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

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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%, 23-66% 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 Konig, 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 Ohshima, 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 Jew's ear mushroom (*Auricularia auricular* Judae) and white jelly fungus (*Tremella fuciformis* Berk) have hypocholesterolemic 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,

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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 Vinijsanun, 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 180 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. safor-caju* and *L. edodes*, 2 min for *A. cylindracea* 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.

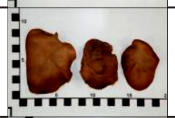
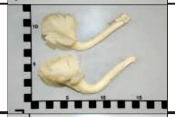
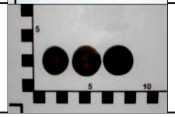
Moisture content analysis: All raw and boiled mushroom samples were analyzed for moisture content by drying in a hot air oven at 100±5°C to a constant weight (AOAC, 2005).

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 KGaA # 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-tetramethychroman-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 $FeCl_3.6H_2O$ 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-tetramethychroman-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 1: Common, local (Thai) and scientific names of selected mushrooms

Common name	Local (Thai) name	Scientific name	Image in cm. scale	Characteristics
Golden needle	Hed Khem Thong	*Flammulina velutipes* (Curt, ex Fr.) Sing		Small button-shaped caps, white long and thin stripes
Jew's Ear or tree ear	Hed Hoo Noo Si Na Tan	*Auricularia auricular*-judae		Similar to an ear with purple to dark brown or black color, soft, jelly-like shape
White Jelly fungus	Hed Hoo Noo Khao	*Tremella fusiformis* Berk		Silky smooth texture after soaking water, like white flower
King Oyster	Hed Nang Rom Loung	*Pleurotus eryngii* (Cand. Ex Fr.) Quel.		Thick, meaty white stem and a small tan cap
Straw mushroom	Hed Fang	*Volvariella volvacea* (Bull. Ex.Fr.) Sing		White, egg-like shaped fruiting body, at early stage and becomes bell shape later
Shiitake	Hed Hom	*Lentinus edodes* (Berk.) Pegler		Tan to dark brown color, umbrella-shaped caps, wide open veils
Sarjor-caju	Hed Nang Fa	*Pleurotus sajor-caju* (Fr.) Sing		Similar shape to *P. cystidiosus*, meaty cap with white to light brown color, white stripes and gills
Yanagimat-sutake	Hed Kon Yee Pun	*Agrocybe cylindracea* (DC: Fr.) Mre. (Bolbitiaceae)		Fruiting bodies have brown closed caps and white long stripes
Abalone	Hed Pao Hue	*Pleurotus cystidiosus* Miller		Grey to dark grey cap, but underside have white color, solid and firm stripe
Barometer Earthstars	Hed Phor	*Astraeus hygrometricus* (Pers.) Morgan		Circle shape with brown to dark brown color, a little hard and solid stage inside

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-picryl-hydrazyl (DPPH; Wako # 047-04051) according to the method of Burits 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:

$$\text{True retention (\%)} = \frac{\text{Antioxidant activity value or polyphenol content per g of cooked mushroom x g of cooked mushroom}}{\text{Antioxidant activity value or polyphenol content per g of raw mushroom x g of raw mushroom}} \times 100$$

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

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

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

$$\text{Loss (\%)} = \frac{\text{(antioxidant activity value or polyphenol content per g of cooked mushroom x g of cooked mushroom) + (antioxidant activity value or polyphenol content per g of mushroom broth x g of mushroom broth)}}{\text{(antioxidant activity value or polyphenol content per g of raw mushroom x g of raw mushroom)}} \times 100$$

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 2: Summary of advantages and disadvantages of the antioxidant activity methods

Methods	Advantages	Disadvantages
FRAP	• Inexpensive, simple • Not require specialize equipment • Fast reaction (4 min), requires short times to quantify result	• Measures the reducing capacity of antioxidants, but not directly radical scavenging activity measurement
ORAC	• Uses biologically relevant free radicals such as peroxyl radical • Integrates both degree and time of antioxidant reaction	• Use expensive equipment • Data variability can be large across equipment • Long analysis time • Temperature-control problems can lead to intra-assay variability
DPPH	• Inexpensive and easy to use • High correlation with ORAC	• Slow reaction, requires long times to quantify results (45 min) • Color interference may lead to underestimation results

Table 3: Antioxidant activity and polyphenol content in raw and boiled edible mushrooms

Name	Condition	Moisture (g/100 g)[1]	Antioxidant activity[1] ORAC[2]	FRAP[3]	DPPH[4]	Polyhenol content (mg GAE[5]/100 g)[1]
F. velutipes	Raw	91.5±1.2	13.60±3.62	1.26±0.20	0.33±0.03	93.4±7.4
	Boiled, 15 s.	92.1±0.6	4.20±0.49**	0.31±0.06**	0.08±0.02**	21.4±4.5**
A. auricular	Raw	92.0±0.9	2.15±0.69	1.36±0.40	0.05±0.01	29.5±11.0
	Boiled, 30 s.	91.6±2.2	0.76±0.23**	0.45±0.09**	0.02±0.00**	10.2±2.5**
T. fusiformis	Raw	87.7±4.3	1.96±0.27	0.47±0.17	0.06±0.01	27.8±5.6
	Boiled, 30 s.	90.3±1.6	0.91±0.04**	0.14±0.07**	0.01±0.00**	13.0±3.5**
P. eryngii	Raw	90.0±0.8	11.69±2.17	0.45±0.07	0.17±0.04	77.6±16.3
	Boiled, 1 min	89.5±0.5	4.82±0.74**	0.26±0.11*	0.11±0.01*	33.8±4.9**
V. volvacea	Raw	90.3±0.9	16.51±0.96	2.32±0.14	0.68±0.10	135.4±16.3
	Boiled, 1 min	90.3±1.3	8.54±1.47**	1.36±0.16**	0.30±0.07**	77.2±13.9**
L. edodes	Raw	91.0±1.2	16.39±4.30	2.65±0.32	0.59±0.12	129.1±15.3
	Boiled, 1 min	89.6±1.1	4.13±1.30**	0.73±0.21**	0.20±0.05**	41.5±8.7**
P. sajor-caju	Raw	93.8±1.9	8.41±2.20	0.45±0.17	0.08±0.02	64.9±5.7
	Boiled, 1 min	92.6±2.2	1.92±0.44**	0.21±0.07*	0.03±0.01*	15.6±3.2**
A. cylindracea	Raw	91.7±1.2	27.72±3.68	4.15±0.89	0.72±0.11	143.4±20.7
	Boiled, 2 min	91.0±1.1	12.24±2.63**	1.89±0.50**	0.40±0.12*	55.0±14.0**
P. cystidiosus	Raw	90.2±1.6	16.50±4.52	2.25±0.63	0.25±0.12	73.2±17.3
	Boiled, 2 min	90.4±1.4	3.59±0.63**	0.54±0.22**	0.08±0.02**	19.0±3.2**
A. hygrometricus	Raw	77.1±3.0	25.10±2.92	4.32±0.19	0.41±0.06	110.2±6.8
	Boiled, 4 min	76.1±3.8	20.79±2.14*	3.24±0.40*	0.30±0.04*	85.2±7.0*

[1]Mean±SD from analysis of five individual samples, in duplicate.
[2]Oxygen radical absorbance capacity (ORAC), expressed as micromole trolox equivalents per gram.
[3]Ferric reducing antioxidant power (FRAP), expressed as micromole trolox equivalents per gram.
[4]1,1-diphenyl-2-picryl-hydrazyl (DPPH), expressed as milligram ascorbic acid equivalents per gram.
[5]Gallic acid equivalent. *Significant differences of antioxidant activity values or polyphenol content between raw and boiled mushroom in the same variety ($p<0.05$), **Significant differences of antioxidant activity values or polyphenol content between raw and boiled mushroom in the same variety ($p<0.01$)

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. cystidiosus* > *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. volvace* and *P. cystidiosus*. 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 0.76-20.79 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%, 23-66% 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 (Patthamakanokporn *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).

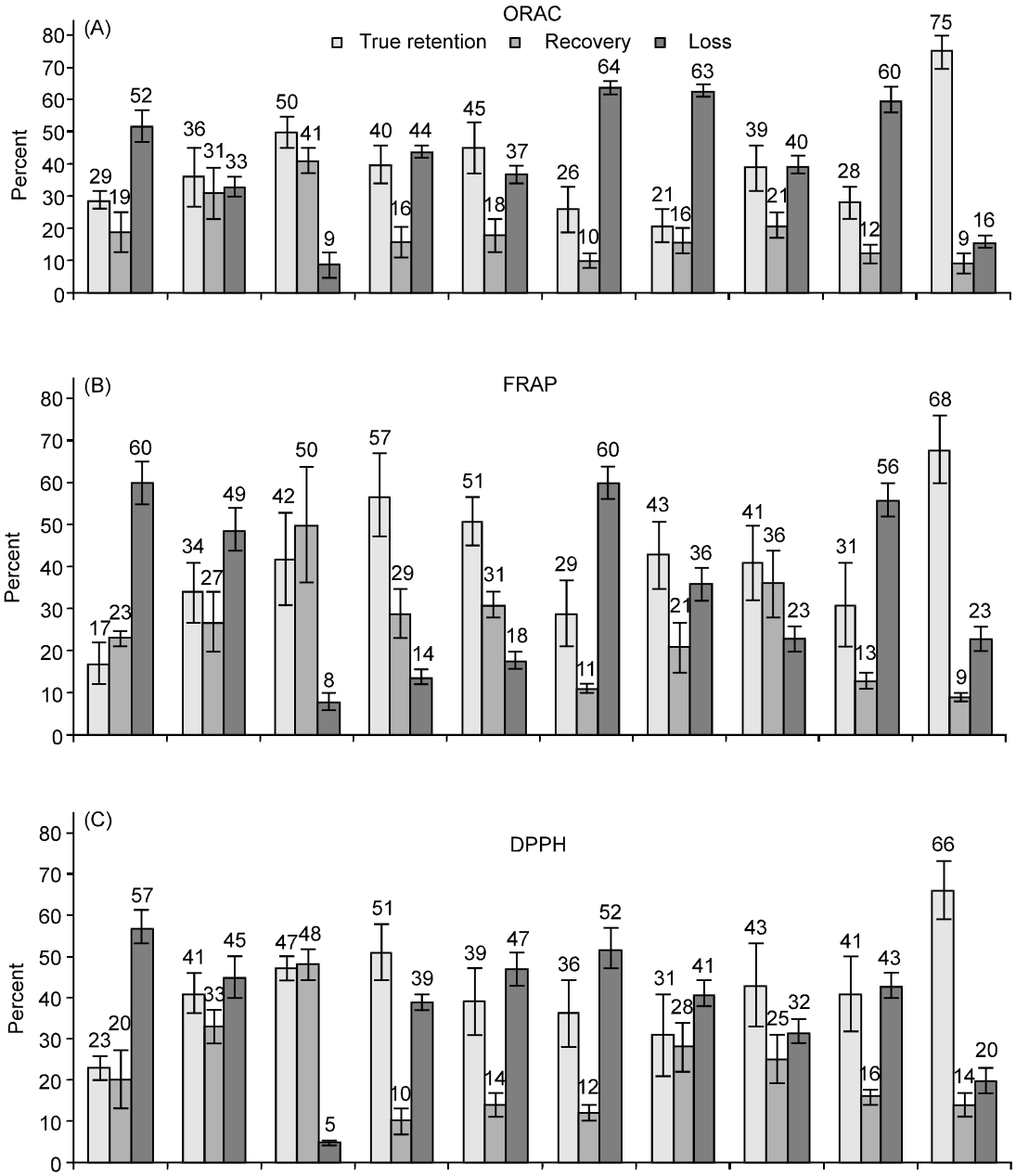


Fig. 1 Contd.

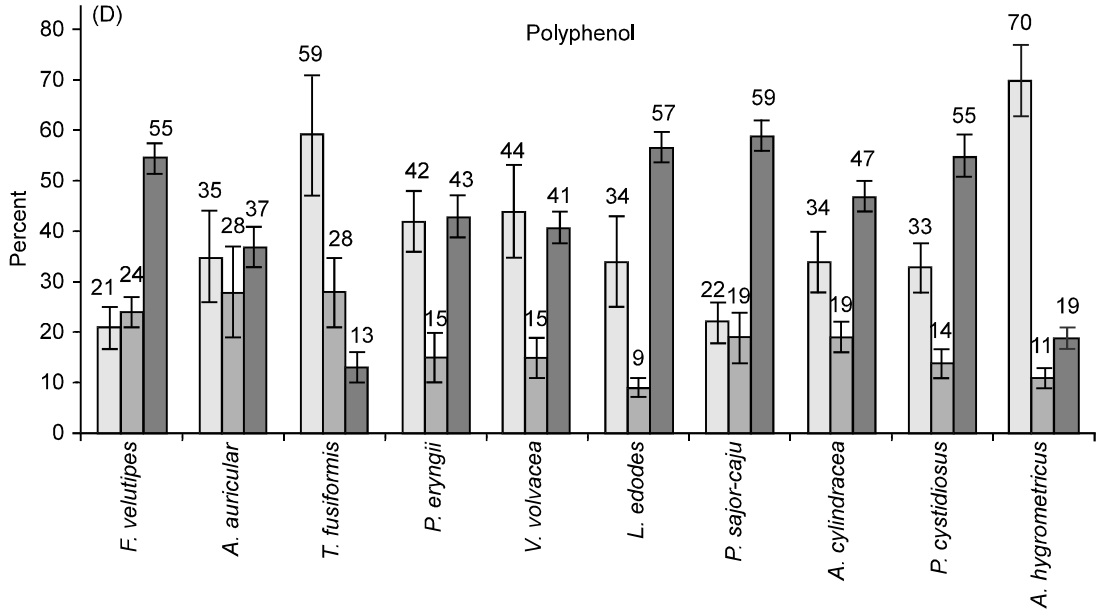


Fig. 1: Percent true retention, % recovery and % loss of ORAC (1A), FRAP (1B), DPPH (1C) and polyphenol (1D) in ten mushroom varieties after boiling. Data are expressed as means ± SD from 5 individual samples, in duplicate

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 Hamauzu, 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 Hamauzu (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 29%, 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*., 2006; 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

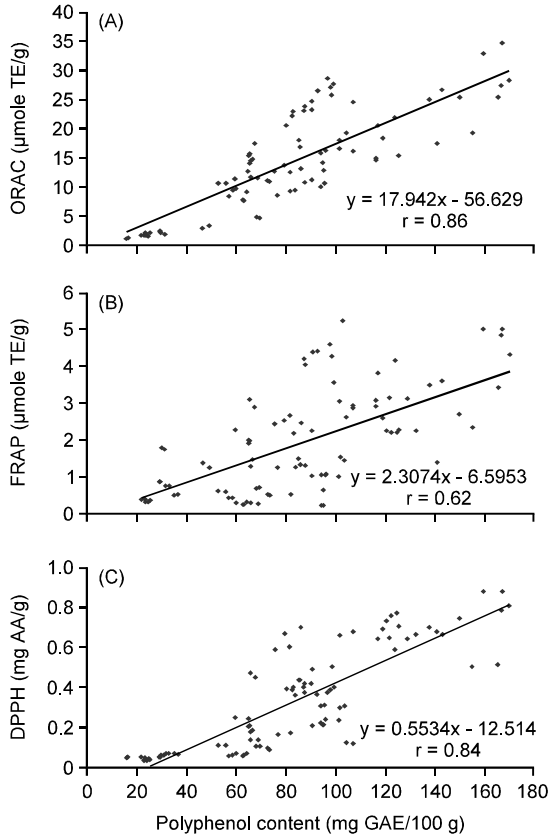


Fig. 2: Correlation among polyphenol content in ten varieties of fresh edible mushrooms and various measurement of antioxidant activity: (A) ORAC (B) FRAP and (C) DPPH

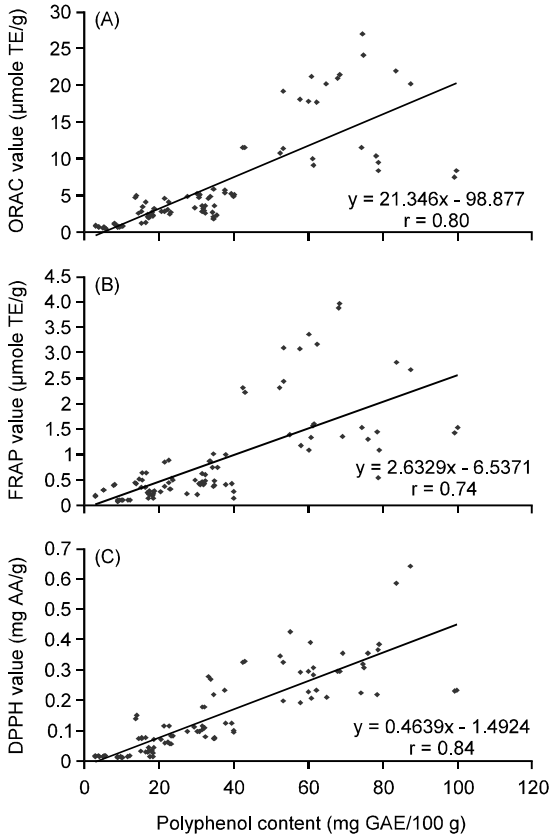


Fig. 3: Correlation among polyphenol content in ten varieties of boiled edible mushrooms and various measurement of antioxidant activity: (A) ORAC (B) FRAP and (C) DPPH

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. cylindracea* (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. hygrometricus* (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. eryngii*. 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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REFERENCES

Alvarez-Parrilla, E., L.A. de la Rosa and N.R. Martinez, 2007. Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico. Ciencia. Tecnol. Aliment., 5: 329-334.

Amarowicz, R., R.B. Pegg and P. Rahimi-Moghaddam, 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food. Chem., 84: 551-562.

Arabshahi-Delouee, S. and A. Urooj, 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica L.*) leaves. Food. Chem., 102: 1233-1240.

Association of Official Analytical Chemists, 2005. Determination of Moisture Content 950.46, Washington, D.C.

Awika, J.M., L.W. Rooney and X. Wu, 2003. Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products. J. Agric. Food. Chem., 51: 6657-6662.

Barros, L., M.J. Ferreira and B. QueirOs, 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portugese wild edible mushrooms and their antioxidant activities. Food. Chem., 103: 413-419.

Benzie, I.F. and J.J. Strain, 1993. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.

Breene, W.M., 1990. Nutritional and medicinal value of specialty mushrooms. J. Food. Protect., 53: 883.

Brenna, V.O. and E. Pagliarini, 2001. Multivariate analysis of antioxidant power and polyphenolic composition in red wines. J. Agric. Food. Chem., 49: 4841-4844.

Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res., 14: 323-328.

Chang, R., 1996. Functional properties of edible mushrooms. Nutr. Rev., 54: S91-93.

Cheung, L.M., P.C.K. Cheung and V.E.C. Ooi, 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food. Chem., 81: 249-255.

Cheung, P.C.K., 1996. The hypocholesterolemic effect of extracellular polysaccharide from the submerged fermentation of mushroom. Nutr. Res., 16: 1953-1957.

Cuendet, M., K. Hostettmann and O. Potterat, 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica. Chimica. Acta., 80: 1144-1152.

Fraga, C.G., M.K. Shigenaga and J.W. Park, 1990. Oxidative damage to DNA during aging: 8-hydroxy-20-deoxyguanosine in rat organ DNA and urine. Proceedings of the National Academy of Sciences of the United States of America, 87: 4533-4537.

Frankel, E.N. and A.S. Meyer, 2000. The problem of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. Sci. Food. Agric., 80: 1925-1941.

Gramza, A., S. Khokhar and S. Yoko, 2006. Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. Eur. J. Lipid. Sci. Technol., 108: 351-362.

Harman, D., 1981. The aging process. Proceedings of the National Academy of Sciences of the United States of America, 78: 7124-7128.

Hertog, M.G., E.J. Feskens and P.C. Hollman, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet, 342: 1007-1011. â

Huang, D.J., B.X. Ou and M. Hampsch-Woodill, 2002. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. J. Agric. Food. Chem., 50: 1815-1821.

Huang, D.J., B.X. Ou and R.L. Prior, 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food. Chem., 53: 1841-1856.

Huang, S.J., S.Y. Tsai and J.L. Mau, 2006. Antioxidant properties of methanolic extracts from Agrocybe cylindracea. Lebensmittel-Wissenschaft und-Technologie., 39: 378-386.

Ismail, A., Z.M. Marjan and C.W. Foong, 2004. Total antioxidant activity and phenolic content in selected vegetables. Food. Chem., 87: 581-586.

Javanmardi, J., C. Stushnoff and E. Locke, 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food. Chem., 83: 547-550.

Kahkonen, M.P., A.I. Hopia and H.J. Vuorela, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food. Chem., 47: 3954-3962.

Kaneda, T. and S. Tokuda, 1966. Effect of various mushroom preparations on cholesterol levels in rat. J. Nutr., 90: 371.

Kiselova, Y., D. Ivanova and T. Chervenkov, 2006. Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother. Res., 20: 961-965.

Law, M.R. and J.K. Morris, 1998. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease. Eur. J. Clin. Nutr., 52: 549-556.

Manzi, P., L. Gambelli and S. Marconi, 1999. Nutrients in edible mushrooms: An inter-species comparative study. Food. Chem., 65: 477-482.

Mekem, S.M. and W.A. Konig, 2001. Chemical study of the essential oil of *Cyperus rotundus*. Phytochemistry, 58: 799-810.

Murphy, E.W., P.E. Criner and B.C. Gray, 1975. Comparisons of methods for calculating retention of nutrients in cooked foods. J. Agric. Food. Chem., 23: 1153-1157.

Ohira, S., T. Hasegawa and K.I. Hayasashi, 1998. Sesquiterpenoids from *Cyperus rotundus*. Phytochemistry, 47: 577.

Patthamakanokporn, O., P. Puwastien and A. Nitithamyong, 2008. Changes of antioxidant activity and total polyphenol compounds during storage of selected fruits. J. Food. Comp. Anal., 21: 241-248.

Perez-Jimenez, J. and F. Saura-Calixto, 2006. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food. Res. Int., 39: 791-800.

Pinelo, M., L. Manzocco and M.J. Nunez, 2004. Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. J. Agric. Food. Chem., 52: 1177-1180.

Pizzale, L., R. Bortolomeazzi and S. Vichi, 2002. Antioxidant activity of sage *(Salvia officinalis* and *S. fruticosa)* and oregano *(Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. J. Sci. Food. Agric., 82: 1645-1651.

Price, K.R., F. Casuscelli and I.J. Colquhoun, 1998. Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. J. Sci. Food. Agric., 77: 468-472.

Prior, R.L., H. Hoang and L. Gu, 2003. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. J. Agric. Food. Chem., 51: 3273-3279.

Prior, R.L., X.L. Wu and K. Schaich, 2005. Standardized methods for the determination of antioxidant capacity and phenolic in foods and dietary supplements. J. Agric. Food. Chem., 53: 4290-4302.

Proteggente, A.R., S. Wisemen and F.H.M.M. van de Put, 2003. The relationship between the phenolic composition and the antioxidant activity of fruits and vegetables. In C.M. Rice-Evans and L. Packer (Eds.), Flavonoids in health and disease (2nd Edn.). Marcel Dekker Inc., New York.

Racchi, M., M. Daglia and C.A. Lanni, 2002. Antiradical activity of water soluble composition in common diet vegetables. J. Agric. Food. Chem., 50: 1272-1277.

Ruiz, A., I. Hermosín-Gutierrez and C. Mardones, 2010. Polyphenols and antioxidant activity of calafate (*Berberis microphylla*) fruits and other native berries from Southern Chile. J. Agric. Food. Chem., 58: 6081-6089.

Shon, Y.H. and K.S. Nam, 2001. Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. J. Ethnopharmacol., 77: 103-109.

Smith, E.M., J.T. Hoi and J.C. Eissenberg, 2007. Feeding Drosophila a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns. J. Nutr., 137: 2006-2012.

Steinmetz, K.A. and J.D. Potter, 1991. Vegetables, fruit and cancer. I. Epidemiology. Cancer Causes Control, 2: 325-357.

Suzuki, S. and S. Ohshima, 1976. Influence of shiitake (*Lentinus edodes*) on human serum cholesterol. Mushroom Sci., 9: 463.

Thaipong, K., U. Boonprakob and K. Crosby, 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food. Comp. Anal., 19: 669-675.

Turkmen, N., F. Sari and S. Velioglu, 2005. The effect of cooking methods on total phenolic and antioxidant activity of selected green vegetables. Food. Chem., 93: 713-718.

Velioglu, Y.S., G. Mazza and L. Gao, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and gain products. J. Agric. Food. Chem., 46: 4113-4117.

Wang, L.F. and H.Y. Zhang, 2003. A theoretical investigation on DPPH radical-scavenging mechanism of edaravone. Bioorganic Med. Chem. Lett., 13: 3789-3792.

Wasser, S.P. and A.L. Weis, 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspective (review). Int. J. Med. Mushrooms, 1: 31-62.

Zhang, D. and Y. Hamauzu, 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food. Chem., 88: 503-509.

Zulueta, A., M.J. Esteve and A. Frigola, 2009. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. Food. Chem., 114: 310-316.