Characterization of Antioxidant Properties of Common Spices and the Effect of Thermal Treatment on their Antioxidant Activity

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Abstract

A total of 16 spices including 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 Roscoe), mustard (Brassica alba 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) were used in this experiment to characterize antioxidant properties including polyphenols and flavonoids. Among the tested spices, clove contained the highest amount of phenolics such as eugenol, acetyleugenol, gallic acid and flavonoids (quercetin, kaempferol glycosides and quercetin glycosides). Phenol contents such as gallic acid, quercetin, piperine, ferulic acid and caffeic acid were found increased after heating treatment. Significant increment was found in gallic acid for clove and allspice, ferulic acid for turmeric, caffeic acid for coriander and piperine for black pepper and white pepper. Most of the phenolic components of spices were found stable during heat treatment at 100°C.

Keywords: Polyphenol; Antioxidant; Spices; Thermal Treatment

Introduction

Polyphenols are the most abundant antioxidants in human diets. The main classes of polyphenols are phenolic acids and flavonoids. Phenolic acids are hydroxy cinnamic acids, hydroxy benzoic acids etc. Flavonoids are represented a subclass of polyphenols with a C6-C3-C6 backbone structure. Phenolic compounds act as antioxidant to scavenge reactive oxygen species and chelate metals. They have been known to possess a wide range of physiological functions, such as antiallergic, anti-inflammatory, antimicrobial, and antioxidant effects [1,2]. Epidemiological studies have indicated an inverse correlation between coronary heart disease risk and intake of flavonoids [3]. Hashim, et al. [4] reported that phenolic compounds effectively suppress hydrogen peroxide-induced oxidative stress.

Spices have been widely used as flavoring, coloring and seasoning agents and as preservatives from the past. The volatile oils of black pepper, clove and nutmeg have inhibitory effects against 25 different genera of bacteria including animal and plant pathogen, food poisoning and spoilage bacteria [5]. Takikawa, et al. [6] reported antibacterial activity of nutmeg against Escherichia coli O157. Spices have also

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been used as folk medicines in many traditional cultures. The powdered nutmeg is used as warming agent, a remedy against dysentery, colic and as a stimulant for the treatment of chronic rheumatism in China. In Indonesia, mace is used as medicine for rheumatism [7]. Spices have inhibitory effect on lipid peroxidation [8,9].

Use of spices are increasing day by day for preparing many kinds of foods such as snacks, chocolates, candies and other delicious dishes. Most of the dishes containing spices are boiled in water for long time at around 100°C. Therefore, active components of spices may be affected by thermal cooking. Phenolic compounds seem to act as major active components of spices. Khatun., et al. [10] observed a highly positive correlation (R² = 0.97) between antioxidant activity and total phenol contents. Shan., et al. [11] reported a positive correlation between total phenol content and antioxidant activity. There are many researches regarding antioxidant activity of spices. Most of these studies were carried out using organic solvents [11] characterized major phenolic components of 25 spices using methanol extract. However, studies relevant to thermal cooking in aqueous system have scarcely seen. Furthermore, studies on the effect of thermal treatment on individual polyphenols of spices are insufficient. Availability of phenolic components in aqueous system and effects of thermal treatment on phenolic components need to evaluate. This study was carried out in 20% ethanol solution as a model system of boiling. Use of commercial spice powder was another characteristic of our experiment, whereas most of the previous experiments were performed using fresh edible part or dried plant materials of spices. Drying and processing may affect active components of spices. During drying and processing, active components of ginger (gingerols) are easily dehydrated to shogaols or degrade to zingerone and shogaols are somewhat unstable, degrade to zingerone [12]. Curcumin, active component of turmeric, is degraded easily in the presence of light [13]. In this experiment, we evaluated available active components from commercial spice powder in 20% ethanol and observed the changes in individual polyphenols of spices during heating for long time.

Materials and Methods

Sodium dihydrogenphosphate, dimethyl sulfoxide (DMSO), ferulic acid, 3,5-dimethoxy-4-hydroxycinnamic acid and gallic acid were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Caffeic acid, chlorogenic acid, p-hydroxycinnamic acid, coumarin, piperine, eugenol and rutin were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Acetyleneugenol was purchased from Tokyo Kasei Kogyo Co., Ltd. and myristicin was purchased from sigma Aldrich Inc. Quercetin, luteolin and luteolin-7-glucoside were obtained from Extrasynthese, Genay, France. All organic solvents were of HPLC grade.

Sixteen spices were used in this experiment as follows: 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 Roscoe), mustard (Brassica alba 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 (powder) were provided by House Foods Co. (Osaka, Japan).

Thermal treatment

Each spice (1g) was thoroughly mixed with 20% ethanol (20 ml) in a screw-capped test tube to prevent an evaporated loss of active components. Then the test tube was placed at 100°C for 1 to 6h in a thermally controlled oven. Sample without heating (0h) was used as control.

Extraction

After thermal treatment, the tubes were allowed to cool, and then the spices were extracted twice with 20 ml of 20% aqueous ethanol by shaking for 30 minutes and centrifuging at 1500×g for 20 minutes at 4°C. The supernatant was combined and filled up to 50 ml in a
volumetric flask, then filtered by 0.45 µm filter (Cosmonice Filter W, 25 mm, Nacalai Tesque Inc., Japan). Individual polyphenol content was measured by using this extract solution.

**Determination of individual polyphenol**

Individual polyphenols were determined using HPLC according to the method of Sakakibara, *et al.* [14]. The HPLC analysis was carried out on a Capcell pak C18 UG120 (5 µm mesh, 4.6 × 250 mm, Shiseido, Tokyo, Japan) with a guard column (4.6 mm Ø × 10 mm), equipped with a Hitachi model L-7100 LaChrom pump and a Hitachi model L-7455 LaChrom diode array detector set at 200 - 600 nm at 35°C temperature. Two mobile phases were used for this analysis. The mobile phase A was consisted of 10% methanol with 90% 50 mM sodium dihydrogen phosphate buffer and the mobile phase B was consisted of 70% methanol with 30% 50 mM sodium dihydrogenphosphate buffer (pH 3.3). The flow rate of mobile phase was 1 ml/min and was distributed as follows: A mobile phase initially 100%, 70% A for next 15 minutes, 65% A for another 30 minutes, 60% A for next 20 min, 50% A for next 5 min, and finally 0% A for 25 min. The injection volume of samples was 10 µl. Phenolic acids (hydroxy benzoic acid and hydroxycinnamic acid), phenolic volatile oils (eugenol, acetylgenolen, myristicin), flavonoids (flavonols, flavones) were identified by comparison of retention time of peak and spectra of corresponding standards. Data were calculated and expressed as µmol/g spice powder. Standard curves were made from each corresponding standard of the known phenolics. Hydrolysis was performed when individual phenol showed similar spectra but different retention time compared to respective standard. It was supposed that these phenols of spices might be an ester or glycoside. Both ester and glycoside were hydrolyzed to form aglycon. However, non-hydrolyzed samples were used for quantitative analysis. Phenolic volatile oils were detected at 280 nm, cinnamic acids, phenolic amides (piperine) and flavones were detected at 320 nm and flavonols were detected at 370 nm.

**Hydrolysis**

Spices were subjected to hydrolysis by a modification of the method of Hertog, *et al.* [15]. Spice powder (50 mg) was placed in test tube and added 4 ml of 62.5% aqueous methanol containing 0.5 mg/ml of tert-butylhydroquinone and 1 ml of 2N HCl. The tube was heated in sealed condition at 90°C for 2 hrs, and then sample was extracted with two volumes of ethyl acetate. The extract was dried under a nitrogen gas stream, dissolved in 500 µl DMSO, then filtered by 0.45 µm filter and analyzed by HPLC.

**Statistical analysis**

Statistical analysis was performed using Student’s t-test. Differences of treatments at p < 0.05 were considered to be significantly different.

**Results and Discussion**

**Qualitative and quantitative determination of phenolic components in spices**

Individual phenolic components and their quantities in spices are shown (Table 1). Phenolic volatile oils, phenolic acids and flavonoids were identified and quantified in spices according to retention time of peak and UV spectral characteristics of phenolic standards.

<table>
<thead>
<tr>
<th>List of the spices</th>
<th>Individual polyphenol content (µmol/g dry weight)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenolic acid</td>
</tr>
<tr>
<td>Clove</td>
<td>Eugenol</td>
</tr>
<tr>
<td></td>
<td>Acetylgenolen</td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
</tr>
<tr>
<td></td>
<td>Other flavonoidc</td>
</tr>
<tr>
<td>Allspice</td>
<td>Eugenol</td>
</tr>
<tr>
<td></td>
<td>Acetylgenolen</td>
</tr>
</tbody>
</table>

As shown in table 1, content of phenolic volatile oils (eugenol and acetyleneugenol) was high in clove and allspice. Clove and allspice contained 236.8, 64.6 µmol/g eugenol and 31.8, 21.1 µmol/g acetyleneugenol, respectively. Shan, et al. [11] reported that clove contained high content of eugenol and acetyleneugenol and hydroxyl group of phenolic volatile oils can play the most important role for high radical-scavenging activity of this spice. Kikuzaki, et al. [16] isolated eugenol, vanillin, cinnamaldehyde etc. from the berries of allspice (Pimenta dioica). In this study, eugenol (1.2 µmol/g) and acetyleneugenol (10.6 µmol/g) were also observed in nutmeg and mace, respectively. Phenolic volatile oil, myristicin, was identified in nutmeg (38.7 µmol/g) and mace (9.4 µmol/g).

Gallic acid 67.8 and 12.5 µmol/g was quantified in clove and allspice, respectively. Shan, et al. (2005) reported that high content of gallic acid in clove and catechol structure of gallic acid can play the most important role for high radical-scavenging activity of this spice. Kikuzaki, et al. [16] isolated eugenol, vanillin, cinnamaldehyde etc. from the berries of allspice (Pimenta dioica). In this study, eugenol (1.2 µmol/g) and acetyleneugenol (10.6 µmol/g) were also observed in nutmeg and mace, respectively. Phenolic volatile oil, myristicin, was identified in nutmeg (38.7 µmol/g) and mace (9.4 µmol/g).

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<table>
<thead>
<tr>
<th>Spice</th>
<th>Major individual polyphenols</th>
<th>Glycosides</th>
<th>Phenolic acid</th>
<th>Flavonoid</th>
<th>Quercetin glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon</td>
<td>Gallic acid 12.5 ± 1.0</td>
<td>67.8 ± 1.0</td>
<td>18.8 ± 0.6</td>
<td>3.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Myristicin 36.8 ± 3.4</td>
<td>36.8 ± 3.4</td>
<td>36.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mustard</td>
<td>Eugenol 1.2 ± 0.2</td>
<td>3,5-dimethoxy-4-hydroxy cinnamic acid ester 36.2 ± 1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumin</td>
<td>Chlorogenic acid 0.9 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>4.1 ± 0.3</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Ginger</td>
<td>Other phenolic acid 8.7 ± 0.9</td>
<td>9.4 ± 0.5</td>
<td>10.6 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fennugreek</td>
<td>Phenolic acid tr</td>
<td>9.4 ± 0.5</td>
<td>10.6 ± 1.2</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Red pepper</td>
<td>Myristicin 9.4 ± 0.5</td>
<td>36.8 ± 3.4</td>
<td>36.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mace</td>
<td>Acetyleneugenol 10.6 ± 1.2</td>
<td>36.8 ± 3.4</td>
<td>36.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coriander</td>
<td>Chlorogenic acid 0.5 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>4.1 ± 0.3</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>Caffeic acid 0.1 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>4.1 ± 0.3</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Cardamom</td>
<td>p-hydroxycinnamic acid 0.5 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>4.1 ± 0.3</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Black Pepper</td>
<td>Piperine 9.6 ± 0.1</td>
<td>9.4 ± 0.5</td>
<td>10.6 ± 1.2</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>White Pepper</td>
<td>Piperine 7.2 ± 0.3</td>
<td>9.4 ± 0.5</td>
<td>10.6 ± 1.2</td>
<td>3.3 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Major phenolic compounds in spices.

Turmeric is commonly used in curry and many snacks for making natural color. It is well known that curcumin is the main phenolic component in turmeric. In 1985, Tonnessen and Karlsen [18] reported that curcumin molecules were extremely unstable at pH more than 7 and were formed ferulic acid, feruloylmethan as the first degradation steps [19]. In this study, we identified ferulic acid (0.7 µmol/g), p-hydroxy cinnamic acid (0.5 µmol/g) suggesting that curcumin was degraded. A little amount of p-hydroxy cinnamic acid (0.1 µmol/g) and a high content of 3,5-dimethoxy-4-hydroxycinnamic acid ester (36.2 µmol/g) were quantified in cardamom and mustard, respectively. Chung., et al [20] isolated 3,5-dimethoxy-4-hydroxycinnamic acid as an ester from brown mustard (Brassica nigra).

Gingerol is known to be main phenolic component of ginger. It is reported that gingerol, pungent principle of ginger, is easily dehydrated to form the corresponding shogaol during drying and processing [12,21]. In this study, only one phenol component (major peak) was identified in ginger. This phenol was quantified as eugenol equivalent and shown as “other phenolic acid”. It might be shogaol because ginger powder was used in our experiment. Therefore, gingerol might be dehydrated to shogaol. Cai., et al [22] reported that gingerols and shogaols were the major phenolic compounds in ginger.

Piperine was the common phenol in black pepper and white pepper. Black pepper and white pepper contained 9.6 and 7.2 µmol/g piperine, respectively (Table 1). Epstein., et al [23] isolated piperine from black pepper. Red pepper is popular spice in many countries. Vanillic acid and eriodictyol were found in red pepper after hydrolysis. A little amount of phenolic acids was observed in aqueous extract solution, but it could not be quantified. Capsaicin is the main component of red pepper and vanillic acid might be degrading component of capsaicin. Parejo., et al [24] isolated eriodictyol-7-O-rutinoside in 50% methanol extract of fennel.

Chlorogenic acid (0.5 - 0.9 µmol/g) and caffeic acid (0.1 - 0.4 µmol/g) were quantified in coriander and cumin (Table 1). In 2001 Justesen and Knuthesen [25] identified quercetin and kaempferol in coriander. Luteolin glycosides (4.1 µmol/g) and luteolin (3.3 µmol/g) were also quantified in cumin. Shan., et al [11] observed caffeic acid and kaempferol in cumin, but we could not find kaempferol in this study.

Quercetin glycoside was quantified in clove (5.3 µmol/g), allspice (3.3 µmol/g) and fennel (0.9 µmol/g). Parejo., et al [24] isolated twelve major phenolic compounds including flavonoids such as isoquercitrin, kaempferol glycosides and rutin from fennel. Quercetin (0.2 µmol/g) and kaempferol glycosides (1.7 µmol/g) were also quantified in clove. Another flavonoid was detected in clove but it could not be confirmed by authentic standard. It was shown as “other flavonoid” (5.8 µmol/g) as quercetin equivalent. In fenugreek, the quercetin equivalent flavonoid that expressed as “other flavonoid” (5.5 µmol/g) was quantified. Shan., et al [11] quantified quercetin (28.4 mg/100g of dry weight) and kaempferol (23.8 mg/100g of dry weight) in clove.

The content of phenolic acid (cinnamic acid, chlorogenic acid, caffeic acid) of spices was higher than many vegetables and fruits such as potato (1.9 µmol/100g, chlorogenic acid), cabbage (11.1 µmol/100g, chlorogenic acid), apple (4.8 - 35 µmol/100g, chlorogenic acid), peach (12 - 15 µmol/100g, chlorogenic acid), asparagus (1.3 - 5.7 16 µmol/100g caffeic acid and 1.7 - 16 µmol/100g cinnamic acid), broccoli (9.4 µmol/100g caffeic acid and 2.8 µmol/100g chlorogenic acid) etc. as reported by Sakakibara., et al [14]. The content of flavonoids of spices is also comparable to that of many vegetables and fruits [14]. The content of quercetin glycosides of clove and allspice were higher than most of vegetables and fruits including onion (92 - 178 µmol/100g), which is called a rich source of quercetin glycosides. Kaempferol glycosides were also higher than radish (27 µmol/100g), broccoli (6.3 µmol/100g), cabbage (1.6 µmol/100g) etc. The content of luteolin and its glycosides of spices were higher than that of vegetables and fruits. Therefore, from the study, it was concluded that spices were rich source of phenolic compounds.

Effect of thermal treatment on individual polyphenols of spices

The effect of thermal treatment on individual polyphenols of spices was investigated and shown in table 2a and 2b. In this study, phenolic volatile oils (eugenol and acetyleneugenol), main components of clove and allspice, did not change after heating (Table 2a). Tomaino.,
et al. [26] also found the antioxidant activity of clove and cinnamon oil did not change after 3 hrs heating at 180°C. The amount of gallic acid increased significantly after heating in both the spices clove and allspice (Table 2a). It is known that gallic acid is easily soluble in water. Therefore, the content of gallic acid might be increased due to release of more amount of gallic acid from disrupted cell wall and cell components after heating.

### Table 2a: Effect of thermal treatment on individual polyphenols of spices.

<table>
<thead>
<tr>
<th>Heating time</th>
<th>Individual polyphenol content (μmol/g spice)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Clove</strong></td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
</tr>
<tr>
<td>0h</td>
<td>67.8 ± 5.0</td>
</tr>
<tr>
<td>1h</td>
<td>67.8 ± 1.0</td>
</tr>
<tr>
<td>3h</td>
<td>95.8 ± 4.6</td>
</tr>
<tr>
<td>6h</td>
<td>105.2 ± 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heating time</th>
<th>Individual polyphenol content (μmol/g spice)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Cumin</strong></td>
</tr>
<tr>
<td></td>
<td>Gallic acid</td>
</tr>
<tr>
<td>0h</td>
<td>12.5 ± 1.0</td>
</tr>
<tr>
<td>1h</td>
<td>16.2 ± 2.0</td>
</tr>
<tr>
<td>3h</td>
<td>22.3 ± 2.6</td>
</tr>
<tr>
<td>6h</td>
<td>28.8 ± 1.4</td>
</tr>
</tbody>
</table>

The results are the mean of 3 determinations ± SD. *: Major individual polyphenols were quantified by external standards of corresponding known individual polyphenols. b: Glycosides were quantified by corresponding aglycon equivalent. c: Unknown/unconfirmed phenolic acids and flavonoid were calculated into total amounts of “other phenolic acid” and “other flavonoid. CR-Changing rate.
It was observed that the amount of quercetin glycosides and kaempferol glycosides decreased but the amount of quercetin (aglycon) increased in clove, showing the glycosides were hydrolyzed to form aglycon quercetin. Quercetin glycosides showed the same tendency in allspice (Table 2a). In 1964 Pratt and Watts [27] reported that flavonoid present in living cells, as glycosides may be breakdown by enzyme, acid or heat treatment to form their aglycon and sugar, being in agreement with our results. We also observed that “other flavonoid” of clove increased gradually after heating (Table 2a). Approximately 2 times increase was found in this flavonoid after 6-h heating.

An increase of radical-scavenging activity and total phenol content was found in clove and allspice [30]. In 2000, Shobana and Akhilender [28] reported that the bound antioxidants might be released due to heat treatments resulting that the higher antioxidant activity compared to that of fresh spices extract. Maeda., et al. [29] reported that thermal treatment might be destroyed the cell wall and sub cellular compartments of vegetables to liberate more amounts of components, or thermal chemical reaction might cause to produce more potent radical-scavenging components.

Myristicin was the main phenol of nutmeg and mace. Myristicin was decreased in nutmeg, but increased significantly in mace after heating (Table 2a). Acetyleugenol, another phenol of mace also increased. Tomaino., et al. [26] reported that the amount of active components (safrol and myristicin) of nutmeg increased after 3 hrs heating. Therefore, myristicin might not be affected by heat. We observed nutmeg and fenugreek were coagulated after heating. Such coagulation was found severely in fenugreek. Phenols of nutmeg and fenugreek were affected badly after 1-h heating (Table 2a and 2b). Our previous findings also coincided with this result. A decrease of the DPPH radical-scavenging activity was observed in nutmeg and fenugreek [30]. The content of amino acids and protein are rich in fenugreek. In 1994, Mansour and El-Adawy [31] reported that fenugreek is a good source of amino acids and protein (albumin and globulin). After heating, protein might have denatured, resulting that extraction ability of phenols was decreased due to coagulation.

In case of ginger, phenol content was constant after 3-h heating but a decrease was observed after 6-h heating (Table 2b), suggesting that phenol of ginger was somewhat stable to heat. Chlorogenic acid was the main phenolic acid of coriander. The amount of chlorogenic acid decreased during heating. On the other hand, amount of caffeic acid increased significantly after heating (Table 2b). It is known that chlorogenic acid is an ester of caffeic acid and easily hydrolyzed to caffeic acid, showing that caffeic acid is more stable to heating than chlorogenic acid. Cuvelier., et al. [32] reported that antioxidative potency of caffeic acid is higher than chlorogenic acid. Chlorogenic acid might have changed to caffeic acid therefore; coriander showed an increasing activity after heating [30]. In case of cinnamon, content of coumarin was constant after 3-h heating but it decreased significantly after 6h heating (Table 2b). A study was conducted with sixteen spices including cinnamon and observed that radical-scavenging activity of this spice decreased after heating [30].

Amount of ferulic acid and p-hydroxy cinnamic acid of turmeric increased gradually after heating (Table 2b). Therefore, degraded products of curcumin of turmeric were stable after heating. Content of chlorogenic acid, luteolin and luteolin-glycoside decreased in cumin after heating (Table 2b). Among these polyphenols, luteolin decreased drastically after 3 to 6-h heating. Though luteolin was an aglycon, it decomposed after heating, suggesting that luteolin itself was unstable during heating in cumin. On the other hand, a significant change of chlorogenic acid was found after 6-h heating but change of luteolin glycoside was insignificant in cumin. The amount of piperine increased gradually in black pepper and white pepper (Table 2b). The DPPH radical-scavenging activity of turmeric (3 times), black pepper (2 times), cardamom and white pepper were observed to increase after 6-h heating [30]. This increasing tendency might be due to increase of active components such as ferulic acid for turmeric, piperine for black and white pepper in extract solution. In 2002, Dewanto., et al. [33] found that thermal processing disrupts the cell membranes and cell walls to release lycopene from the insoluble portion of tomato, resulting that the antioxidant activity increased in tomato. A little amount of flavonoid (quercetin glycoside) was present and insignificant decreasing tendency was observed in fennel. Phenol of mustard (3,5-dimethoxy-4-hydroxycinnamic acid ester) was found to change slightly. Therefore, these phenols seem to be somewhat stable in heating.

**Conclusion**

From the results of this study, the content of simple phenols and flavonoids of spices were higher than many vegetables and fruits. Spices may be a potential source of phenols besides fruits and vegetables. Most of phenols of spices were relatively stable during thermal cooking at around 100°C even for long time. Phenolic volatile oils are usually evaporated after heating, but our experiment by using screw-capped vial might be reduced the evaporation. Therefore, it can suggest that cover can help to protect the loss of volatile phenolic components during cooking of spicy dishes. Gallic acid for clove and allspice, ferulic acid for turmeric and piperine for black pepper and white pepper increased with thermal treatment in aqueous solution. In conclusion, spices would be expected as a valuable food ingredient for the maintenance of human health.

**Bibliography**


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