Effects of Cooking on Antioxidant Activities and Polyphenol Content of Edible Mushrooms Commonly Consumed in Thailand

Aikkarach Kettawan, Kunlaya Chariekhla, Ratchanee Kongkachuchai and Rin Charoen Siri
Laboratory of Food Chemistry, Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

Abstract: The effect of boiling on ten edible mushroom varieties was analyzed for antioxidant activities by three different assays (ORAC, FRAP and DPPH) and polyphenol content. The results indicated that the boiling process significantly decreased both antioxidant activities and polyphenol content in all mushroom varieties. Antioxidant activities and the polyphenol content of boiled mushrooms can be ranked from high to low as A. hygrometricus > A. Cylindracea > V. volvacea > L. edodes > P. eryngii. Percent true retention of ORAC, FRAP, DPPH and polyphenol content in cooked tissue of all mushrooms ranged from 21-75%, 17-68%, 23-69% and 21-70%, respectively with the highest value in A. hygrometricus. Antioxidant activities and polyphenol contents were released into the cooking water was approximately 9-31%, 9-50%, 10-48% and 10-39% for ORAC, FRAP, DPPH and polyphenol, respectively. Percent loss of ORAC, FRAP, DPPH values and polyphenol content caused by thermal effect were about 9-64%, 8-60, 5-57% and 13-59%, respectively. Significantly positive correlations were observed between polyphenol and antioxidant activities in both raw and cooked mushrooms. The difference of characteristic structure and shape of each mushroom variety could affect the different loss of antioxidant activities and the polyphenol during the cooking process. Since mushrooms have been used as food and food flavoring material in soups for many years, consuming boiled mushroom tissues as well as their broths to recover some polyphenol and antioxidant activity is recommended.

Key words: Boiling, mushroom, antioxidant activity, ORAC, DPPH, FRAP, polyphenol

INTRODUCTION
Environmental factors, food and genetic traits can cause several chronic degenerative diseases, such as cardiovascular disease, cancer, immune-system decline and cataracts (Smith et al., 2007). These diseases are also associated with oxidative damaging effects induced by free radicals, such as Reactive Oxygen Species (ROS) accumulation in the human body (Fraga et al., 1990; Harman, 1981). There is much evidence that the consumption of plant foods, such as fruits and vegetables, provides protection against various diseases, especially chronic degenerative diseases (Hertog et al., 1993; Steinmetz and Potter, 1991; Law and Morris, 1998). This protection can be explained by the free-radical scavenging capacity of antioxidants in plant foods. Plant foods are a good source of polyphenols, which have been reported to be effective radical scavengers and inhibitors of lipid peroxidation (Ohira et al., 1998; Mekem and Kong, 2001). Many Thai foods are composed of plants and vegetables as the main ingredients that could be beneficial to human health. Mushrooms are also popular in Thai food because of their flavors and textures. Mushrooms contain low amounts of fat, high amounts of protein and high amounts of dietary fiber (Manzi et al., 1999), which could be suitable in consumption for good health. The medicinal properties of mushrooms have been recognized for a long time. For example, shiitake mushrooms (Lentinula edodes [Berk.] Sing) have been reported to have antitumor effects (Breene, 1990) and a blood cholesterol lowering effect in both animal tests (Kaneda and Tokuda, 1966) and human studies (Suzuki and Onshima, 1976), which can decrease the risk of cardiovascular diseases (Chang, 1996). Yanagimatsutake mushrooms (Agrocybe cylindracea [DC.: Fr.]: Mre.) might play a role in the prevention of cancer and may act as antitumor, antihypercholesterolemia and antihyperlipidemia agents (Shon and Nam, 2001; Wasser and Weis, 1999). The Jews ear mushroom (Auricularia auricular Judae) and white jelly fungus (Tremella fuciformis Berk) have hypcholesterolemic activity in rats (Cheung, 1996). These edible mushrooms are very popular in Thailand. However, the data on the polyphenol content, antioxidant activities and the changes after cooking of these edible mushrooms have been limited when compared with other plant foods. Several assays have been developed to estimate the total antioxidant capacity in foods. The test methods of antioxidant capacity are basically divided into two groups of reaction mechanisms: the hydrogen atom transfer reaction and the single electron transfer reaction (Huang et al., 2005; Prior et al., 2005). However,
no single method or official standardized method is adequate for evaluating the antioxidant capacity of foods because different methods can give widely diverging results. Thus, it was suggested that each evaluation on antioxidant activity should be done with various methods based on different mechanisms and measurement techniques (Zulueta et al., 2009; Frankel and Meyer, 2000). The aim of this study was to determine the effect of boiling on polyphenol contents and antioxidant activities by three different methods, including the correlation between polyphenol content and antioxidant activities in edible mushrooms.

MATERIALS AND METHODS
Sample collection and preparation: All mushroom varieties in this study were verified for types and species by associate professor Taworn Vinjisanun, a taxonomic specialist of mushrooms in the Department of Agricultural Science at Mahidol University. The information about each mushroom variety is shown in Table 1. Ten edible mushroom varieties were purchased from 5 representative markets in Bangkok, Thailand. Approximately 500 g of each mushroom variety obtained from each market was washed with tap water to remove sand, dirt and debris and then rinsed again with 300 ml deionized water. Each mushroom sample was divided into 2 portions, one portion was retained fresh (raw), while the second portion of approximately 160 g was cooked by boiling in deionized water at a ratio 1.3 w/w of mushroom to deionized water. The boiling process was done according to a standardized cooking time for each kind of mushroom, which was determined by five cooking specialists by testing each kind of mushroom with different boiling times and observing its texture after boiling. Standardized cooking times were 15 s. for F. velutipes, 30 s. for A. auricular and T. fuciformis, 1 min for P. eryngii, V. volvacea V. P. sauro-caju and L. edodes, 2 min for A. cymbidaceae and P. cystidiosus and 4 min for A. hygrometricus. Cooked mushroom and cooking water (broth) of each mushroom sample were weighed to be used for calculating the percent of true retention, percent recovery and percent loss of polyphenol contents and antioxidant activities. In this study, polyphenol contents and antioxidant activities in raw, boiled and broth samples of each mushroom variety were determined in duplicate analysis for five individual samples from five different markets. All raw and boiled mushroom samples were individually homogenized by an electric blender (400 W 1.75 L, HR2021/75, Philips, Thailand) and stored at -20°C until analyzed.

Moisture content analysis: All raw and boiled mushroom samples were analyzed for moisture content by drying in a hot air oven at 100±5°C to a constant weight (AOAC, 2005).

Sample extraction for determination of total antioxidant activity: The sample size is 0.5 g for raw or cooked homogenized mushroom or 10 g for mushroom broth. Each sample was extracted with 20 ml of 50% acetone (Merck KGA #1.00014.2500) in deionized water (v/v) on a mechanical shaker at 400 rpm at room temperature (25-28°C) for 1 h. Afterwards, the mixture was centrifuged at 4,400 g for 15 min. Finally, the supernatant was collected and analyzed for total antioxidant activity by ORAC, FRAP and DPPH assays.

Oxygen Radical Absorbance Capacity (ORAC) assay: The ORAC assay measures the ability of antioxidant compounds in test materials to protect against oxidation induced by the peroxyl radical generator AAPH. The antioxidant activity of the sample extract was measured according to the method described by Huang et al. (2002). AAPH, 2,2-azobis (2-amidinopropane) dihydrochloride (Aldrich # 440914), was used as a peroxyl radical generator. Trolox, 6-hydroxy-2,5,7,8-tetramethylenchroman-2-carboxylic acid (Aldrich # 238813), a hydrophilic derivative of vitamin E, was used as a standard. The standard curve concentration was kept linear between 6.25 and 100 µmol by diluting Trolox with 75 mM phosphate buffer (pH 7.2). Reaction mixtures containing 3 ml of 4.19 µM Fluorescein solution in 75 mM phosphate buffer (pH 7.2) was mixed with either 0.5 ml of extracted sample, a Trolox standard solution, or a blank (75 mM phosphate buffer, pH 7.2) and pre-incubated for 10 min at 37°C. Exactly 0.5 ml of 153 mM AAPH solution was added, then the immediate loss of fluorescence was measured by a spectrofluorometer (Perkin-Elmer LS 55 luminescence spectrometer) at the excitation and emission wavelengths of 493 and 515 nm, respectively. The results were expressed as micromole Trolox equivalent per gram of sample (µmol TE/g).

Ferric Reducing Antioxidant Power (FRAP) assay: FRAP was evaluated by a spectrophotometer method according to Benzie and Strain (1993). A total of 1 ml of each extracted sample was added to 3 ml of FRAP reagent, which was prepared freshly as required by mixing 300 mM acetate buffer pH 3.6, 10 mM HCL containing TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM FeCl3.6H2O in the ratio of 10:1:1 (v/v/v). The mixture was incubated for 4 min at 37°C. Trolox, 6-hydroxy-2,5,7,8-tetramethylenchroman-2-carboxylic acid (Aldrich # 238813), was used as a standard. Trolox standard solution was prepared by diluting Trolox powder in deionized water and the standard curve was linear between 6.25 and 100 µmol concentration. Test solution absorbance was measured at 593 nm by spectrophotometer (Shimadzu UV-1601 UV-VIS) and compared with that of the Trolox standard solution. The results were expressed as micromole Trolox equivalent per gram of sample (µmol TE/g).
<table>
<thead>
<tr>
<th>Common name</th>
<th>Local (Thai) name</th>
<th>Scientific name</th>
<th>Image in cm. scale</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden needle</td>
<td>Hed Khem Thong</td>
<td>Flammulina velutipes (Curt, ex Fr.) Sing</td>
<td></td>
<td>Small button-shaped caps, white long and thin stripes</td>
</tr>
<tr>
<td>Jew’s Ear or tree ear</td>
<td>Hed Hoo Noo Si Na Tan</td>
<td>Auricularia auricular-judaee</td>
<td></td>
<td>Similar to an ear with purple to dark brown or black color, soft, jelly-like shape</td>
</tr>
<tr>
<td>White Jelly fungus</td>
<td>Hed Hoo Noo Khao</td>
<td>Tremella fusiformis Berk</td>
<td></td>
<td>Silky smooth texture after soaking water, like white flower</td>
</tr>
<tr>
<td>King Oyster</td>
<td>Hed Nang Rom Loung</td>
<td>Pleurotus eryngii (Cand. Ex Fr.) Quel.</td>
<td></td>
<td>Thick, meaty white stem and a small tan cap</td>
</tr>
<tr>
<td>Straw mushroom</td>
<td>Hed Fang</td>
<td>Volvariella volvacea (Bull. Ex.Fr.) Sing</td>
<td></td>
<td>White, egg-like shaped fruiting body, at early stage and becomes bell shape later</td>
</tr>
<tr>
<td>Shiitake</td>
<td>Hed Horm</td>
<td>Lentinus edodes (Berk.) Pegler</td>
<td></td>
<td>Tan to dark brown color, umbrella-shaped caps, wide open veils</td>
</tr>
<tr>
<td>Sarjor-caju</td>
<td>Hed Nang Fa</td>
<td>Pleurotus sajor-caju (Fr.) Sing</td>
<td></td>
<td>Similar shape to P. cystidiosus, meaty cap with white to light brown color, white stripes and gills</td>
</tr>
<tr>
<td>Yanagimat-sutake</td>
<td>Hed Kon Yee Pun</td>
<td>Agrocybe cylindracea (DC: Fr.) Mre. (Bolbitiaceae)</td>
<td></td>
<td>Fruiting bodies have brown closed caps and white long stripes</td>
</tr>
<tr>
<td>Abalone</td>
<td>Hed Pao Hue</td>
<td>Pleurotus cystidosus Miller</td>
<td></td>
<td>Grey to dark grey cap, but underside have white color, solid and firm stripe</td>
</tr>
<tr>
<td>Barometer Earthstars</td>
<td>Hed Phor</td>
<td>Astraeus hygrometricus (Pers.) Morgan</td>
<td></td>
<td>Circle shape with brown to dark brown color, a little hard and solid stage inside</td>
</tr>
</tbody>
</table>
DPPH free radical scavenging assay: The hydrogen atom-or electron-donation ability of the sample extracts was measured by the bleaching of purple-colored methanol solution of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH; Wako # 047-04051) according to the method of Burris and Bucar (2000) and Cuendet et al. (1997) with some modifications. The supernatant of the extracted sample was diluted to various concentrations with absolute methanol. A total of 1 ml of each diluted sample solution was added with 1 ml of 0.2 mM DPPH radical solution in methanol, while the sample blank of each diluted sample solution was added with 1 ml of absolute methanol. The mixture was left at room temperature for 45 min in the dark and then, the absorbance of the solution was measured at 517 nm with a Helios Beta UV-Vis Spectrophotometer. Ascorbic acid was used as a standard by dissolving and diluting with deionized water. The standard curve was linear between 20-300 mg/ml concentration. Antioxidant activity was expressed as mg of ascorbic acid equivalents per gram of sample (mg AA/g).

Determination of polyphenol content: Polyphenol content was determined according to the method of Brenna and Pagliarini (2001). Approximately 3 g of raw or boiled homogenized sample or 10 ml of mushroom broth was extracted with 25 ml of 50% N,N-dimethylformamide (DMF; Carlo Erba # 444923) by a mechanical shaker at 400 rpm at room temperature (25-28°C) for 18 hr. The mixture was centrifuged at 130 g for 10 min and the supernatant was used for polyphenol content analysis. A total of 25 ml of each sample extraction was mixed with 125 µl of 10% Folin-Ciocalteu reagent and 100 µl of 0.5 M of aqueous sodium hydroxide. The mixture was left in the dark at room temperature for 15 min. The absorbance of colored product was measured at 750 nm by a microplate reader (TECAN sunrise microplate reader, Austria). Gallic acid was used as a standard by diluting with 50% DMF to a 10-80 µg/ml concentration. The polyphenol content was expressed as milligram gallic acid equivalents per 100 g sample (mg GAE/100 g).

Calculation of percent true retention, percent recovery and percent loss: True retention of antioxidant activity and polyphenol content in boiled mushrooms was calculated as described by Murphy et al. (1975) using the following formula:

\[
\text{True retention (\%) = } \frac{g \text{ of cooked mushroom } \times g \text{ of cooked mushroom}}{g \text{ of raw mushroom } \times g \text{ of raw mushroom}} \times 100
\]

The recovery of antioxidant activity and polyphenol content in mushroom broth was calculated using the following formula:

\[
\text{Recovery (\%) = } \frac{\text{Antioxidant activity value or polyphenol content}}{\frac{g \text{ of mushroom broth } \times g \text{ of mushroom broth}}{100}} \times \frac{\text{Antioxidant activity value or polyphenol content}}{\frac{g \text{ of raw mushroom } \times g \text{ of raw mushroom}}{100}}
\]

The loss of antioxidant activity and polyphenol content was calculated using the following formula:

\[
\text{Loss (\%) = } \frac{\text{Antioxidant activity value or polyphenol content}}{\frac{g \text{ of cooked mushroom } \times g \text{ of cooked mushroom}}{100}} \times \frac{\text{Antioxidant activity value or polyphenol content}}{\frac{g \text{ of raw mushroom } \times g \text{ of raw mushroom}}{100}}
\]

Statistical analysis: The data of moisture contents, polyphenol contents and antioxidant activities were expressed as the means ± Standard Deviations (SD) from five individual samples of duplicate analysis. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 17 for Windows 98, SPSS Inc. The mean values of antioxidant activity and polyphenol content between raw or boiled samples were analyzed by one-way Analysis of Variance (ANOVA). Pearson’s correlation coefficient (r) was used to calculate the relationship between the polyphenol contents and the antioxidant activity values (ORAC, FRAP and DPPH assays) of raw or boiled mushrooms. The difference was considered statistically significant at the level of p<0.05 and p<0.01.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP</td>
<td>Inexpensive, simple</td>
<td>Measures the reducing capacity of antioxidants, but not directly radical scavenging activity measurement</td>
</tr>
<tr>
<td>ORAC</td>
<td>Uses biologically relevant free radicals such as peroxyl radical, integrates both degree and time of antioxidant reaction</td>
<td>Use expensive equipment, data variability can be large across equipment, long analysis time, temperature-control problems can lead to intra-assay variability</td>
</tr>
<tr>
<td>DPPH</td>
<td>Inexpensive and easy to use, high correlation with ORAC</td>
<td>Slow reaction, requires long times to quantify results (45 min), color interference may lead to underestimation results</td>
</tr>
</tbody>
</table>

Table 2: Summary of advantages and disadvantages of the antioxidant activity methods.
Table 3. Antioxidant activity and polyphenol content in raw and boiled edible mushrooms

<table>
<thead>
<tr>
<th>Name</th>
<th>Condition</th>
<th>Moisture (g/100 g)</th>
<th>Antioxidant activity ORAC⁻¹</th>
<th>FRAP⁻²</th>
<th>DPPH⁻³</th>
<th>Polyphenol content (mg GAE/100 g)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. velutipes</td>
<td>Raw</td>
<td>91.6±1.2</td>
<td>13.60±0.62</td>
<td>1.29±0.20</td>
<td>0.33±0.03</td>
<td>93.4±7.4</td>
</tr>
<tr>
<td></td>
<td>Boiled, 15 s</td>
<td>92.5±0.6</td>
<td>4.26±0.49**</td>
<td>0.31±0.06**</td>
<td>0.08±0.02**</td>
<td>21.4±5.5**</td>
</tr>
<tr>
<td>A. auricular</td>
<td>Raw</td>
<td>82.0±0.9</td>
<td>2.15±0.69</td>
<td>1.39±0.40</td>
<td>0.05±0.01</td>
<td>29.5±11.0</td>
</tr>
<tr>
<td></td>
<td>Boiled, 30 s</td>
<td>91.6±2.2</td>
<td>0.79±0.23**</td>
<td>0.45±0.06**</td>
<td>0.02±0.00**</td>
<td>10.2±2.5**</td>
</tr>
<tr>
<td>T. fusiformis</td>
<td>Raw</td>
<td>87.7±4.3</td>
<td>1.96±0.27</td>
<td>0.47±0.17</td>
<td>0.06±0.01</td>
<td>27.8±5.6</td>
</tr>
<tr>
<td></td>
<td>Boiled, 30 s</td>
<td>90.3±1.6</td>
<td>0.91±0.04**</td>
<td>0.14±0.07**</td>
<td>0.01±0.00**</td>
<td>13.0±3.5**</td>
</tr>
<tr>
<td>P. eryngii</td>
<td>Raw</td>
<td>80.0±0.8</td>
<td>11.69±2.17</td>
<td>0.45±0.07</td>
<td>0.17±0.04</td>
<td>77.6±10.3</td>
</tr>
<tr>
<td></td>
<td>Boiled, 1 min</td>
<td>80.5±0.5</td>
<td>4.82±0.74**</td>
<td>0.26±0.11*</td>
<td>0.11±0.01*</td>
<td>33.8±4.9**</td>
</tr>
<tr>
<td>V. volvacea</td>
<td>Raw</td>
<td>83.5±0.9</td>
<td>18.5±0.96</td>
<td>2.32±0.14</td>
<td>0.68±0.10</td>
<td>155.4±18.3</td>
</tr>
<tr>
<td></td>
<td>Boiled, 1 min</td>
<td>80.3±1.3</td>
<td>8.54±1.47**</td>
<td>1.36±0.16**</td>
<td>0.30±0.07**</td>
<td>77.2±13.9**</td>
</tr>
<tr>
<td>L. edodes</td>
<td>Raw</td>
<td>81.9±1.2</td>
<td>18.39±1.30</td>
<td>2.05±0.32</td>
<td>0.59±0.12</td>
<td>129±15.3</td>
</tr>
<tr>
<td></td>
<td>Boiled, 1 min</td>
<td>69.8±1.1</td>
<td>4.13±1.30**</td>
<td>0.73±0.21**</td>
<td>0.20±0.05**</td>
<td>41.5±8.7**</td>
</tr>
<tr>
<td>P. Cajan</td>
<td>Raw</td>
<td>93.8±1.9</td>
<td>8.41±2.20</td>
<td>0.45±0.17</td>
<td>0.08±0.02</td>
<td>64.9±6.7</td>
</tr>
<tr>
<td></td>
<td>Boiled, 1 min</td>
<td>92.6±2.2</td>
<td>1.92±0.44**</td>
<td>0.21±0.07*</td>
<td>0.03±0.01*</td>
<td>15.6±3.2**</td>
</tr>
<tr>
<td>A. cylindracea</td>
<td>Raw</td>
<td>91.7±1.2</td>
<td>27.72±5.86</td>
<td>4.15±0.89</td>
<td>0.72±0.11</td>
<td>143.4±20.7</td>
</tr>
<tr>
<td></td>
<td>Boiled, 2 min</td>
<td>91.0±1.1</td>
<td>12.24±2.63**</td>
<td>1.89±0.50**</td>
<td>0.40±0.12*</td>
<td>55.0±14.0**</td>
</tr>
<tr>
<td>P. cystidicus</td>
<td>Raw</td>
<td>90.2±1.8</td>
<td>15.50±5.22</td>
<td>2.25±0.83</td>
<td>0.29±0.12</td>
<td>73.2±17.3</td>
</tr>
<tr>
<td></td>
<td>Boiled, 2 min</td>
<td>90.4±1.4</td>
<td>3.50±0.63**</td>
<td>0.54±0.27**</td>
<td>0.08±0.02**</td>
<td>19.9±3.2**</td>
</tr>
<tr>
<td>A. hygrometricus</td>
<td>Raw</td>
<td>77.1±3.0</td>
<td>25.10±5.92</td>
<td>4.32±0.19</td>
<td>0.41±0.09</td>
<td>110.2±8.6</td>
</tr>
<tr>
<td></td>
<td>Boiled, 4 min</td>
<td>76.3±3.8</td>
<td>20.79±2.14*</td>
<td>3.24±0.40*</td>
<td>0.30±0.04*</td>
<td>85.2±7.0*</td>
</tr>
</tbody>
</table>

¹Means±SD from analysis of five individual samples, in duplicate.
²Oxygen radical absorbance capacity (ORAC), expressed as micromole trolox equivalents per gram.
³Ferric reducing antioxidant power (FRAP), expressed as micromole trolox equivalents per gram.
⁴1,1-diphenyl-2-picrylhydrazyl (DPPH), expressed as milligram ascorbic acid equivalents per gram.

RESULTS

Antioxidant activity in raw mushrooms: The antioxidant activities of ten mushroom varieties determined by ORAC, FRAP and DPPH assays are shown in Table 3. The ORAC values of raw mushroom ranged from 1.96-27.72 μmol TE/g, with a ranking from high to low as A. Cylindracea > A. hygrometricus > V. volvacea > P. cystidicus > L. edodes. The FRAP values ranged from 0.45-4.32 μmol TE/g with the greatest value in A. hygrometricus, followed by A. Cylindracea, L. edodes, V. volvacea and P. cystidicus. The DPPH values of raw mushroom ranged from 0.05-0.72 mg AA/g, with ranking from high to low as A. Cylindracea > V. volvacea > L. Edodes > A. hygrometricus > F. velutipes, as shown in Table 3.

Polyphenol content in raw mushrooms: The polyphenol content of ten varieties of raw mushroom ranged from 27.8-143.4 mg GAE/100 g as shown in Table 3 and the ranking from high to low concentration was A. Cylindracea > V. volvacea > L. edodes > A. hygrometricus > F. velutipes, which was in the same order as that of DPPH scavenging activity. This result agreed with Huang et al. (2006) that A. cylindracea had a high antioxidant capacity in scavenging DPPH radicals and also a high content of polyphenols.

The loss of antioxidant activity and polyphenol content of mushrooms by boiling process: Antioxidant activities and polyphenol contents of boiled mushrooms with standardized cooking time are shown in Table 3. ORAC, FRAP, DPPH and the polyphenol content of boiled mushrooms ranged from 0.70-20.79 μmol TE/g, 0.14-3.24 μmol TE/g, 0.01-0.40 mg AA/g and 10.2-85.2 mg GAE/100 g, respectively and were ranked from high to low as A. hygrometricus > A. Cylindracea > V. volvacea > L. edodes > P. eryngii. The percent of true retention, percent recovery and percent loss of ORAC, FRAP, DPPH and polyphenols of mushrooms in each variety are shown in Fig. 1A, 1B, 1C and 1D, respectively. Percent true retention, which indicated the amounts of ORAC, FRAP, DPPH and total polyphenol content retained in each variety of mushrooms after boiling, ranged from 21-75%, 17-68%, 23-68% and 21-70%, respectively, with A. hygrometricus showing the highest percentage of true retention. Meanwhile, percent recovery, which indicated the amounts of antioxidant activities and total polyphenol that leached from raw mushrooms into mushroom broth, ranged from 9-31%, 9-50%, 10-48% and 10-39% for ORAC, FRAP, DPPH and polyphenol content, respectively. Finally, percent loss of ORAC, FRAP, DPPH values and polyphenol content caused by the thermal effect of boiling were about 9-64%, 8-60, 5-57% and 13-59%, respectively, with the highest percent loss in L. edodes.

DISCUSSION

Several plant food components contain phytochemicals, such as vitamin C, tocopherols, carotenoids and
polyphenols and their combined interactions contribute to the overall antioxidant activity of foods (Javanmardi et al., 2003; Pizzale et al., 2002). These compounds provide different mechanisms, such as radical scavenging and power reducing. Although each food sample shows high activity with a single activity measuring method, it does not always present similar results with all other methods (Pathamakanokporn et al., 2008; Pinelo et al., 2004; Arabshahi-Delouee and Urooj, 2007). Thus, three methods, such as ORAC, FRAP and DPPH, which are the most widely used to determine antioxidant activity of various food samples based on different principles and mechanisms (Wang and Zhang, 2003; Perez-Jimenez and Saura-Calixto, 2006; Amarowicz et al., 2004; Barros et al., 2007), were selected for the measurement of antioxidant activities in our present study. The advantages and disadvantages of these antioxidant activity methods are shown in Table 2 (Prior et al., 2003; Awika et al., 2003; Zulueta et al., 2009; Thaipong et al., 2006).
The data on the polyphenol content, antioxidant activities and the changes after cooking of edible mushrooms have been limited when compared with other plant foods. Our results indicated that the boiling process significantly decreased antioxidant activities and polyphenol contents in all mushrooms varieties (p<0.05 and p<0.01), which agreed with several studies that stated that any cooking process, such as boiling, baking and microwaving, reduced both the polyphenol content and the antioxidant activity in selected vegetables (Ismail et al., 2004; Zhang and Hamazuzu, 2004; Turkmen et al., 2005). In this study, there were no significant differences in moisture contents between raw and cooked mushrooms in each variety, implying that the decrease of antioxidant activities and polyphenol contents after boiling are not affected by the moisture content. Zhang and Hamazuzu (2004) reported that five minutes of boiling or microwave cooking retained 28.1% and 28.4% of the polyphenol content in broccoli, respectively, which agreed with our study. We found significant amounts of polyphenol and antioxidant activities in the cooking water of mushrooms, thus indicated that the cause of those loss was that polyphenols were largely leached into the cooking water. Racchi et al. (2002) found that the boiling process reduced the antioxidant activity in mushroom juice. Therefore, the antioxidant activity leached from mushroom tissue into cooking water may be lost during continuously cooking. The percent recovery and percent loss in this study indicated that the cooking process could induce the significant loss of antioxidant activity due to heat treatment and antioxidants largely leaching into the cooking water. However, the cooking time hardly related to the loss of antioxidant activity and polyphenol content of mushrooms tissue in different mushroom varieties. For example, four minutes of standardized boiling time for A. hygrometricus retained 75%, 68%, 66% and 70% of ORAC, FRAP, DPPH and polyphenol, respectively, while 15 s. of standardized boiling time for F. velutipes retained only 26%, 17%, 23% and 21% of those amounts, respectively. The loss of these antioxidants and their activities may be affected by specific characteristics, such as shape, color and texture, of the mushrooms in each variety. The characteristics and images of each mushroom variety, as shown in Table 1, indicated that A. hygrometricus has a brown circle shape (like a stone) and a little hard and solid stage inside, while F. velutipes has small button-shaped caps with white long and thin stripes. Therefore, the large surface area of F. velutipes directly exposed to cooking water is very easy to leaching and the loss of antioxidants and their activities by heat. This reason supported the report of Price et al. (1998) that the different loss of flavonols between boiled onion and broccoli tissues caused from the difference of their surface areas presented into the cooking water. Previously, several studies found a correlation between polyphenol content and antioxidant activity in plant foods (Ruiz et al., 2010; Gramza et al., 2008; Kiselova et al., 2006), while other studies did not (Kahkonen et al., 1999). For the example, Velioglu et al. (1998) found a strong relationship between polyphenol content and antioxidant activity in 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat and several fruits, vegetables and medicinal plants, while Ismail et al. (2004) reported no correlation between polyphenol content and antioxidant activity in...
Fig. 2: Correlation among polyphenol content in ten varieties of fresh edible mushrooms and various measurement of antioxidant activity. (A) ORAC (B) FRAP and (C) DPPH

Fig. 3: Correlation among polyphenol content in ten varieties of boiled edible mushrooms and various measurement of antioxidant activity. (A) ORAC (B) FRAP and (C) DPPH

selected vegetables, including kale, spinach, cabbage, swamp cabbage and shallots. In this study, the correlation between polyphenol content and antioxidant activities was evaluated in both raw mushroom and cooked mushroom tissues. Figure 2 (A-C) showed a linear correlation of polyphenol content in raw mushrooms versus ORAC, FRAP and DPPH values with positive correlations $r = 0.86, 0.62$ and $0.84$, respectively at $p<0.01$. Because ORAC and DPPH assays measured free radical scavenging activity whereas FRAP measured the reducing power of antioxidants (Huang et al., 2002; Cuendet et al., 1997; Perez-Jimenez and Saura-Calixto, 2006) and because polyphenol has strong free radical-scavenging activity (Proteggente et al., 2003) and the most antioxidant phytochemicals found in mushrooms (Alvarez-Parrilla et al., 2007), the correlation between polyphenol and ORAC ($r = 0.86$) or DPPH ($r = 0.84$) values were close and higher than the correlation between polyphenol and FRAP ($r = 0.62$) values. This result indicated that the polyphenols in selected mushrooms inhibited free radicals by the scavenging activity mechanism rather than the reducing power mechanism. Our results agreed with the report of Cheung et al. (2003) that high polyphenol content increased the amount of antioxidant activity with a positive correlation between the total polyphenol content in the mushroom and DPPH. Similar results were also found in boiled mushrooms with a linear relationship between polyphenol contents and ORAC, FRAP or DPPH values with positive correlations $r = 0.80, 0.74$ and $0.84$, respectively, at $p<0.01$, as shown in Fig. 3 (A-C). These results indicated that the boiling process induced the loss of antioxidant content and activities but did not change the correlation between polyphenol and antioxidant activities.

Conclusion: Among ten varieties of raw mushrooms, A. cylinndracea (Yanagimatsutake) had the highest amount of antioxidant activity and polyphenol content. The boiling process strongly decreased both the antioxidant activity and the polyphenol content in mushroom tissues by releasing large amounts of such content into the broth and also via the thermal effect. Boiled A. hygroptericus (Barometer Earthstars) had the highest percent retention.
of antioxidant activity and polyphenol content among all the other mushroom varieties. The antioxidant activities (ORAC, FRAP and DPPH) and polyphenol contents of boiled mushrooms were ranked from high to low as *A. hygrometricus* > *A. Cylindracea* > *V. volvacea* > *L. edodes* > *P. eryngi*. The characteristic structure of the mushroom itself could affect the loss of antioxidant activity and the polyphenol content during the cooking process. Since mushrooms have been used as food and food flavoring material in soups for many years, consuming boiled mushroom tissues as well as their broths to recover some polyphenol and antioxidant activity is recommended.

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