

Influence of customized cooking methods on the phenolic contents and antioxidant activities of selected species of oyster mushrooms (*Pleurotus* spp.)

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Abstract Nutritional value of cooked food has been considered to be lower compared to the fresh produce. However, many reports showed that processed fruits and vegetables including mushrooms may retain antioxidant activity. *Pleurotus* spp. as one of the edible mushroom are in great demand globally and become one of the most popular mushrooms grown worldwide with 25-fold increase in production from 1960–2009. The effects of three different cooking methods (boiling, microwave and pressure cooking) on the antioxidant activities of six different types of oyster mushrooms (*Pleurotus eryngii*, *P. citrinopileatus*, *P. cystidiosus*, *P. flabellatus*, *P. floridanus* and *P. pulmonarius*) were assessed. Free radical scavenging (DPPH) and reducing power (TEAC) were used to evaluate the antioxidant activities and the total phenolic contents were determined by Folin-Ciocalteu reagent. Pressure cooking improved the scavenging abilities of *P. floridanus* (>200 %), *P. flabellatus* (117.6 %), and *P. pulmonarius* (49.1 %) compared to the uncooked samples. On the other hand, the microwaved *Pleurotus eryngii* showed 17 % higher in the TEAC value when compared to the uncooked sample. There was, however, no correlation between total phenolic content and antioxidant activities. There could be presence of other bioactive components in the processed mushrooms that may have contributed to the antioxidant

activity. These results suggested that customized cooking method can be used to enhance the nutritional value of mushrooms and promote good health.

Keywords Oyster mushroom · *Pleurotus* · Cooking method · Antioxidant activity

Introduction

Oxidation is necessary for physiological processes in living system. Free radicals, also known as reactive oxygen species (ROS) are produced during numerous physiological processes. Excessive production of reactive oxygen species leads to oxidative damage and this has been implicated in aging process and many life-threatening human diseases including diabetes, inflammation and cancers (Gogavekar et al. 2012; Song and Van Griensven 2008). Living organisms have endogenous antioxidants that act as major defense against oxidative damage caused by the free radicals. However, these antioxidants are often insufficient to prevent the damage. Mushrooms have been recognized as sources of antioxidants as they contain beneficial components and secondary metabolites that can protect against oxidative damage. The antioxidants found in mushrooms are mainly phenolic compounds reported to have protective role against chronic diseases related to oxidative stress (Ferreira et al. 2009). Today, mushrooms are being considered as functional food mainly because of their nutritional values and medicinal importance (Stamets 2005; Elmastas et al. 2007; Khan and Tania 2012).

Mushrooms have been part of the human diet for thousands of years. Globally cultivated mushrooms such as *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus* spp. have become popular and the industry is expanding with world production greater than two million tonnes annually (Gogavekar et al.

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2012). *Pleurotus* spp. also known as oyster mushrooms are widely cultivated all over the world using various substrates. The production of *Pleurotus* mushrooms increased from 35.0 thousand tonnes to 875.6 thousand tonnes from 1960–2009 (Chang and Wasser 2012). *Pleurotus* spp. have been reported to have anticancer, antimicrobial, antihypertensive, antihyperlipidemic, antidiabetic, hepatoprotective effect and antiallergic properties (Khan and Tania 2012). The inclusion of mushrooms including *Pleurotus* spp. in daily diet may contribute to protection against oxidative damage. Mushrooms however cannot be consumed raw or uncooked. Further, they are perishable and need to be processed.

Most vegetables and some fruits need to be processed before consumption for safety and quality reason and only selected few are consumed uncooked. Generally, food products are heat processed to increase the shelf life. In the last few decades, attention on naturally occurring antioxidants in foods has increased because of the adverse effect of synthetic antioxidants and the practice of minimum usage of artificial food additives. Nutritional value or texture of food could be affected or lost during the heat treatments or storage as most of the bioactive compounds and naturally occurring antioxidants are susceptible to heat (Amin and Lee 2005; Puupponen-Pimia et al. 2003; Pokorny and Schmidt 2001). Recent studies showed that appropriate cooking methods for different vegetables may maintain the antioxidant properties or improve their nutritional value (Galor et al. 2008; Ng et al. 2011). Thermal processing of pepper, peas, broccoli, tomatoes and sweet corn enhanced the antioxidant activity (Nicoli et al. 1999; Dewanto et al. 2002a, b). (Choi et al. 2006) also reported that heat treatment and duration of heating on *Lentinula edodes* significantly enhanced the overall antioxidant activities. Although the importance of antioxidants present in food changes during or after processing, relatively little has been published on the changes in antioxidants after the food processing. Thus, the type of mushroom processing used in this study was boiling, microwave and high pressure cooking. To our knowledge, there are no reports on antioxidant level assessment of microwaved and/or pressure cooked of *Pleurotus* mushrooms. The objective of this study was to evaluate the antioxidant activities of selected oyster mushrooms after different cooking methods. Antioxidant activities were assayed by scavenging abilities on 1-1-diphenyl-2-picrylhydrazyl (DPPH) and Trolox equivalent antioxidant capacity (TEAC). The phenolic contents of cooked mushrooms were also determined.

Materials and methods

Chemicals

Chemicals and reagents were of analytical grade. Gallic acid, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-

carboxylic acid (Trolox), 2,2-azino-di [3-ethylbenzthiazoline sulfonate] (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich[®] Inc (USA). Folin-Ciocalteu's phenol reagent, hydrochloric acid (HCl), potassium persulfate (K₂O₈S₂) and sodium carbonate anhydrous (Na₂CO₃) were obtained from Merck. Absolute ethanol was purchased from Fisher Scientific[®] UK Ltd (UK).

Mushroom samples and processing

Fresh fruiting bodies of six different oyster mushrooms namely *Pleurotus citrinopileatus* Singer (yellow oyster), *P. cystidiosus* O.K. Mill. (abalone), *P. flabellatus* Sacc. (pink oyster), *P. floridanus* (Mont.) Singer (white oyster) and *P. pulmonarius* (Fr.) Quel. (grey oyster) were purchased from local mushroom farmers (Ganofarm Sdn Bhd) while *P. eryngii* (DC.) Quel. (king oyster; Korea) was purchased from a hypermarket in Kuala Lumpur. The mushrooms were identified by experts in Mushroom Research Centre, University of Malaya. The mushrooms (600 g each) were randomly sampled, washed with tap water and dried on paper towel prior to weighing. Four portions each of 150 g mushroom were divided and subjected to various cooking methods: microwave-mushrooms were added to 100 ml of distilled water and cooked in a commercial 1000 W microwave for 5 min; boiled samples- mushrooms were boiled in 100 ml distilled water on hot plate for 5 min; pressure-cooked- mushrooms in 100 ml of distilled water were autoclaved for 15 min at 121 °C. During the cooking process, the samples were covered in order to minimize evaporation of water. All the cooked samples including the water were cooled to room temperature (25±2 °C) and homogenized in Waring Commercial blender (New Hartford, CT, USA). For the uncooked samples, the mushrooms were homogenized with 100 ml distilled water. All the samples were centrifuged at 5000 rpm for 15 min to obtain clear supernatants and freeze dried. The lyophilized extracts were stored at -40 °C prior to analysis.

Antioxidant assays

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay is an electron-based assay involved antioxidant and oxidant in the reaction mixture where the decolorization of the oxidant (ABTS in this study) was determined through the reduction of radical cation (Re et al. 1999). The TEAC assay was selected because it has not been reported previously by others using this method for pressure cooking and microwave mushrooms.

The assay was determined using method outlined by (Re et al. 1999). The ABTS^{•+} reagent was prepared by mixing 5 ml of 7 mM ABTS^{•+} solution with 89 µl of 140 mM K₂S₂O₈. The mixture was added and kept in the dark at room

temperature for 16 h. After 16 h, 95 % ethanol was used to adjust the absorbance of the ABTS^{•+} reagent to 0.70±0.05 at 734 nm. One hundred microlitre of ABTS^{•+} reagent was added to 10 µL of mushroom extract. The mixture was allowed to stand for 1 min and absorbance was measured at 734 nm. Trolox (water-soluble Vitamin E analogue) was used as standard. The TEAC values were calculated based on final concentration and expressed as µmol Trolox equivalent per 150 g of uncooked mushroom (µmol Trolox equiv/150 g).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The diphenyl-1-picryl-hyrazyl (DPPH) radical scavenging assay is widely used to test the free radical scavenging ability of

various plants, food and vegetables. The scavenging ability is measured based on the number of molecules of DPPH reduced by one molecule of the reductant (Reis et al. 2012). Determination of free radical scavenging activity was carried out according to the method described by (Gerhauser et al. 2003). Five microlitre mushroom aqueous extract (5 mg/ml) was mixed with 195 µL of methanolic solution containing DPPH radical. The mixture was shaken vigorously and left to stand for 3 h in the dark. The absorbance was determined at 515 nm with a spectrophotometer. Ascorbic acid was used as standard, water was used as blank and mixture without the sample extract was used as a control. The radical scavenging activities were expressed as percentage of DPPH quenched (%) using the following formula.

$$\text{Scavenging activity (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100\%}{}$$

Total phenolic content (TPC) determination

The Folin-Ciocalteu reagents measured the reducing capacity of the mixture reagents of phosphomolybdic and phosphotungstic acids and used to estimate phenolic contents and other reducing agents (Huang et al. 2005). The concentration of phenolic compounds was determined using method outlined by (Oki et al. 2002). Fifty microlitre of mushroom aqueous extract (5 mg/ml) was mixed with 100 µL 10 % aqueous sodium carbonate solution. After 3 min, 50 µL of 10 % Folin-Ciocalteu reagent was added to the mixture. Mixture was incubated for 1 h in the dark and the absorbance was measured at 750 nm. Gallic acid was used as standard and mixture without extract was used as control. The total content of phenolic in mushroom were calculated based on final concentration and expressed as mg gallic acid equivalent per 150 g of uncooked mushroom (mg GAE/150 g).

Statistical analysis

All the data were recorded as mean ± standard deviation (triplicate values). Data were analysed using SPSS for Windows (ver. 17.) as a one-way analysis of variance (ANOVA), Turkey's post hoc tests were carried out to test any significant differences ($p < 0.05$) between uncooked and cooked mushrooms. Person's correlation analysis was carried out to determine the correlation between the assays.

Results and discussion

Yield of mushroom extracts

The yield of cooked and uncooked mushroom extracts are presented in Table 1. Generally, the yields for all uncooked mushrooms were in the range of extraction efficiency of 1.49 to 2.59 %. Microwaved *P. pulmonarius* gave the lowest yield with extraction efficiency of 1.49 %. Among all the cooking methods studied, *P. cystidiosus* gave higher yields of extracts compared to the other processed mushrooms.

Antioxidant assays

Trolox equivalent antioxidant capacity (TEAC) assay

Boiling is the most commonly used in food processing using water as the medium to transfer heat. The boiling method significantly decreased ($p < 0.05$) the antioxidant activity in all mushrooms studied except for *P. citrinopileatus* (123.21±3.62 µmol Trolox equiv/150 g) and *P. pulmonarius* (114.42±1.33 µmol Trolox equiv/150 g). Further these two mushrooms did not show any significant difference ($p < 0.05$) in TEAC levels when compared to uncooked sample (Table 1). (Kanagasabapathy et al. 2011) reported that the aqueous extracts of *P. sajor-caju* (synonyms *P. pulmonarius*) exhibited higher TEAC values (29.45±0.87 mM Trolox equiv/g of fresh mushroom) than in the present study. Further, *P. cystidiosus* showed lower antioxidant activity (34.42±9.52 µmol Trolox

Table 1 Extraction efficiency, antioxidant activities and total phenolic content of mushroom under different cooking methods

Mushrooms/processing	Extraction efficiency		Antioxidant activities		Total phenolic content (mg GAE/150 g)
	Lyophilized extract (g)	Percentage ^a	TEAC ($\mu\text{mol TE}/150 \text{ g}$)	DPPH (%) ^b	
<i>Pleurotus citrinopileatus</i> (Yellow oyster)					
Raw	2.47	1.65	114.43 \pm 5.48 ^a	18.95 \pm 1.98 ^a	14.14 \pm 0.25
Boiling	2.73	1.82	123.21 \pm 3.62 ^a	18.80 \pm 1.74 ^a	16.44 \pm 0.34
Microwave	2.88	1.92	88.04 \pm 1.47	10.55 \pm 1.23 ^b	10.88 \pm 0.18
Pressure cooked	2.38	1.59	64.08 \pm 2.64	9.70 \pm 2.67 ^b	8.88 \pm 0.48
<i>Pleurotus cystidiosus</i> (Abalone mushroom)					
Raw	3.89	2.59	102.27 \pm 6.43	14.15 \pm 0.94	9.02 \pm 0.04 ^a
Boiling	3.72	2.48	34.32 \pm 9.52 ^a	12.31 \pm 0.70 ^b	10.97 \pm 0.12
Microwave	3.43	2.29	37.91 \pm 7.28 ^a	4.87 \pm 0.79	7.62 \pm 0.22
Pressure cooked	3.68	2.45	74.30 \pm 0.20	14.38 \pm 0.53	9.21 \pm 0.08 ^a
<i>Pleurotus eryngii</i> (King oyster)					
Raw	2.76	1.84	73.30 \pm 5.50 ^{ab}	50.54 \pm 2.36	12.35 \pm 0.14
Boiling	2.78	1.85	53.72 \pm 8.10 ^c	11.47 \pm 1.72 ^a	7.91 \pm 0.11
Microwave	2.89	1.93	85.98 \pm 6.53 ^a	15.05 \pm 0.59 ^{ab}	9.63 \pm 0.30 ^a
Pressure cooked	2.96	1.97	59.70 \pm 3.05 ^{bc}	18.81 \pm 1.23 ^b	9.81 \pm 0.19 ^a
<i>Pleurotus flabellatus</i> (Pink oyster)					
Raw	2.68	1.79	138.77 \pm 7.46	4.60 \pm 0.49 ^a	12.10 \pm 0.29 ^a
Boiling	2.81	1.87	85.37 \pm 6.63 ^{ab}	3.21 \pm 0.45 ^a	12.21 \pm 0.05 ^a
Microwave	2.95	1.97	80.85 \pm 5.22 ^a	6.50 \pm 0.49	9.26 \pm 0.10
Pressure cooked	2.99	1.99	99.08 \pm 6.76 ^b	10.01 \pm 0.91	11.06 \pm 0.41
<i>Pleurotus floridanus</i> (White oyster)					
Raw	2.91	1.94	139.15 \pm 4.91	0.46 \pm 0.00 ^{ab}	17.17 \pm 0.34
Boiling	2.87	1.91	69.71 \pm 6.29 ^{ab}	0.46 \pm 0.00 ^a	11.29 \pm 0.07
Microwave	2.98	1.99	65.80 \pm 8.31 ^{ac}	1.45 \pm 0.47 ^b	12.11 \pm 0.26
Pressure cooked	2.69	1.79	59.29 \pm 5.09 ^{bc}	6.65 \pm 0.89	14.33 \pm 0.13
<i>Pleurotus pulmonarius</i> (Grey oyster)					
Raw	2.92	1.95	117.24 \pm 2.45 ^a	6.72 \pm 1.07 ^a	13.53 \pm 0.30 ^a
Boiling	2.74	1.83	114.42 \pm 1.33 ^a	16.56 \pm 0.18 ^b	13.36 \pm 0.13 ^a
Microwave	2.24	1.49	49.44 \pm 4.72 ^b	14.43 \pm 1.04 ^b	6.76 \pm 0.02
Pressure cooked	2.89	1.93	45.40 \pm 1.34 ^b	10.02 \pm 2.11 ^a	12.49 \pm 0.20

For each mushroom, values were expressed as mean \pm SD and values within the same column followed by same letters are not significantly different ($p > 0.05$)

^a The extraction efficiency percentage of lyophilized extract was calculated based on the amount of raw sample used (150.00 g)

^b for DPPH assay, the IC₅₀ ascorbic standard is 18.94 $\mu\text{g}/\text{ml}$

equiv/150 g) which was 67 % when compared to the uncooked sample (107.27 \pm 6.43 $\mu\text{mol Trolox equiv}/150 \text{ g}$).

When mushrooms were microwaved, *P. eryngii* showed 17 % higher (85.98 \pm 6.53 $\mu\text{mol TE equiv}/150 \text{ g}$) antioxidant activity compared to the uncooked sample (73.70 \pm 5.50 $\mu\text{mol TE equiv}/150 \text{ g}$) (Table 1). However, the other mushrooms exhibited 23–63 % lower antioxidant activities compared to their respective uncooked sample (Table 1) whereas *P. cystidiosus* showed a significant ($p > 0.05$) decrease of 63 % in the antioxidant activity (37.91 \pm 7.28 $\mu\text{mol TE equiv}/150 \text{ g}$) compared to the uncooked sample (102.27 \pm 6.43 $\mu\text{mol TE equiv}/150 \text{ g}$).

All the mushrooms that were pressure cooked showed significant decrease ($p < 0.05$) in their TEAC value (45.40 to 99.08 $\mu\text{mol TE equiv}/150 \text{ g}$) when compared to the respective uncooked samples (73.30 to 138.77 $\mu\text{mol TE equiv}/150 \text{ g}$). *Pleurotus pulmonarius* showed the lowest decrease of antioxidant activity (relative percentage of 61 %) relative to the uncooked sample. (Ng et al. 2011) reported that pressure cooking did not cause any significant decline in the antioxidant property in broccoli, bitter melon, Chinese long bean and water convolvulus. In this study, it was observed that pressure cooking did affect Trolox equivalent antioxidant capacity. To our knowledge, no similar studies with mushrooms have been reported.

Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

The DPPH free radical scavenging assay of mushrooms subjected to boiling, microwave and pressure-cooking compared to uncooked sample are shown in Table 1. The highest increase in antioxidant activity of 151 % was observed when *Pleurotus pulmonarius* was boiled for 5 min compared to uncooked samples (16.56±0.18 %). Antioxidant activity of *Pleurotus citrinopileatus*, *P. flabellatus* and *P. floridanus* did not vary after boiling. *Pleurotus cystidiosus* and *P. eryngii* showed lower levels of antioxidant activity with relative percentage of 12 and 77 % respectively, than their uncooked samples (Table 1). Lee et al. (Lee et al. 2007) reported the scavenging abilities of hot water extracts (heated at reflux for 1 h) of *P. citrinopileatus* was 20.7–2.3 % at 20 mg/ml. In this study, the scavenging ability of *P. citrinopileatus* in this study after 5 min of boiling process, however, was 18.8 % at 5 mg/ml. (Jagadish et al. 2009) reported that the DPPH free radical scavenging activity of boiled (100 °C, 1 h) *Agaricus bisporus* showed a decrease of 8.3–53.1 % when compared to the uncooked 15.6–65.8 % at the concentration range of 100–800 µg/ml. The longer duration of boiling might have contributed to the decrease in scavenging abilities of bioactive compounds in the varieties of mushrooms (Vasanthi et al. 2009; Borowski et al. 2008).

Pleurotus floridanus, *P. flabellatus* and *P. pulmonarius* showed significant increases ($p<0.05$) in antioxidant activity (1.45±0.47; 6.50±0.49 % and 14.43±1.04 respectively) after microwave cooking compared to the uncooked sample (0.46±0.00; 4.60±0.49 and 6.72±1.07 % respectively) (Table 1). *Pleurotus citrinopileatus*, *P. cystidiosus* and *P. eryngii*, however, had lower antioxidant activity compared to the corresponding uncooked sample with relative percentage of 44, 65 and 70 % respectively. (Kettawan et al. 2011) and (Kim et al. 2009) reported that the reduction of antioxidant activities might be affected by the texture, color or shape of each mushroom variety. Both *Pleurotus cystidiosus* and *P. eryngii* have thick, meaty, solid and firm characteristics where the microwave heat may not have penetrated the mushroom tissue or disrupted the cell wall to liberate the antioxidant compounds. However, (Hayat et al. 2009) reported microwave treatment could accelerate release of high amount of phenolic compounds by thermal destruction of cell wall and sub cellular compartments of citrus peels. (Sun et al. 2012) reported that microwave cooking had shown better retention of certain phenolic acids in *Boletus* mushrooms than pressure-cooking, steaming, boiling and frying.

For the mushrooms subjected to pressure cooking, *P. floridanus* again showed the highest scavenging ability (>200 %) when compared to all the mushrooms processed and uncooked. The improvement in antioxidant activity could be due to the release of active antioxidants from the fibrous

complexes during pressure cooking (low moisture level and high temperature). *Pleurotus flabellatus* and *P. pulmonarius* also showed significantly increase ($p<0.05$) in DPPH radical scavenging activities of 10.01±0.91 and 10.02±2.11 % respectively. *Pleurotus citrinopileatus* and *P. eryngii* had lower scavenging abilities after the pressure cooking with relative percentage of 50 and 67 % (Table 1) compared to their respective uncooked samples. (Choi et al. 2006) reported that prolonged heating time and higher temperature enhanced the DPPH radical scavenging activity of *Lentinula edodes*. *Pleurotus pulmonarius*, *P. floridanus* and *P. flabellatus* have thinner and softer tissue structure compared to *P. eryngii* and *P. cystidiosus* which have thick and firm tissue structure. Therefore, application of high temperature in pressure cooking may release high amounts of phenolic compounds from disruption of mushroom tissues (Nicoli et al. 1997; Martins et al. 2000). Thermal reaction could also lead to production of stronger radical-scavenging antioxidants (Jiménez-Monreal et al. 2009). Moreover, it was reported longer pressure cooking time of common beans (*Phaseolus vulgaris*) enhanced their free radical scavenging activity (Rocha-Guzman et al. 2007).

Total phenolic content

Total phenolic content of mushroom extracts after boiling were in the range of 7.91 to 16.44 mg GAE/150 g compared to the activities in uncooked samples which range from 9.02 to 17.17 mg GAE/150 g. *Pleurotus cystidiosus* showed highest increase in total phenolic content (10.97±0.12 mg GAE equiv/150 g) after this cooking treatment with respect to its uncooked sample (9.02±0.04 mg GAE equiv/150 g) (Table 1). Total phenolic content of *P. eryngii*, *P. flabellatus* and *P. floridanus* were lower than their uncooked sample with relative percentage of 36, 24 and 34 % respectively. *Pleurotus pulmonarius* showed no significant difference ($p<0.05$) in the total phenolic content compared to the uncooked sample after boiling. Noorlidah et al. (2011) reported the total phenolic contents of hot water extract of five *Pleurotus* spp (*P. cystidiosus*, *P. eryngii*, *P. flabellatus*, *P. floridanus* and *P. pulmonarius*) were higher than the values reported in this study. In that study, those mushrooms were subjected to 30 min of boiling treatment (Noorlidah et al. 2011) while the mushrooms were subjected to 5 min of boiling in this study. Duration of cooking affected the release of phenolic compounds possibly due to the texture of mushrooms and this in turn could contribute to the antioxidant activity (Kettawan et al. 2011). (Puttaraju et al. 2006) reported higher total phenolic content in water extract (5 min boiling) *Pleurotus pulmonarius* and *Pleurotus djamor* (synonym *P. flabellatus*) (14.3 and 13.3 mg GAE/g sample respectively), compared to the levels observed in the present study (13.36 mg GAE/150 g and 12.21 mg GAE/150 g). *Pleurotus eryngii* has solid and

firm fruiting body and was subjected to 5 min boiling in the present study, the total phenolic contents was lower than that reported after 30 min of boiling by (Noorlidah et al. 2011). Thus, prolonged boiling might help the release of polyphenols into the boiling water. (Kettawan et al. 2011) reported significant amount of polyphenol and antioxidant activities in boiled water of edible mushroom and this could be attributed to the antioxidants which largely leach from mushroom tissue into the boiling water with increase in cooking time. Thus, boiling mushrooms to prepare soup and gravies would be a good choice of cooking method to optimize antioxidant intake.

All mushrooms which were subjected to microwave treatment with the exception of *P. cystidiosus* showed significant ($p < 0.05$) reduction in the total phenolic content compared to the respective uncooked samples (Table 1). *Pleurotus pulmonarius* showed the lowest phenolic contents (6.76 ± 0.02 mg GAE/150 g) than uncooked sample (13.53 ± 0.30 mg GAE/150 g) with relative percentage of 50 % (Table 1). The pressure cooking method significantly decreased ($p < 0.05$) the total phenolic content for most of the mushrooms. *P. cystidiosus*, however, showed no significant difference ($p < 0.05$) in its phenolic content with respect to uncooked samples. To our knowledge, no similar studies on total phenolic contents of microwaved- and pressure-cooked *Pleurotus* spp. have been reported. The phenolic compounds play an important role in antioxidant activity, stabilizing lipid oxidation, inhibition of carcinogenesis and mutagenesis in humans (Nicholson and Hammerschmidt 1992). Recently, (Kanagasabapathy et al. 2011) reported 22 compounds in *P. sajor-caju* which include mainly triterpenoids, fatty acids, ergosterol, linoleic acid, cinnamic acid and nicotinamide. Further, the linoleic acid, cinnamic acid and nicotinamide were shown to have antioxidant activities. In the study by (Kim et al. 2008), *Pleurotus ostreatus* was reported to contain phenolic compounds such as gallic acid, homogentisic acid, protocatechuic acid and naringin. Therefore, similar phenolic compounds could be found in other *Pleurotus* spp. and may contribute to their antioxidant activities.

Correlation analysis

Several studies found correlation of total phenolic content in hot water extracted mushrooms and antioxidant activities. (Kanagasabapathy et al. 2011) reported strong correlation ($R^2 = 0.8181$) between TPC and DPPH free radical scavenging activity of the culinary-medicinal mushrooms. This was in accordance to the findings by (Kanagasabapathy et al. 2011) who reported positive correlation ($R^2 = 0.7205$) between TPC and TEAC in *P. pulmonarius*. On the other hand, (Lee et al. 2007) reported a moderate correlation ($R^2 = 0.425$) between TPC and DPPH free radical scavenging activity of *P. citrinopileatus*. But in our study, there was poor correlation

between antioxidant activities (DPPH, TEAC) and total phenolic content of extracts from different cooking methods. Various authors (Jayakumar et al. 2009; Ferreira et al. 2009) have reported presence of ascorbic acid, α -tocopherol, β -carotene, rutin, chrysin, phenolic compounds (include syringic acid, p-coumaric acid, ferulic acid, 5-O-caffeoylquinic acid, naringin and tannic acid), tocopherols, ascorbic acid and β -carotene in various *Pleurotus* spp. Therefore, the other bioactive components may contribute to the antioxidant activity.

Conclusion

The cooking method, mushroom characteristics and variety affected the content and bioavailability of naturally occurring antioxidants or formation of novel compounds that influenced the antioxidant activities. Pressure cooking increased the antioxidant activity of *Pleurotus flabellatus*, *P. floridanus* and *P. pulmonarius*. This suggested that customized cooking method including pressure cooking might increase the health beneficial effects associated with increase of antioxidant activities.

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References

- Amin I, Lee WY (2005) Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *J Sci Food Agric* 85: 2314–2320
- Borowski J, Szajdek A, Borowska EJ (2008) Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.). *Eur Food Res Technol* 246:459–465
- Chang ST, Wasser SP (2012) The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *Int J Med Mushrooms* 14:95–134
- Choi Y, Lee SM, Chun J, Lee HB, Lee J (2006) Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. *Food Chem* 99:381–387
- Dewanto V, Wu XZ, Adom KK, Liu RH (2002a) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem* 50:3010–3014
- Dewanto V, Wu X, Liu RH (2002b) Processed sweet corn has higher antioxidant activity. *J Agric Food Chem* 50:4959–4964
- Elmastas M, Isildak O, Turkecul I, Temur N (2007) Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J Food Comp Anal* 20:337–345
- Ferreira IC, Barros L, Abreu RM (2009) Antioxidants in wild mushrooms. *Curr Med Chem* 16:1543–60
- Galor SW, Wong KW, Benzie IFF (2008) The effect of cooking on Brassica vegetables. *Food Chem* 110:706–710
- Gerhauer C, Klimo K, Heiss E, Neumann I, Gamal-Eldeen A, Knauft J, Liu GY, Sitthimonchai S, Frank N (2003) Mechanism-based in vitro

- screening of potential cancer chemopreventive agents. *Mutat Res: Fund Mol Mech Mutagen* 523–524:163–172
- Gogavekar SS, Rokade SA, Ranveer RC, Ghosh JS, Kalyani DC, Sahoo AK (2012) Important nutritional constituents, flavour components, antioxidant and antibacterial properties of *Pleurotus sajor-caju*. *J Food Sci Tech*. doi:10.1007/s13197-012-0656-5
- Hayat K, Hussain S, Abbas S, Farooq U, Ding B, Xia S, Jia C, Zhang X, Xia W (2009) Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. *Sep Purif Technol* 70:63–67
- Huang DJ, Ou BX, Prior RL (2005) The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 53:1841–1856
- Jagadish LK, Krishnan VV, Shenbhagaraman R, Kaviyaran V (2009) Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) imbach before and after boiling. *Afr J Biotechnol* 8(4):54–661
- Jayakumar T, Thomas PA, Geraldine P (2009) In-vitro antioxidant activities of an ethanolic extract of oyster mushroom, *Pleurotus ostreatus*. *Innov Food Sci Emerg Technol* 10:228–234
- Jiménez-Monreal AM, Garcia-Diz L, Martinex-Tome M, Mariscal M, Murcia MA (2009) Influence of cooking methods on antioxidant activity of vegetables. *J Food Sci* 74(3):H97–H103
- Kanagasabapathy G, Malek SRA, Kuppusamy UR, Vikineswary S (2011) Chemical composition and antioxidant properties of extracts of uncooked fruiting bodies of *Pleurotus sajor-caju* (Fr.) Singer. *J Agric Food Chem* 59:2618–2626
- Kettawan A, Chanlekha K, Kongkachuichai R, Charoensiri R (2011) Effect of cooking on antioxidant activities and polyphenol content of edible mushrooms commonly consumed in Thailand. *Pak J Nutr* 10:1094–1103
- Khan MA, Tania M (2012) Nutritional and medicinal importance of *Pleurotus* mushrooms: an overview. *Food Rev Int* 28(3):313–329
- Kim MY, Segun P, Ahn JK, Kim JJ, Chun SC, Kim EH, Seo SH, Kang EY, Kim SL, Park YJ, Ro HM, Chung IM (2008) Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *J Agric Food Chem* 56:7265–7270
- Kim JH, Kim SJ, Park HR, Choi JI, Ju YC, Nam KC, Kim SJ, Lee SC (2009) The different antioxidant and anticancer activities depending on the color of oyster mushrooms. *J Med Plants Res* 3:1016–2020
- Lee YL, Huang GW, Liang ZC, Mau JL (2007) Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. *LWT* 40:823–833
- Martins SIFS, Jongen WMF, van Boekel MAJS (2000) A review of Maillard reaction in food in implications to kinetic modeling. *Trends Food Sci Technol* 11(9–10):364–373
- Ng ZX, Chai JW, Kuppusamy UR (2011) Customised cooking method improves total antioxidant activity in selected vegetables. *Int J Food Sci Nutr* 62:158–163
- Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. *Annu Rev Phytopathol* 30:369–389
- Nicoli MC, Anese M, Parpinel MT, Franceschi S, Lericri CR (1997) Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett* 114:71–74
- Nicoli MC, Anese M, Parpinel M (1999) Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci Technol* 10:94–100
- Noorlidah A, Ismail SM, Aminudin N, Shuib AS, Lau BF (2011) Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. *eCAM*. Volume 2012 (2012), Article ID 464238. doi:10.1155/2012/464238.
- Oki T, Masuda M, Furuta S, Nishiba Y, Terahara N, Suda I (2002) Involvement of anthocyanins and other phenolic compounds in radical scavenging activity of purple fleshed sweet potato cultivars. *J Food Sci* 10:94–100
- Pokorny J, Schmidt S (2001) Natural antioxidant functionally during food processing. In: Pokorny J, Yanishileva N, Gordon M (ed) *Antioxidants in food: practical application*. Woodhead Publishing Ltd, pp 331–354.
- Puttaraju NG, Venkateshaiah SU, Dharmesh Urs SMN, Somasundaram R (2006) Antioxidant activity of indigenous edible mushrooms. *J Agric Food Chem* 54:9764–9772
- Puupponen-Pimia R, Hakkinen ST, Aarni M, Suortti T, Lampi AM, Euro M (2003) Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *J Sci Food Agric* 83:1389–1402
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231–1237
- Reis FS, Martins A, Barros L, Ferreira ICFR (2012) Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples. *Food Chem Toxicol* 50:1201–1207
- Rocha-Guzman NE, Gonzalez-Laredo RF, Ibarra-Berumen CA, Gallegos-Infante JA (2007) Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. *Food Chem* 100:31–35
- Song W, Van Griensven LJLD (2008) Pro- and antioxidative properties of medicinal mushroom extracts. *Int J Med Mushrooms* 10:315–324
- Stamets P (2005) Notes on nutritional properties of culinary-medicinal mushrooms. *Int J Med Mushrooms* 7(1–2):103–110
- Sun LP, Bai X, Zhuang YL (2012) Effect of different cooking methods on total phenolic contents and antioxidant activities of four *Boletus* mushrooms. *J Food Sci Technol*. doi:10.1007/s13197-012-0827-4
- Vasanthi HR, Mukherjee S, Das DK (2009) Potential health benefits of broccoli: a chemico-biological overview. *Min Rev Med Chem* 9: 749–759