligularia stenocephala*, *Artemisia princeps* var. *orientalis*, *Petasites japonicus* and *Aster scaber* showed very strong protective activities (>90%) regardless of heat treatment, as shown in Fig. 2.

When vegetables were dried with hot-air (70°C), very strong antioxidant activities (>90%) against lipid peroxidation were observed in *Aruncus americanus*, *Artemisia princeps* var. *orientalis*, *Petasites japonicus*, *Aster scaber*, *Coriandrum sativum*, *Cucurbita moschata* and *Plantago*

*asiatica*. Vegetables retaining more than 50% of their antioxidant activity after heat treatment were *Ligularia stenocephala*, *Aralia continentalis*, *Lactuca sativa*, *Solanum tuberosum* (skin), *Pimpinella brachycarpa*, *Aralia elata*, *Artium lappa*, *Petasites japonicus*, *Lactuca sativus*, *allium cepa* (skin), *Ixeris dentate*, *Lycopersicon esculentum* and *Chrysanthemum coronarium*. Moreover, among the hot air-dried vegetables mentioned above, antioxidant activities of *Cucurbita moschata* (leaf) and *Chrysanthemum coronarium* were enhanced by up to 50% above their freeze-dried counterparts. These results suggest that new material with antioxidant activity may be formed in these vegetables during heat processing. The IC<sub>50</sub> values of vegetables possessing strong antioxidant activities (more than 90%) in freeze-drying or hot-air drying are shown in Table 3. Their IC<sub>50</sub> values ranked between 0.049 and 0.623 mg/mL. These values are lower than that of the control antioxidant, ascorbic acid (0.790 mg/mL). *Aruncus america-*

**Table 3.** IC<sub>50</sub> value of methanol extract of edible plants

Common name	Scientific name	IC <sub>50</sub> (mg dry wt/mL)
Mugwort	<i>Artemisia princeps</i> var. <i>orientalis</i>	0.093
Rough aster	<i>Aster scaber</i>	0.136
Onion	<i>Allium cepa</i> (skin)	0.219
Butterbur	<i>Petasites japonicus</i>	0.260
Nungaesengma	<i>Aruncus americanus</i>	0.261
Water dropwort	<i>Oenanthe stolonifera</i> (leaf)	0.284
Kondalbi	<i>Ligularia stenocephala</i>	0.379
Sowthistle	<i>Ixeris dentata</i>	0.403
Burdock	<i>Artcium lappa</i>	0.559
Sedum, raw	<i>Sedum sarmentosum</i>	0.623
Vitamin C	Ascorbic acid	0.790



**Fig. 2.** Native edible plants showing great antioxidant activity both in freeze drying and in hot-air drying.

*nus*, *Artemisia asiatica*, *Petasites japonicus* and *Aster scaber* required very low concentrations for 50% inhibition of lipid peroxidation of brain tissue. *Artemisia princeps* var. *orientalis* has been used, for centuries as a Chinese medicinal plant rather than as a vegetable. *Aster scaber* (compositae) is widespread and cultivated as a culinary vegetable in Korea. *Aster* species have also been used to treat bruises, snakebite, headache, and dizziness in traditional Chinese medicine (24).

The methanol extract from *Aster scaber* was fractionated systematically with solvents. The IC<sub>50</sub> values of the solvent fractions of *Aster scaber* were 0.028~0.142 mg/mL. The IC<sub>50</sub> value of the butanol fraction of methanol extract of *Aster scaber* was 0.028 mg/mL, providing the strongest *in vitro* protection against lipid peroxidation in brain tissue. Potent antioxidants in *Aster scaber* are reported to be triterpene glycosides (25,26) and caffeoyl quinic acid (27).

Vegetables could be grouped according to the effect of processing on their antioxidant activities by more than 30%; Group I vegetables had higher activities when freeze-dried rather than hot air dried, as shown in Fig. 3; group II vegetables had similar activity regardless of drying method, and are shown in Fig. 2; group III vegetables had higher activity when hot air dried than when freeze-dried, as shown in Fig. 4.

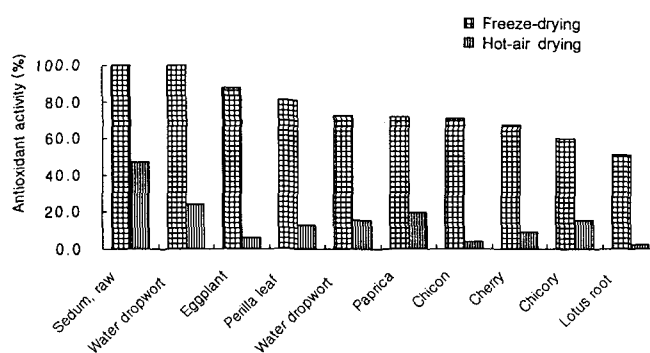


Fig. 3. Native edible plants showing greater antioxidant activity in freeze-drying than in hot-air drying.

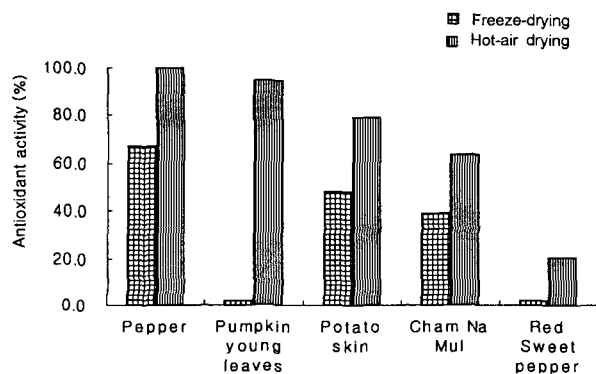


Fig. 4. Native edible plants showing greater antioxidant activity in freeze-drying than in hot-air drying.

Results of group I were expected, because antioxidant components in most vegetables such, as ascorbic acid and flavonoids, are water-soluble and heat-unstable. The extent of decreases in antioxidant activity was reported to be affected by several factors such as heating time and cooking methods. The longer the heating time, the greater the loss in antioxidant activity of vegetable juices (16). Cooking procedures such as boiling (15 min), microwaving (at 800 W, for 1.3 min, in water), and frying (for 2.5~3 min in sunflower oil) resulted in a lowered conjugated quercetin content in tomatoes, which was attributed to flavonoid breakdown and/or to the extraction of conjugated quercetin by hot water and sunflower oil (28).

Group II vegetables maintained their antioxidant activities after heating at 70°C. The preservation of antioxidant activities in Group II vegetables, after dry-heat treatment at 70°C, can be attributed to the presence of thermostable phytochemicals. These phytochemicals may not be easily broken down under such a mild condition as 80°C (18,19). Lavelli et al. (18) reported that the antioxidant activity of air-dried tomatoes (80°C) was not decreased, compared to that of fresh tomatoes, because carotenoid levels were substantially unchanged upon drying, and phenolic reducing compounds increased, even though the drying process decreased the ascorbic acid content. Various cooking or processing methods can affect the antioxidant activity of vegetables. Stewing tomatoes (8 min) was reported to retain the levels of individual carotenoids (29). Pasteurization (121°C, 42 s) and canning of tomato were shown to cause no damage to  $\beta$ -carotene (30). Since Nice et al. (31) reported that bound phenolic compounds exhibit a heat-stable antioxidant activity in peas, group II vegetables may also contain such bound phenolic antioxidants. Further research is needed to further elucidate the mechanism of antioxidant stability in foods.

Although the loss of nutrients as a consequence of food processing has been widely documented, up to now very little data has been available on increases in antioxidant activity following heat processing. Heat treatment enhances the antioxidant activity of lignans in sesame seeds due to structural changes. In the case of Group III vegetables, we suppose that heat treatment increases antioxidant activity or decreases prooxidant activity. It has been reported that food processing, such as cooking or grinding, might improve lycopene bioavailability by breaking down cell walls (32,33). Dewanto et al. (19) reported that thermal treatment, at 88°C, increased the total antioxidant activity of tomatoes by increasing the bioaccessible lycopene content while leaving the total phenolics and total flavonoids unchanged. Also, it was reported that thermal processing of sweet corn (115°C for 25 min) elevated the

total antioxidant activity by 44% and increased phytochemical content, such as ferulic acid by 550% and total phenolics by 54%, despite a loss of 25% vitamin C. Most antioxidant activity in edible plant comes from the combination of phytochemicals, such as phenolics and flavonoids (34). Phenolic acids occur in plants as metabolic intermediates and may accumulate in the vacuoles (35). Thermal processing may release more bound phenolic acids by breaking down cellular constituents.

Although disruption of cell walls releases oxidative and hydrolytic enzymes that can destroy antioxidants in fruits and vegetables (35), thermal processing at 80°C deactivates these enzymes thereby avoiding the destruction of phenolic acids. Decreases in prooxidant activity was addressed by Gazzani et al. (16) who proposed that prooxidant activity in some vegetable juices is due to peroxidase which is inactivated at high temperature because it, like most oxidants, is thermo-labile.

There are several possible explanations for the increased antioxidant activity in the Group III vegetables subjected to heat. First, it may be due to an increase in the bioaccessible phytochemicals as a consequence of an increased release of phytochemicals from the matrix making them more susceptible to extraction. Second, the increased activity could be due to chemical changes resulting in new or more active antioxidants. Thirdly, the increased antioxidant activity could be due to the additive and synergistic effects of other phytochemicals such as phenolics and flavonoids (34).

Our results demonstrated that although antioxidant activity is decreased by heat treatment in most plants, it is increased in others; although the antioxidant content was not measured. The observations of enhanced antioxidant activity in some edible plants due to thermal processing are contrary to the notion that hot-air dried vegetables always have lower nutritional value than freeze-dried ones. This research contributes important data for evaluating optimal processing methods, using cost/benefit analysis, in the food processing and nutraceutical industries.

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