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Evaluation of peroxynitrite-scavenging capacities of several commonly used fresh spices

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ABSTRACT

Peroxynitrite-induced nitration of protein tyrosine residues is considered as one of the major pathological causes of several human diseases, e.g. cardiovascular disorders. Therefore, it appears that attenuation of peroxynitrite-induced nitration by certain foods could be beneficial to human health. Certain spices, used widely in folk medicine for cardiovascular disorders, conceivably protect against the activities of peroxynitrite. Seven culinary spices, including chilli, garlic, ginger, leek, onion, shallot and Welsh onion, were selected for this study. The peroxynitrite-scavenging capacities of these aqueous spice extracts were evaluated on the basis of their ability to attenuate peroxynitrite-induced nitrotyrosine formation in albumin. All of the spices had abilities to attenuate the peroxynitrite-mediated protein nitration. Ginger had outstanding peroxynitrite-scavenging ability. The phenolics and flavonoids in certain spices had abilities to suppress the peroxynitrite-mediated tyrosine nitration reaction. This indicates that these compounds could act as peroxynitrite-scavengers.

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1. Introduction

Peroxynitrite ($ONOO^-$), a product of the reaction of nitric oxide (NO) with superoxide anion (O_2^-), is formed in tissues with perfusion injury and inflammation. Peroxynitrite is a potent and versatile oxidant that can oxidise and nitrify DNA. Such cytotoxicity can damage a large variety of biomolecules such as proteins, lipids, and DNA. Protein tyrosine residues are especially susceptible to peroxynitrite-dependent nitration. The stable end-product, 3-nitrotyrosine, is considered to be a biomarker of peroxynitrite-specific protein damage. Nitration of tyrosine residue destroys the tertiary structure of protein, renders the degradation of damaged protein by proteosomes, and interferes with the signalling pathway, which affects cell proliferation and apoptosis (Virag, Szabo, Gergely, & Szabo, 2003). 3-Nitrotyrosine is found in tissue specimens from several diseases, including cardiovascular disorders, degenerative neurological disorders and certain renal diseases. This suggests that peroxynitrite is involved in the pathological processes of these diseases (Ischiropoulos & Beckman, 2003; Pennathur et al., 2004). Undoubtedly, inherent suppression of the actions of peroxynitrite *in vivo* is important for the human body to eliminate the consequences of peroxynitrite-induced reaction. The inhibition of sinister peroxynitrite activity *in vivo* relies solely upon the actions of certain nonenzymatic compounds because humans lack a specific enzyme needed to decompose peroxynitrite (Klotz & Sies, 2003). Therefore, in addition to certain endogenous molecules, such as glutathione, ascorbate and albumin, the consumption of foods which are rich in peroxynitrite-scavengers appears to be a promising strategy to boost inherent protection against peroxynitrite damage in humans.

Because of their health-promoting properties and use as folk medicines, many plants, such as chilli, ginger, and *Allium* spices, serve, not only as culinary seasonings, but also as vegetables in Asian dishes. The health-promoting properties of spices, such as anti-atherosclerotic, anti-cancer, and anti-inflammatory activities, have been attributed mainly to their antioxidant constituents. To the best of our knowledge, previous researches pertaining to the relative health benefits of spice intake have focussed on their ability to scavenge reactive oxygen species, such as superoxide, hydrogen peroxide, and the hydroxyl radical (Pedraza-Chaverri, Medina-Campos, Avali-Lombardo, Zuniga-Bustos, & Orozco-Ibarra, 2006; Prakash, Singh, & Upadhyay, 2007). However, limited information is available about the ability of certain dietary spices to scavenge the biologically relevant reactive nitrogen species, especially peroxynitrite. Preventing the formation of peroxynitrite, directly scavenging peroxynitrite, and repairing any peroxynitrite-damaged biomolecules have been recognised as three important mechanisms (Arteel, Briviba, & Sies, 1999). According to our previous study, spices were shown to have the ability to eliminate

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NO production by suppressing the expression of inducible nitric oxide synthase (Tsai, Tsai, & Ho, 2005). However, it appears that comprehensive and comparative information regarding the peroxynitrite-scavenging ability of various spices is somewhat scant. In the present study, the peroxynitrite-scavenging abilities of seven commonly used spices, including chilli, garlic, ginger, leek, onion, shallot and Welsh onion, were assessed on the basis of their inhibitory effect on the formation of peroxynitrite-mediated protein nitration. Additionally, the phenolic and flavonoid contents, the NO and O_2^- scavenging activities and the antioxidant capacities, including trolox equivalent antioxidant capacity (TEAC), DPPH-radical-scavenging capacity, and oxygen-radical absorbance capacity (ORAC), of the spices were determined.

2. Materials and methods

2.1. Chemicals used for investigation

Peroxynitrite was purchased from Upstate Co. (Lake Placid, NY, USA). Folin–Ciocalteau phenol reagent, gallic acid, catechin, sodium nitroprusside, sulphanilamide, naphthylethylenediamine, nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulphate, bovine serum albumin (BSA), anti-3-nitrotyrosine antibodies and nitro blue tetrazolium chloride/5-bromo-4-chloro-3′-indolyphosphate *p*-toluidine salt (NBT/BCIP) liquid-substrate systems were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical-grade purity.

2.2. Preparation of aqueous spice extracts

Seven fresh spices – chilli, garlic, ginger, leek, onion, shallot and Welsh onion – were purchased from a traditional market in Hsinchu City (Hsinchu, Taiwan). The edible portions were cut into small pieces and blended with a minimal amount of cold double-distilled water. After being filtered through several layers of gauze, the filtrates were centrifuged at 12,000 *g* for 20 min at 4 °C to remove the solid debris. The supernatant was then collected and freeze-dried. The extraction yields for such supernatants are listed in Table 1. In the following experiments, the weighed extract solid was dissolved in phosphate-buffered saline (PBS) to the indicated concentration.

2.3. Measurement of total flavonoids

The content of total flavonoids was measured by a colorimetric method. Briefly, 0.25 ml of an appropriately diluted sample was added to a tube containing 1 ml of double-distilled water. Next, 0.075 ml of 5% $NaNO_2$, 0.075 ml of 10% $AlCl_3$ and 0.5 ml of 1 M NaOH were added at 0, 5 and 6 min, sequentially. Finally, the volume of the reacting solution was adjusted to 2.5 ml with double-distilled water. The absorbance of the solution at a wavelength of 510 nm was detected using a spectrophotometer (Ultrospec 2100 *pro*; Amersham Pharmacia Biotech Co., Piscataway, NJ, USA). The total flavonoids content in each spice extract was then calculated using a standard curve prepared with catechin and expressed as milligrammes of catechin equivalents (CE) per gramme of extract solids.

2.4. Measurement of total phenolic compounds

The quantity of total phenolics in the aqueous extracts was measured by a Folin–Ciocalteau colorimetric method and expressed as milligrammes of gallic-acid equivalents (GAE) per gramme of aqueous extract. Briefly, aqueous extract stock was diluted with double-distillated water to a final concentration of 10 mg/ml. Folin–Ciocalteau phenol reagent was then added to the sample and held for 3 min. Next, 2 ml of 10% (w/v) aqueous sodium carbonate were added and the mixture allowed to stand at room temperature for 1 h. The absorbance of the developed colour was measured by a spectrophotometer at 765 nm. The total content of phenolic compounds in each spice extract was then calculated using a standard curve prepared with gallic acid.

2.5. Measurement of trolox equivalent antioxidant capacity

The trolox equivalent antioxidant capacity (TEAC) of each spice extract was measured with a commercial total antioxidant status detecting kit (Randox Laboratories Ltd., Antrim, UK). This assay was based on 2,2′-azinobis(3-ethylbenzothiazoline sulphonate) (ABTS) incubated with metmyoglobin and hydrogen peroxide to produce the radical cation, $ABTS^+$. $ABTS^+$ has a stable blue–green colour and can be measured at 600 nm. Antioxidants in the sample suppress the colour development proportionally to their concentration. The total antioxidant capacity of each extract was determined, following the instructions, and calculated, based on the suppressive effect related to a 1 mM trolox standard.

2.6. Measurement of DPPH-radical-scavenging capacity

The DPPH-radical-scavenging capacity of spice extracts was measured as described previously (Ho, Tsai, Tsai, & Lin, 2008). Briefly, 20 µl of serially diluted sample extract was pipetted into the wells of a 96-well flat-bottomed plate; 200 µl of 0.2 mM DPPH methanolic solution were added to each well and the plate was shaken with a plate shaker for 5 min. The change in absorption at wavelength of 540 nm, subsequent to the addition of DPPH·, was then measured by an enzyme-linked immunosorbent assay reader (EL800; BIO-TEK Instruments Inc., Winooski, VT, USA). The scavenging capacity of spice extract was expressed as the IC_{50}, i.e., the concentration of the tested spice extract required to quench 50% of the DPPH radical present.

Table 1
Extracting yield and total flavonoids content of aqueous spice extracts.

Spice	Botanical name	Extracting yield	Total flavonoids[A,B]	
		(%)	(mg CE/g extract solids)	(mg CE/100 g fresh spices)
Garlic	*Allium sativum*	18.8	0.19 ± 0.00[f]	3.64 ±0.00[e]
Leek	*Allium tuberosum*	2.41	4.87 ± 0.08[b]	11.7 ± 0.21[c]
Onion	*Allium cepa*	6.16	0.33 ± 0.01[e]	2.01 ± 0.07[f]
Shallot	*Allium ascalonicum*	13.1	0.68 ± 0.01[d]	9.02 ± 0.16[d]
Welsh onion	*Allium fistulosum*	2.49	0.64 ± 0.01[d]	1.59 ± 0.03[g]
Chili	*Capsicum annuum*	6.87	3.48 ± 0.06[c]	23.9 ± 0.38[a]
Ginger	*Zingiber officinale*	2.36	6.14 ± 0.05[a]	14.5 ± 0.12[b]

[A] The values are expressed as means ± S.D. of triplicate tests. Means not sharing a common letter in the same column were significantly different ($p < 0.05$) when analysed by ANOVA and Duncan's multiple-range test.
[B] The total flavonoids content was expressed as milligrammes of catechin equivalents (CE) per gramme of extract solid and per 100 g of fresh spice, respectively.

2.7. Measurement of oxygen-radical absorbance capacity

The oxygen-radical absorbance capacity (ORAC) assay was performed as described previously (Ho et al., 2008). Briefly, 25 μl of optimally diluted sample extract, trolox standard and PBS blank were pipetted into wells of a 96-well flat-bottomed plate; 150 μl of fluorescein solution were added to each well and the plate was incubated at 37 °C for 30 min in the dark. Next, 25 μl of 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) solution were added to the wells as a source of peroxyl radical, after which the solution's fluorescence readings were taken every 2 min for a total of 120 min with a microplate multimode detector (Zenyth 3100; Anthos Labtec Instruments Inc., Wals, Austria). Finally, the difference between the area under the fluorescence decay curve for each sample and the corresponding area for the blank was determined to calculate the ORAC value.

2.8. Measurement of NO-scavenging activity

The NO-scavenging activity of the sample extract was measured as follows: 50 μl of serially diluted sample extract were pipetted into a 96-well flat-bottomed plate. Next, 50 μl of 10 mM sodium nitroprusside dissolved in PBS were added to each well and the plate was incubated under light at room temperature for 90 min. Finally, an equal volume of Griess reagent (1% of sulphanilamide and 0.1% of naphthylethylenediamine in 2.5% HPO_3) was added to each well to measure the nitrite content. The scavenging capacity (%) of the sample extracts to NO was expressed as $[1 - (A_{sample} - A_{sample\ blank})/(A_{control} - A_{control\ blank})] \times 100\%$, where $A_{sample} - A_{sample\ blank}$ is the difference in the absorbance of a sample, with or without 10 mM sodium nitroprusside, and $A_{control} - A_{control\ blank}$ is the difference in the absorbance of the PBS control, with or without 10 mM sodium nitroprusside.

2.9. Measurement of superoxide anion ($O_2^{\cdot-}$)-scavenging activity

The $O_2^{\cdot-}$-scavenging activity of sample extract was determined as follows: briefly, 25 μl of different concentrations of sample extract, 62.5 μl of 300 μM NBT and 62.5 μl of 936 μM NADH were added separately into wells. Next, 62.5 μl of 30 μM phenazine methosulphate were added into each well, and the absorbance at 540 nm was read every 30 s for a total of 5 min with an enzyme-linked immunosorbent assay reader (EL800). The scavenging capacity (%) of the sample extracts to $O_2^{\cdot-}$ was expressed as $[1 - (A_{sample\ at\ 5\ min} - A_{sample\ at\ 0\ min})/(A_{PBS\ at\ 5\ min} - A_{at\ 0\ min})] \times 100\%$, where $A_{sample\ at\ 5\ min} - A_{sample\ at\ 0\ min}$ was the difference in the absorbance of a sample at 5 and 0 min, respectively, and $A_{PBS\ at\ 5\ min} - A_{at\ 0\ min}$ was the difference in the absorbance of the PBS control at 5 and 0 min.

2.10. Measurement of peroxynitrite-scavenging capacity

The peroxynitrite-scavenging capacity of the aqueous spice extracts was evaluated on the basis of the inhibition of peroxynitrite-induced nitrotyrosine formation in BSA. The nitration of tyrosyl residue in BSA was performed as previously reported (Ho et al., 2008). Briefly, a diluted extract solution was mixed with 200 μg/ml of BSA in PBS. Peroxynitrite was then added, and the reaction solution was incubated for 30 min at 37 °C. Next, the formation of 3-nitrotyrosine was determined by immunoblot analysis. Briefly, 10 μl of reaction solution were loaded into, and separated on, a 10% SDS–polyacrylamide gel and then transferred to polyvinylidene fluoride filters. The filters were then blocked, probed with anti-3-nitrotyrosine antibodies, incubated with secondary antibody conjugated to alkaline phosphatase and detected using an NBT/BCIP solution. Finally, the relative intensities of the bands on the filters were quantified with a software-supported photoimager (ImageMaster VDS; Amersham Pharmacia Biotech Co., Piscataway, NJ, USA).

2.11. Statistical analysis

All results presented herein are expressed as means ± S.D.s for at least three independent tests for each spice extract. The significance of the differences between the treatments was analysed by ANOVA and followed by Duncan's multiple-range test for multiple comparisons. The correlation between two variants was analysed by application of the Pearson test. All of the statistical analyses were performed by means of SPSS software with the level of significant difference between compared data sets being set at $p < 0.05$ (SPSS for Windows; version 10.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Flavonoid content of the fresh spices

The flavonoid content of the aqueous spice extracts is shown in Table 1. The flavonoid content of the spice extracts varied greatly. Ginger had the highest flavonoid content (6.14 mg CE/g of extract solid). Except for leeks, all of the *Allium* spice extracts had low flavonoid contents (<1 mg CE/g of extract solid). For comparison with other studies, the flavonoid content was also expressed on the basis of fresh weight. The flavonoid content of the *Allium* spices, especially garlic, onion, and Welsh onion, was six times lower than that of the chilli.

3.2. Antioxidant capacities of the aqueous spice extracts

Many different methods have been established for evaluating the antioxidant capacity of biological samples. They are roughly classified into two categories based upon the nature of the reaction that the method involved. The methods involving an electron-transfer reaction include the TEAC and the DPPH-radical-scavenging assay. The ORAC assay belongs to the group of methods that evaluate the antioxidant capacity via the hydrogen-atom transfer reaction (Huang, Ou, & Prior, 2005). The antioxidant capacities of the spice extracts, by different assay methods, are presented in Table 2. The phenolic content of the aqueous spice extracts also varied greatly. The leek (21.2 mg GAE/g of extract solid) and the ginger (20.5 mg GAE/g of extract solid) had the highest phenolic contents. The onion (7.09 mg GAE/g of extract solid) and the garlic (9.08 mg GAE/g of extract solid) had the lowest phenolic contents. The order of antioxidant capacity, based on the TEAC assay, was leek and ginger > chilli > shallot > onion > Welsh onion > garlic. The DPPH-scavenging capacity of the spices was presented as IC_{50}. Of the seven spices tested, the most active one was the ginger, which had an IC_{50} value of 0.92 mg/ml. The garlic had an IC_{50} value greater than l0 mg/ml. This indicates that the garlic is a poor DPPH-radical scavenger. In this study, we used an ORAC assay to determine the hydrogen-atom transfer capacity of the tested spices and found that the magnitude of the resultant ORAC value for the tested spices was similar to the TEAC values. Almost all of the results of the four antioxidant assay methods correlated significantly with each other, except for the correlation between the total phenolic content and the DPPH-radical-scavenging assays (data not shown).

3.3. NO-scavenging capacities of the aqueous spice extracts

The results of the NO-scavenging capacities of the aqueous spice extracts are shown in Fig. 1. All of the spice extracts had the NO-scavenging capacity, and all exhibited a linear dose–re-

Table 2
Antioxidant capacities of aqueous spice extracts as determined by means of total phenolics, TEAC, DPPH·-scavenging and ORAC assays[A].

Spice	Total phenolics[B] (mg GAE/g extract solids)	TEAC[C] (mmol TE/g extract solids)	DPPH-scavenging[D] (mg extract solids/ml)	ORAC[C] (mmol TE/g extract solids)
Garlic	9.08 ± 0.27[e]	4.8 ± 0.3[d]	>10.00[a]	105 ± 6[d]
Leek	21.2 ± 0.34[a]	107 ± 15.2[a]	1.75 ± 0.06[d]	203 ± 1[b]
Onion	7.09 ± 0.12[f]	20.4 ± 0.8[cd]	8.78 ± 0.43[b]	107 ± 9[d]
Shallot	10.5 ± 0.41[d]	31.3 ± 4.5[c]	3.71 ± 0.03[c]	138 ± 9[c]
Welsh onion	11.1 ± 0.33[c]	15.7 ± 6.7[cd]	3.82 ± 0.03[c]	134 ± 4[c]
Chili	11.0 ± 0.24[c]	78.0 ± 18.9[b]	1.59 ± 0.02[d]	139 ± 8[c]
Ginger	20.5 ± 0.20[b]	106 ± 12.1[a]	0.92 ± 0.03[e]	215 ± 5[a]

[A] The values are expressed as means ± S.D. of triplicate tests. Means not sharing a common letter in the same column were significantly different ($p < 0.05$) when analysed by ANOVA and Duncan's multiple-range test.
[B] The total phenolics content was expressed as milligrammes of gallic-acid equivalents (GAE) per gramme of extract solid.
[C] The TEAC and ORAC were expressed as millimoles of trolox equivalents (TE) per g of extract solid.
[D] The DPPH-radical-scavenging capacity was expressed as the IC_{50}, which denoted the concentration of spice extracts that scavenged 50% of DPPH radical present.

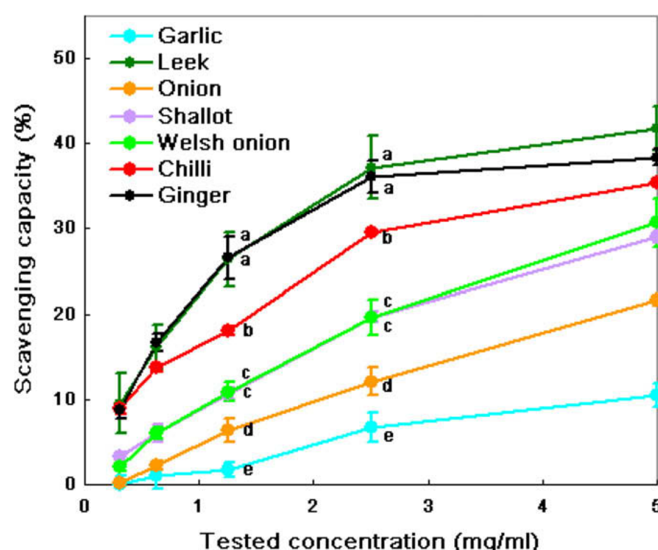

Fig. 1. NO-scavenging capacity of the aqueous spice extracts. The values are expressed as means ± S.D. of triplicate tests. Means not sharing a common letter at the same tested concentration were significantly different ($p < 0.05$) when analysed by ANOVA and Duncan's multiple-range test.

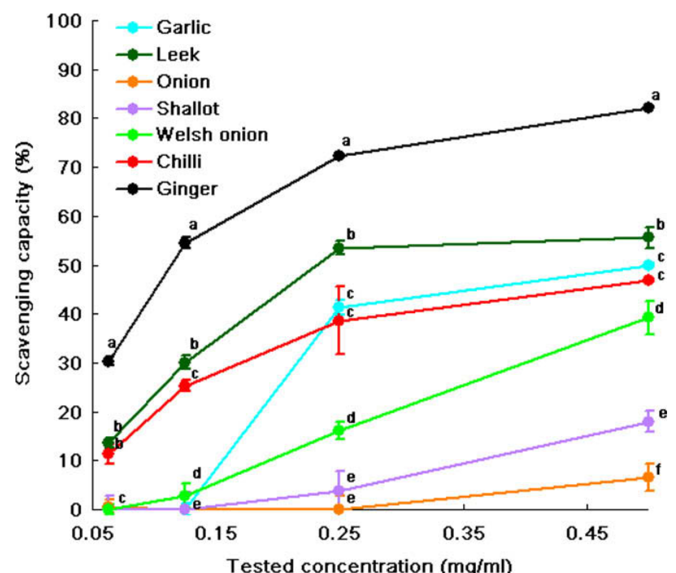

Fig. 2. $O_2^{\cdot-}$-scavenging capacity of the aqueous spice extracts. The values are expressed as means ± S.D. of triplicate tests. Means not sharing a common letter at the same tested concentration were significantly different ($p < 0.05$) when analysed by ANOVA and Duncan's multiple-range test.

sponse curve at concentrations less than 2.5 mg/ml. At a concentration of 2.5 mg/ml, the order of the NO-scavenging capacity was leek (37.2%) and ginger (36.1%) > chilli (29.6%) > shallot (14.6%) and Welsh onion (14.6%) > onion (12.1%) > garlic (6.7%).

3.4. $O_2^{\cdot-}$-scavenging capacities of the aqueous spice extracts

The $O_2^{\cdot-}$-scavenging capacities of the tested spices are shown in Fig. 2. Except for the garlic, all of the spices had a typical dose–response curve. Garlic extracts at concentrations below 0.125 mg/ml showed no $O_2^{\cdot-}$-scavenging capacity. However, garlic extracts had a moderate $O_2^{\cdot-}$-scavenging capacity (40–50%) at concentrations of 0.25 and 0.5 mg/ml. Most of the spice extracts showed a linear dose–response curve at concentrations below 0.25 mg/ml. Therefore, we compared the $O_2^{\cdot-}$-scavenging capacities at concentrations of 0.125 mg/ml, and the order of the results was ginger (54.5%) > leek (30.0%) > chilli (25.3%) > Welsh onion (2.8%) > shallot (−0.5%), onion (−0.6%) and garlic (−0.9%).

3.5. Peroxynitrite-scavenging capacities of the aqueous spice extracts

The peroxynitrite-scavenging capacities of the aqueous spice extracts were evaluated on the basis of their ability to attenuate the nitrotyrosine formation induced by peroxynitrite in albumin. As shown in Fig. 3, after addition of peroxynitrite to BSA, a significant nitrotyrosine formation was observed. Also, the level of peroxynitrite-induced nitrotyrosine formation under such conditions was attenuated by the addition of various spice extracts. At a concentration of 5 mg/ml, the aqueous extracts of ginger, leek and chilli almost completely inhibited the formation of nitrotyrosine after addition of peroxynitrite to BSA. Furthermore, the peroxynitrite-scavenging capacities of spice extracts were evaluated by using a lower concentration (1 mg/ml) of the spice extract. Thus, we were able to rank the spices according to their ability to attenuate the formation of nitrotyrosine. The order of the results was ginger (98.4%) > leek (75.1%) > chilli (62.6%) > Welsh onion (44.0%) and garlic (43.7%) > shallot (40.2%) > onion (24.7%).

3.6. Correlation between flavonoid contents and antioxidant, radical-scavenging and peroxynitrite-scavenging capacities

Correlations amongst the total flavonoid contents, the antioxidant capacities, the NO-scavenging activity, $O_2^{\cdot-}$-scavenging activity, and peroxynitrite-scavenging activities of the spice extracts are shown in Table 3. We found that the flavonoid content of a particular spice not only highly correlated with the antioxidant capac-

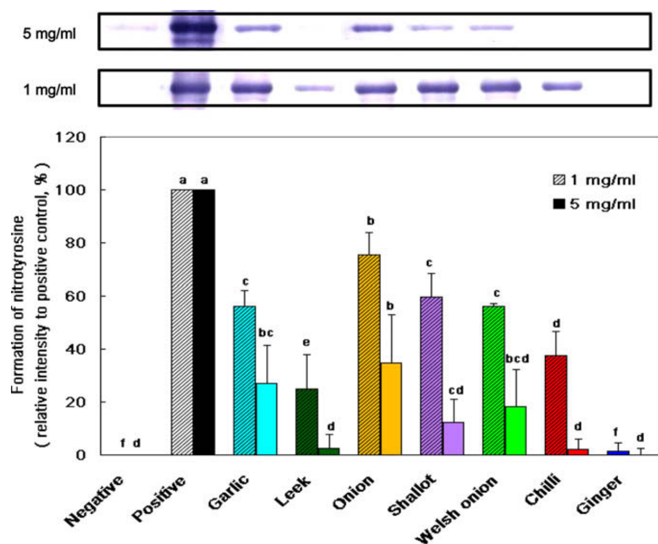

Fig. 3. Peroxynitrite-scavenging capacity of the aqueous spice extracts. The peroxynitrite-scavenging capacity of spice aqueous extract was evaluated, based on the inhibitory activity to peroxynitrite-induced nitrotyrosine formation in BSA. The values are expressed as means ± S.D. of triplicate tests. Means not sharing a common letter at the same tested concentration were significantly different ($p < 0.05$) when analysed by ANOVA and Duncan's multiple-range test.

Table 3
Correlation coefficients of total phenolics and flavonoids with antioxidant, radical-scavenging and peroxynitrite-scavenging capacities.

	Total phenolics	Total flavonoids
	Coefficient (probability)	
TEAC	0.88* (0.009)	0.98* (0.000)
DPPH-scavenging	−0.72 (0.069)	−0.78* (0.037)
ORAC	0.98* (0.000)	0.93* (0.003)
NO-scavenging[a]	0.77* (0.044)	0.80* (0.030)
O_2^--scavenging[b]	0.78* (0.040)	0.79* (0.034)
Peroxynitrite-scavenging[c]	0.91* (0.005)	0.96* (0.001)

* Presents significant correlation ($p < 0.05$).
[a] The NO-scavenging capacity of different spice extract at tested concentration of 2.5 mg/ml was used in correlative analysis.
[b] The O_2^--scavenging capacity of different spice extract at tested concentration of 0.125 mg/ml was used in correlative analysis.
[c] The peroxynitrite-scavenging capacity of different spice extracts at tested concentration of 1 mg/ml was used in correlative analysis.

ities, but also highly correlated with the NO, O_2^-, or peroxynitrite-scavenging activities. Similarly, except for DPPH·-scavenging activity, total phenolics also highly correlated with the antioxidant capacities, NO-scavenging, O_2^--scavenging, or peroxynitrite-scavenging activities.

4. Discussion

In this study, we evaluated the peroxynitrite-scavenging abilities of several different spices by measuring their inhibition effects on peroxynitrite-mediated tyrosine nitration. Of the tested spices, ginger extracts had the best effect. Leek and chilli extracts had moderate scavenging activities, whereas extracts of onion, shallot, garlic and Welsh onion had poor scavenging activities. Most importantly, the peroxynitrite-scavenging abilities of spice extracts correlated significantly with their phenolic or flavonoid contents. According to previous study, a high correlation between the peroxynitrite-scavenging abilities and the phenolic contents of methanolic extracts of some dried spices was also observed (Ho et al., 2008). These results indicate that the phenolic and flavonoid compounds of certain spices were responsible for the inhibition effects on peroxynitrite-mediated tyrosine nitration *in vitro*.

Phenolic compounds can attenuate peroxynitrite-induced tyrosine nitration, either by donating electron to reduce peroxynitrate, or by acting as an alternative substance of nitration (Kerry & Rice-Evans, 1999). When reacting with peroxynitrite, catechol-containing flavonoids yield oxidative products, whereas flavonones form nitrated products (Pollard et al., 2006). The peroxynitrite-scavenging activity of flavonoids depends largely on the structural features, including the number of hydroxyl groups, a catechol group in the B-ring, a hydroxyl group at the 3-position, and the 2,3 double bond (Santos & Mira, 2004). Moreover, the peroxynitrite-scavenging activity of flavonoids can be accurately predicted by calculating the enthalpy for tyrosyl radical repair by flavonoids (Sadeghipour, Terreux, & Phipps, 2005) and the energy of the highest occupied molecular orbital and net charge upon C3′ and C4′ (Calgarotto et al., 2008). Although divergent results were presented in some cases, the structural features and electrical variables needed for high peroxynitrite-scavenging activity were identified. In most cases, they are crucial, affecting other antioxidant activity (Amić et al., 2007). Therefore, high correlations between the peroxynitrite-scavenging abilities and antioxidant activities were observed.

Although the major active components responsible for the peroxynitrite-scavenging activities of the spice extracts were not identified in this study, many studies have provided useful information for further investigation. Two major pungent phenolics, 6-gingerol and gingerone, have been identified as powerful peroxynitrite-scavengers and are regarded as contributors to ginger's peroxynitrite-scavenging ability (Ippoushi, Azuma, Ito, Horie, & Higashio, 2003; Shin, Kim, Chung, & Jeong, 2005). Sinapoyl, feryloyl glycoside, capsaicin and dihydrocapsaicin are predominant antioxidant compounds in chilli (Materska & Perucka, 2005). Therefore, these compounds are expected to have peroxynitrite-scavenging abilities and are being investigated in our laboratory. Kaempferol and quercetin are the most abundant flavonoids in the green leaves and bulbs of *Allium* spices, respectively (Aoyama & Yamamoto, 2007), and their contents have been shown to reflect their antioxidant activities, based on the inhibition of lipid peroxidation and scavenging of the DPPH radical (Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003). The high kaempferol content of leeks (Nuutila et al., 2003) might explain why the peroxynitrite-scavenging ability of leeks is stronger than that of the other tested *Allium* spices. Quercetin 3,4-diglycoside and quercetin 4-glycoside account for approximately 90% of the total flavonol content of *Allium* species (Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2008). Although the peroxynitrite-scavenging activity of flavonoids is weakened by glycosylation, we believe that these two quercetin glycosides (quercetin 3,4-diglycoside and quercetin 4-glycoside), because of their abundance, would be the best peroxynitrite-scavengers in the aqueous extracts of *Allium* species.

In addition to flavonoids, organosulphur compounds are also recognised as an important class of antioxidant components of *Allium* plants (Yin & Cheng, 1998). Some organosulphur compounds have been shown to indirectly improve the antioxidant status of some pathological conditions by elevating antioxidant enzyme activities, such as catalase, superoxide dismutase and glutathione peroxidase (Borek, 2001; Hsu, Huang, Hung, & Yin, 2004). However, the antioxidant ability, especially radical-scavenging, of organosulphur compounds, is still controversial. Xiao and Parkin (2002) reported that thiosulphinates and related compounds are probably key contributors to the H_2O_2, O_2^- and DPPH-radical-scavenging activities of onion and garlic. Moreover, because the peroxynitrite-scavenging capacity of raw garlic could not be eliminated by heating, thiosulphinates (heat-susceptible substances) were reasonably excluded as peroxynitrite-scavenging compounds (Pedraza-Chaverri, Medina-Campos, & Segoviano-Murillo, 2007).

In addition, *s*-allylcysteine showed a lower peroxynitrite-scavenging ability than that of the reference compound penicillamine (Medina-Campos et al., 2007). It seems that organosulphur compounds did not make a contribution to the peroxynitrite-scavenging action of *Allium* spices.

The peroxynitrite-scavenging ability of plant phenolic compounds, especially flavonoids, is crucial to the cardioprotective effects associated with high fruit and vegetable intakes (MaCarty, 2008). The present study provides, for the first time, evidence that the peroxynitrite-scavenging ability of fresh spices depends upon their phenolic content, especially the flavonoid content. Although onion was not the most powerful peroxynitrite-scavenging spice tested in the present study, it is a major source of flavonoids, especially flavonols, in Western diets. Approximately 2% of the flavonol content of onion was detected in the urine and blood after consumption. This indicates that flavonoids in onion are readily bioavailable (Mullen, Boitier, Stewart, & Crozier, 2004). Therefore, we conclude that the phenolics and flavonoids absorbed from these spices, after consumption, might have peroxynitrite-scavenging ability *in vivo*.

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