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Effect of Thermal Treatment on Radical-scavenging Activity of Some Spices

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Changes in the radical-scavenging activities and the total phenol content of sixteen spices (clove, allspice, cinnamon, nutmeg, mustard, cumin, ginger, fennel, fenugreek, black pepper, red pepper, mace, coriander, turmeric, cardamom and white pepper) were determined for different heating times (1, 3 and 6 h) at 100℃. Most of the spices showed high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity (4–1353 μmol Trolox eq./g), peroxy radical-scavenging activity (31–1019 μmol Trolox eq./g), and total phenol content (5–1267 μmol gallic acid eq./g). Clove was found to have the highest radical-scavenging activity followed by allspice and cinnamon. After heating, both DPPH and peroxy radical-scavenging activities as well as the total phenol content increased in most of the spices. A distinct increase in the activities was found in some spices such as black pepper, red pepper and turmeric. A high correlation coefficient was found between the total phenol content and peroxy radical-scavenging activity.

Keywords: radical-scavenging activity, spices, phenol, thermal treatment

Introduction

Free radicals (superoxide, nitric oxide, hydroxyl radicals, *etc.*) and other reactive species (hydrogen peroxide, peroxynitrile, hypochlorous acid, *etc.*) are produced in the body, primarily as a result of metabolism. These species cause chronic diseases such as cancer, cardiovascular diseases, diabetes, *etc.* (Halliwell *et al.,* 1992; Aruoma, 1994). Free radicals create chain reactions, which cause cell membrane damage, DNA mutation, lipid and protein damages, and immune cell damage and cell death. Natural antioxidants are known to scavenge free radicals, enhance the immune system, prevent diseases and improve general health and life quality. Epidemiological studies show an inverse correlation between cardiovascular disease risk and dietary antioxidant consumption (Waring, 2001). Well known antioxidants include a number of enzymes (superoxide dismutase, catalase, glutathione peroxidase, *etc.*), vitamin C, vitamin E, carotenoids, phenolic compounds, *etc.* Phenolic compounds are the major components in spices. Phenolic compounds act as antioxidants to scavenge reactive oxygen species and to chelate metals. Active components of spices such as curcumin (turmeric), capsaicin (red pepper), eugenol (clove), linalool (coriander), zingerone (ginger) and cuminaldehyde (cumin) inhibit lipid peroxidation (Nagababu and Lakshmaiah, 1992; Noguchi *et al.,* 1994; Reddy and Lokesh, 1992). Anderson *et al.* (2004) reported that cinnamon may act as an antioxidant, potentiate insulin action, and may be beneficial in the control of glucose intolerance and diabetes. Curcumin acts as an anti-carcinogen and anti-mutagenic agent (Nagabhushan and Bhide, 1987).

Spices are commonly used in a raw chopped form or a ground paste in various Asian foods. Dishes containing spices, such as curries, are usually cooked at around 100℃. There are many studies regarding antioxidative activity of spices. However, very few studies on thermal cooking of aqueous systems have been published, since the active components of spices are fat-soluble. Shobana and Naidu (2000) evaluated antioxidant activity of spices using water and alcohol (1:1) as the extraction solvent. The water and ethanol extracts of clove have antioxidative potentiality such as free radical-scavenging, superoxide anion radical-scavenging and metal chelating activities (Gulçin *et al.,* 2004). The water extract and the ethanol extract of fennel showed 91.6% and 98.6% inhibition of lipid peroxidation, respectively (Oktay *et al.,* 2003). In this study, we evaluated the change in the antioxidant activity of spice powders mixed with 20% ethanol during thermal cooking. Most dishes containing spices are boiled in water for a long time; however, active components of spices are barely soluble in water. So this study was carried out in a 20% ethanol solution as a model system of boiling.

Spices have been used as important constituents of food from the past for preservation and tasting. However, investigations pertaining to spices lag behind those into other foods such as vegetables, fruits, herbs, *etc.* Spices are expected to not only be a source of flavor and increase the palatability of the dish, but they also are expected to be a source of natural antioxidants. Spices are usually consumed after thermal cooking. Therefore, radical-scavenging activity of spices may be affected by

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thermal cooking. There have been few studies regarding the effect of thermal treatment of spices. The effect of thermal treatment on radical-scavenging activity of spices has not been studied fully. So, the change in the radical-scavenging activity of spices after thermal treatment needs to be evaluated.

In this study, we investigated the radical-scavenging activity of spices before heating and the changes of radical-scavenging activity of spices after heating for a long time. The changes in the total phenol content of spices after thermal cooking were also evaluated.

Materials and Methods

Reagents Tris(hydroxymethyl)aminomethane (Tris), dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 2,2′-Azobis (2- amidinopropane) dihydrochloride (AAPH) and 2′-deoxyguanosine (2′-dG) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ethylenediaminetetraacetic acid disodium salt (EDTA) and 6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). All organic solvents were of HPLC grade.

This study was done with sixteen kinds of spices. Red pepper (*Capsicum annum* L.), turmeric (*Curcuma longa* L.), white pepper (*Piper nigrum* L.), black pepper (*Piper nigrum* L.), cardamom (*Elettaria cardamomum* MATON), allspice (*Pimenta dioica* L.), ginger (*Zingiber officinale* L. ROSCOE), mustard (*Brassica alba* L. BOISS), cinnamon (*Cinnamomum zeylanicum* BERYN), fennel (*Foeniculum vulgare* MILL), fenugreek (*Trigonella foenumgraecum* L.), cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.), clove (*Eugenia caryophyllata* THUNB), nutmeg (*Myristica fragrans* HOUTT) and mace (*Myristica fragrans* HOUTT). These spices were provided by House Foods Co. (Osaka, Japan).

Thermal treatment Each spice (1 g) was thoroughly mixed with 20% aqueous ethanol (20 mL) in a tight-capped test tube to prevent loss of active components by evaporation. Then the test tube was heated at 100℃ for 1, 3 and 6 h in a thermal controlled oven. One treatment was also done without heating as a control experiment.

Extraction After thermal treatment, the tube was allowed to cool, then the active compounds were extracted twice with 20 ml of 20% aqueous ethanol by shaking for 30 min and centrifuging at 3,000× *g* for 20 min at 4℃. The supernatant was combined and filled up to 50 ml in a volumetric flask, and filtered using a 0.45-μm filter (Cosmonice Filter W, 13 mm, Nacalai Tesque). DPPH radical-scavenging activity, peroxy radical-scavenging activity and total phenol content were measured using this extract solution. Three measurements were performed for each sample, and the results were expressed as the mean value±SD.

Measurement of DPPH radical-scavenging activity DPPH radical-scavenging activity was measured by the colorimetric method. Each sample (200 μL) was mixed with 800 μL of Tris-HCl buffer (100 mM, pH 7.4), then 1 mL of 500 μM DPPH in ethanol was added to start the reaction. The mixture was vigorously shaken and was left for 20 min at room temperature in the dark. After 20 min, the absorbance of the mixture was measured by a UV-2100PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm. The DPPH radical-scavenging activity was determined by using the difference of the DPPH radical absorbance between a blank and a sample. The activity was expressed as μmol Trolox eq./g.

Measurement of peroxy radical-scavenging activity by 2′-dG method Peroxy radical-scavenging activity was measured according to the method of Sakakibara *et al.* (2002) with slight modification. The sample solution (10 μL) was mixed with 1 mM of 2′-dG (50 μL) solution and the reaction was started by adding 500 μL of 50 mM AAPH solution. The reaction mixture was left at 37℃ for 1 h. Then, 10 μL of the reaction mixture was injected for HPLC analysis. The HPLC analysis was carried out on a Capcell pak C18 UG120 (5-μm mesh, 4.6×250 mm, Shiseido, Tokyo, Japan) equipped with a Shimadzu LC-6A pump and a Shimadzu SPD-10AV UV-VIS detector set at 254 nm at ambient temperature. The mobile phase consisted of 6.5% methanol and 93.5% 20 mM potassium phosphate buffer (pH 4.5) containing 0.1 mM EDTA, and the flow rate was 1 mL/min. The radical-scavenging activity was determined by taking the difference in the suppression of 8-hydroperoxy-2′-deoxyguanosine (8-OOHdG) formations between a blank and a sample. The activity was expressed in units of μmol Trolox eq./g.

Determination of total phenol content The total phenol content was determined according to the method of Singleton and Rossi (1965). The sample (200 μL) was mixed with 7.5% Na_2CO_3 (800 μL), and then 1 ml of Folin-Ciocalteu reagent was added to reaction mixture. The mixture was vigorously shaken and was left for 30 min at room temperature. After 30 min, the absorbance of the mixture was measured using a UV-2100PC UV-VIS spectrophotometer (Shimadzu) at 765 nm. The total phenol content was expressed as μmol gallic acid eq./g.

Statistical analysis Statistical analysis was performed using Microsoft Excel®. Differences between the treatments were determined using a t-test. Differences between the treatments of at least $p<0.05$ were considered to be significantly different.

Results and Discussion

Change in DPPH radical-scavenging activity during heating The DPPH radical-scavenging activity of 16 spices was measured using the colorimetric method (Table 1). First, we describe the activity before heating. Out of the sixteen spices, clove showed the highest radical-scavenging activity (1353.3±103.0 μmol Trolox eq./g), followed by allspice (546.4±12.0 μmol Trolox eq./g) and cinnamon (364.0±10.3 μmol Trolox eq./g). The lowest activity was observed in white pepper (3.6±0.3 μmol Trolox eq./g). These were higher than the activities of many vegetables such as carrot (31.9±4.9 μmol Trolox eq./100 g), cucumber (47.0±1.1 μmol Trolox eq./100 g), lettuce (104±17.6 μmol Trolox eq./100 g), onion (138.2±20.5 μmol Trolox eq./100 g), tomato (213.4±13.9 μmol Trolox eq./100 g), *etc.*

Table 1. Change in DPPH radical-scavenging activity of some spices after different heating time at 100℃.

Spices	DPPH radical-scavenging activity (μmol Trolox eq./g)			
	Heating time			
	0h	1h	3h	6h
Clove	1353.3±103.0	1536.4±132.2 (1.1)*	1572.1±120.7 (1.2)*	1671.1±157.2 (1.2)*
Allspice	546.4±12.0	611.3±13.9 (1.1)*	665.8±22.3 (1.2)*	796.2±37.9 (1.5)**
Cinnamon	364.0±10.3	356.5±29.4 (1.0)	354.5±33.3 (1.0)	303.3±27.8 (0.8)
Nutmeg	50.9±4.6	28.7±1.6 (0.6)**	38.5±3.5 (0.8)**	45.3±2.6 (0.9)
Mustard	38.2±3.0	34.1±1.4 (0.9)	35.2±2.4 (0.9)	39.3±3.2 (1.0)
Cumin	32.7±0.5	32.6±0.7 (1.0)	33.3±0.9 (1.0)	33.1±1.0 (1.0)
Ginger	31.8±1.2	26.9±1.4 (0.8)	25.5±1.9 (0.8)	26.9±1.5 (0.8)*
Fennel	20.6±0.7	23.5±1.4 (1.1)	25.4±1.3 (1.2)	24.8±1.0 (1.2)*
Fenugreek	20.5±2.0	11.6±1.1 (0.6)*	10.3±0.9 (0.5)*	14.8±1.5 (0.7)
Black pepper	19.5±1.5	31.7±2.3 (1.6)*	39.6±3.9 (2.0)**	45.1±3.1 (2.3)**
Red pepper	18.1±1.2	19.4±0.5 (1.1)	19.1±1.5 (1.1)	20.1±1.4 (1.1)
Mace	18.1±0.9	19.6±1.4 (1.1)	22.2±1.1 (1.2)	23.9±0.9 (1.3)*
Coriander	16.4±0.6	22.3±0.3 (1.4)*	24.8±2.0 (1.5)*	24.9±2.3 (1.5)*
Turmeric	9.6±0.7	14.5±0.3 (1.5)*	23.3±1.2 (2.4)**	28.3±0.4 (2.9)***
Cardamom	7.5±0.4	8.4±0.7 (1.1)*	11.0±1.1 (1.5)*	12.0±1.2 (1.6)**
White pepper	3.6±0.3	3.2±0.3 (0.9)	4.2±0.4 (1.2)	5.2±0.4 (1.4)**

The values are the means±SD of 3 determinations. Data in parentheses are relative value to the activity at 0 h. *$p<0.05$, **$p<0.01$, ***$p<0.001$

as reported by Yamaguchi *et al.* (2001). The activity range of other spices such as nutmeg, mustard, cumin and ginger was 31.8–50.9 μmol Trolox eq./g. The activity of fennel, fenugreek, black pepper, red pepper, mace, coriander, turmeric and cardamom was less than 21.0 μmol Trolox eq./g. Shobana and Naidu (2000) reported the relative antioxidant activities of some spices; the order of the activities was clove, cinnamon, ginger, pepper and onion.

Next, changes in the DPPH radical-scavenging activity of spices for different heating times are shown in Table 1. After heating, a significant change in the activity occurred in many spices. An increase in radical-scavenging activity was found in clove, allspice, fennel, black pepper, mace, coriander, turmeric, cardamom and white pepper. The activity increased three times in turmeric and two times in black pepper after 6-h heating. The activity of other spices increased slightly (1.2–1.5 times). An insignificant change in cinnamon, mustard, cumin and red pepper was observed.

The major active components of spices such as eugenol (clove, cinnamon, allspice), myristicin (mace, nutmeg), curcumin (turmeric), capsaicin (red pepper) are usually fat-soluble. But the present study was carried out in an aqueous solution containing 20% ethanol. So, the active components of spices might not dissolve completely in this solution before heating. After heating, the solubilities of the active components probably increased because of decomposition of the cell wall and by passing of the solvent into the cell. For this reason, an increase in the radical-scavenging activity of spices might be observed after heating. Shobana and Naidu (2000) reported that the bound antioxidants might be released due to heat treatment, resulting in the higher antioxidant activity compared that in that of fresh spices extract. Maeda *et al.* (1992) suggested that thermal treatment might destroy the cell wall and the subcellular compartments of vegetables to liberate greater amounts of components, or thermal chemical reactions might produce more potent radical-scavenging components. Dewanto *et al.* (2002) found that thermal processing disrupts the cell membranes and cell walls to release lycopene from the insoluble portion of tomato, which might cause the antioxidant activity of tomato to increase. Tomaino *et al.* (2005) found the antioxidant activity of clove and cinnamon oil did not change after 3-h heating at 180℃, but the amounts of the active components (safrol and myristicin) of nutmeg increased. A significant quantitative loss in the active components of turmeric was found after boiling of mixed spices (Srinivasan *et al.,* 1992). Takamura *et al.*

Table 2. Change in peroxy radical-scavenging activity of some spices after different heating time at 100℃.

Spices	Peroxy radical-scavenging activity (μmol Trolox eq./g)			
	Heating time			
	0h	1h	3h	6h
Clove	1018.7±99.2	1054.7±83.7 (1.0)	1235.7±16.4 (1.2)	1215.8±120.4 (1.2)
Allspice	343.5±32.7	466.5±4.0 (1.4)*	533.5±53.1 (1.6)*	567.7±22.1 (1.7)**
Cinnamon	417.4±19.2	469.7±39.6 (1.1)	455.9±9.1 (1.1)	420.0±27.7 (1.0)
Nutmeg	104.3±6.8	84.9±4.6 (0.8)	105.6±5.5 (1.0)	117.4±9.2 (1.1)
Mustard	119.7±3.3	104.7±9.8 (0.9)	132.8±11.8 (1.1)	127.8±12.7 (1.1)
Cumin	126.3±8.2	127.0±8.7 (1.0)	130.6±12.9 (1.0)	139.6±7.2 (1.1)
Ginger	53.3±4.9	50.8±1.8 (1.0)	58.8±3.1 (1.1)	56.1±1.9 (1.1)
Fennel	104.4±10.2	109.3±8.5 (1.0)	108.8±4.6 (1.0)	115.1±4.2 (1.1)
Fenugreek	141.4±6.8	100.5±9.1 (0.7)*	102.0±0.9 (0.7)*	115.6±4.1 (0.8)*
Black pepper	65.8±1.3	111.8±0.9 (1.7)**	118.0±7.9 (1.8)**	149.2±13.2 (2.3)**
Red pepper	34.8±2.8	58.6±5.6 (1.7)*	56.9±3.2 (1.6)*	80.9±6.4 (2.3)**
Mace	58.6±4.8	68.3±4.3 (1.2)*	81.1±8.0 (1.4)**	74.8±6.6 (1.3)*
Coriander	49.1±3.9	71.4±3.9 (1.5)*	71.1±5.5 (1.4)*	67.4±3.8 (1.4)*
Turmeric	48.4±3.7	88.2±7.5 (1.8)**	112.1±11.1 (2.3)**	142.6±13.0 (2.9)**
Cardamom	31.0±1.9	27.7±2.7 (0.9)	44.7±2.0 (1.4)*	47.0±3.1 (1.5)*
White pepper	48.7±3.7	49.2±0.6 (1.0)	50.6±1.6 (1.0)	45.4±2.0 (0.9)

The values are the means±SD of 3 determinations. Data in parentheses are relative value to the activity at 0 h. *p<0.05, **p<0.01, ***p<0.001

(2002) reported a decrease of the radical-scavenging activity of curry paste and cooked curry, possibly due to decomposition or evaporation of the active compounds, since the spices were heated with butter at high temperature.

In the present study, a decrease in the radical-scavenging activity was observed in nutmeg, ginger and fenugreek. After heating, coagulation of these spices was observed. Therefore, the extraction ability might be decreased by coagulation after heating, resulting in a reduction in the radical-scavenging activities of these spices.

Change in peroxy radical-scavenging activity during heating In this study, AAPH was used to produce molecular peroxy radical via AAPH-peroxyl radical. This peroxy radical reacts with 2′-dG to form 8-OOHdG. Consequently, the water soluble and fat-soluble active components suppress the formation of 8-OOHdG. Therefore, the 2′-dG oxidation method could be expected to be an index for evaluating the prevention of oxidative genetic damage by the suppression of 8-OOHdG formations (Sakakibara *et al.,* 2002).

The peroxy radical-scavenging activity of spices before heating is shown in Table 2. All spices showed a considerably high peroxy radical-scavenging activity. Clove (1018.7±99.2μmol Trolox eq./g), allspice (343.5±32.7μmol Trolox eq./g), cinnamon (417.4±19.2μmol Trolox eq./g) showed higher activities than those of other spices and the lowest activity was found in cardamom (31.0±1.9 μmol Trolox eq./g). The activity range of other spices such as nutmeg, mustard, cumin, ginger, fennel, fenugreek, black pepper, red pepper, mace, coriander, turmeric and white pepper was 34.8–141.4μmol Trolox eq./g. Gulçin *et al.* (2004) reported that the water extract and ethanol extract of clove buds had a similar level of superoxide radical-scavenging activity and this activity was stronger than BHA, BHT and tocopherol. Both water and ethanol extracts of fennel seeds showed to have DPPH radical-scavenging, superoxide anion radical-scavenging, hydrogen peroxide scavenging and metal chelating activities (Oktay *et al.,* 2003). Curcumin, tetrahydrocurcumin and dihydroxytetrahydrocurcumin were reported to exhibit a significant inhibitory effect on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced O_2 generation in different leukocytes *in vitro* and *in vivo* (Nakamura *et al.,* 1998).

Next, the changes of peroxy radical-scavenging activity of spices after heating are presented in Table 2. A significant increase in the activity was found in allspice, black pepper, red pepper, mace, coriander, turmeric and cardamom. On the other hand, no significant change in

Table 3. Change in total phenol content of some spices after different heating time at 100℃.

Spices	Total phenol content (μmol Gallic acid eq./g)			
	Heating time			
	0h	1h	3h	6h
Clove	1267.0±125.0	1226.0±45.0 (1.0)	1335.0±85.0 (1.1)	1395.0±135.0 (1.1)
Allspice	421.5±10.0	460.0±35.0 (1.1)	515.0±15.0 (1.2)*	569.5±10.0 (1.4)***
Cinnamon	272.5±10.0	279.0±20.0 (1.0)	259.0±15.0 (1.0)	210.5±10.0 (0.8)
Nutmeg	49.0±5.0	35.0±1.0 (0.7)*	45.0±5.0 (0.9)	45.0±5.0 (0.9)
Mustard	53.0±5.0	50.0±2.0 (0.9)	55.0±5.0 (1.0)	59.0±5.0 (1.1)
Cumin	49.5±2.0	50.0±1.5 (1.0)	50.0±2.0 (1.0)	50.0±1.5 (1.0)
Ginger	20.0±0.5	15.0±0.5 (0.8)**	15.0±0.0 (0.8)*	15.0±0.5 (0.8)*
Fennel	46.1±5.0	45.0±5.0 (1.0)	45.0±5.0 (1.0)	42.6±0.5 (0.9)
Fenugreek	52.0±1.5	30.0±5.0 (0.6)**	30.0±1.5 (0.6)**	45.0±5.0 (0.9)
Black pepper	27.5±5.0	43.0±5.0 (1.6)*	52.5±5.0 (1.9)*	55.0±5.0 (2.0)*
Red pepper	32.5±2.0	40.0±1.5 (1.2)*	46.2±2.0 (1.3)*	45.4±5.0 (1.3)*
Mace	20.0±2.0	25.0±0.5 (1.3)	30.0±1.0 (1.5)*	30.0±0.5 (1.5)*
Coriander	18.5±1.0	24.5±0.5 (1.3)*	28.0±2.5 (1.5)*	27.5±2.5 (1.5)*
Turmeric	14.5±1.0	25.0±2.5 (1.7)*	47.5±2.5 (3.3)**	59.0±1.0 (4.1)***
Cardamom	7.5±0.0	9.0±0.0 (1.2)**	11.0±0.5 (1.5)**	12.0±1.0 (1.6)**
White pepper	4.5±0.5	4.0±0.5 (0.9)	4.5±0.5 (1.0)	5.5±0.5 (1.2)

The values are the means±SD of 3 determinations. Data in parentheses are relative value to the content at 0 h. *p<0.05, **p<0.01, ***p<0.001

the DPPH radical-scavenging activity of red pepper during thermal treatment was observed (Table 1), but an increase in the activity was observed after 6-h heating by the 2′-dG method. After heating, the peroxy radical-scavenging activities of allspice, black pepper, mace, coriander, turmeric and cardamom showed the same tendency as the results of the DPPH method (Table 1). There was no change in the activities of clove, cinnamon, nutmeg, mustard, cumin, ginger, fennel and white pepper. In the case of fenugreek, a significant decrease was observed. The reason why the peroxy radical-scavenging activity increased and decreased after heating could be explained by the same factors described above.

Change in total phenol content during heating Plant phenolic compounds are expected to play a role for chemo preventive action of cancer, chronic disease and coronary heart diseases. They can act as free radical scavengers and metal ion chelators and are widely used as antioxidants. Some of them have a higher antioxidant activity than the common antioxidants, vitamins C and E (Rice-Evans *et al.*, 1997). Among polyphenols, caffeic acid, ferulic acid and vanillic acid are widely distributed in the plant kingdom (Larson, 1988). Hashim *et al.* (2005) reported that phenolic compounds effectively suppress hydrogen peroxide-induced oxidative stress.

First, the result before heating is described. In this study, we measured the total phenol content as shown in Table 3. The highest content was found in clove and the lowest was in white pepper (Table 3). The polyphenol contents in clove (1267.0±125.0μmol gallic acid eq./g), allspice (421.5±10.0μmol gallic acid eq./g) and cinnamon (272.5±10.0μmol gallic acid eq./g) were considerably higher than that in other spices. The correlation between the content of phenolic compounds and DPPH or peroxy radical-scavenging activity was evaluated as shown in Figs. 1 and 2.

The DPPH radical-scavenging activity of all spices was highly correlated (R^2=0.99) with total phenol content (Fig. 1A). With the exception of clove, allspice and cinnamon, the correlation coefficients of the tested spices were low (R^2=0.55) (Fig. 1B). On the other hand, peroxy radical-scavenging activity was highly correlated with their total phenol contentd (Fig. 2AB). The correlation coefficients were R^2=0.97 (“all spices”) and R^2=0.80 (“except three spices”). From these results, the active components of spices are considered to be mostly polyphenol compounds. Several polyphenols did not show any DPPH radical-scavenging activity. Taira *et al.* (1992) reported that the strong DPPH-activities of clove, allspice and nutmeg might be related to their phenolic antioxidant

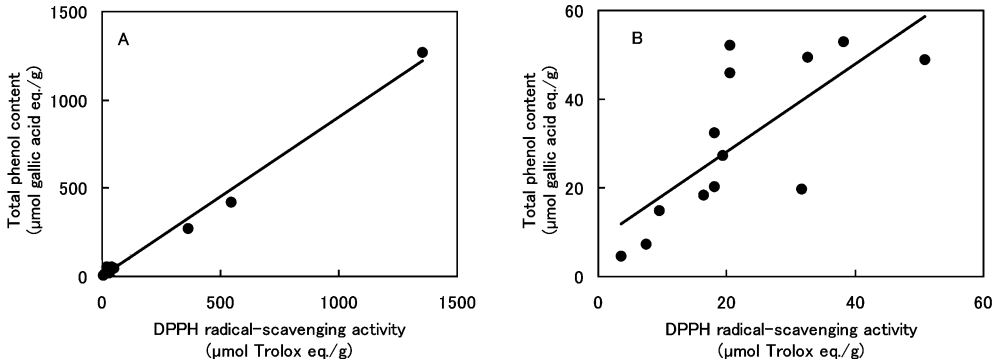

Fig. 1. Correlation between DPPH radical-scavenging activity and total phenol content. (A) All spices (B) All spices except clove, allspice and cinnamon.

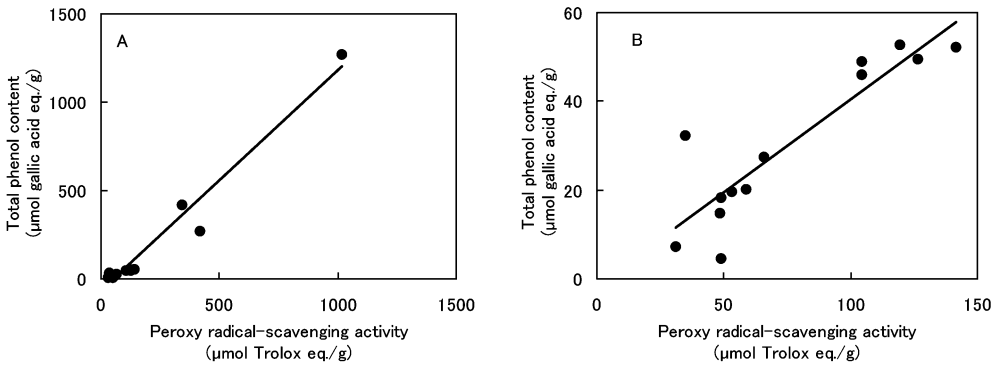

Fig. 2. Correlation between peroxy radical-scavenging activity and total phenol content. (A) All spices (B) All spices except clove, allspice and cinnamon.

components such as eugenol, isoeugenol, *etc.* Dorman *et al.* (2000) identified 16–18 components from clove and nutmeg essential oils, and found that 94% of phenylpropanoids obtained in clove oil, and eugenol (91%) was the main component of that phenylpropanoids. Kikuzaki *et al.* (1999) isolated a phenyl propanoid, *threo*-3-chloro-1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol from the berries of allspice (*Pimenta dioica*) with five known compounds, eugenol, vanillin, 4-hydroxy-3-methoxycinnamaldehyde, 3,4-dimethoxycinnamaldehyde and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol showing that these components inhibited an auto-oxidation of linolic acid in a water-alcohol assay system. Twelve major phenolic compounds including flavonoid such as isoquercitrin, kaempferol glycoside and rutin were isolated from fennel (Parejo *et al.,* 2004). Quercetin and kaempferol were found in coriander (Justesen and Knuthesen, 2001). Furthermore, positive correlation between phenolic content and antioxidant activity was found for coriander seed (Wangensteen *et al.,* 2004). Capsaicin (red pepper), gingerol and and shogaol (ginger) were reported to be responsible for the antioxidant activity of these spices (Nakatani, 1996; Kikuzaki and Nakatani, 1993; Epstein *et al.,* 1993). In addition, five phenolic amides of pepper were also shown to be responsible for antioxidant activity of black pepper. Chung *et al.* (1997) identified 3,5-dimethoxy-4-hydroxycinnamic acids as an ester from brown mustard (*Brassica nigra*). Parejo *et al.* (2004) found a high correlation between phenolic content and DPPH radical-scavenging activity. However, Oktay *et al.* (2003) reported finding no such relation between these parameters.

Next, the result after heating is described. The change in the total polyphenol content after heating is presented in Table 3. After heating, there was a positive and significant change in the total phenol contents of allspice, black pepper, red pepper, mace, coriander, turmeric and cardamom. Among them, allspice, red pepper, mace, coriander and cardamom increased approximately 1.5 times, black pepper increased 2 times and turmeric increased 4 times. A significant negative change was found in nutmeg, ginger and fenugreek. It is well known that glycosides are hydrolyzed to form their aglycone. Pratt and Watts (1964) reported that flavonoid present in living cells as glycosides may be breakdown by enzyme, acid or heat treatment to form their aglycone and sugar. Some aglycones are known to have a more active potential for antioxidant activity than their glycosides. Quercetin (aglycone) is more active than its glycoside rutin (Shahidi *et al.,* 1992).

In the present study, the spices were heated at 100℃. Therefore, in this situation, glycoside could be hydrolyzed to be form aglycone, thus the antioxidant activity may increase after heating. It seems that some active components are degraded by heating to form less active

components. Tonnesen and Karlsen (1985a) reported that curcumin is extremely unstable in alkaline solution and is degraded to ferulic acid and feruloyl methane (Tonnesen and Karlsen, 1985b). So, the pH might also be a factor in reducing the antioxidant activities of spices.

From the results of this study, it is clear that spices have strong antioxidant activities even in a 20% ethanol extract solution. The radical-scavenging activities of spices remained after boiling, suggesting that the active components are relatively stable during thermal cooking at about 100℃. The antioxidant components of spices could be measured more accurately by the 2′-dG method than by the DPPH method. Further studies are in progress to identify the individual polyphenol components and the effect of heating on the individual polyphenols of spices. In conclusion, spices are expected to be a valuable food constituent for promoting health in our daily lives.

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