

Impact of optimised cooking on the antioxidant activity in edible mushrooms

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Abstract This study aimed to investigate the effect of four cooking methods with different durations on the in vitro antioxidant activities of five edible mushrooms, namely *Agaricus bisporus*, *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus eryngii*. Among the raw samples, *A. bisporus* showed the highest total antioxidant activity (reducing power and radical scavenging), total flavonoid, ascorbic acid and water soluble phenolic contents. Short-duration steam cooking (3 min) increased the total flavonoid and ascorbic acid while prolonged pressure cooking (15 min) reduced the water soluble phenolic content in the mushrooms. The retention of antioxidant value in the mushrooms varied with the variety of mushroom after the cooking process. The cooking duration significantly affected the ascorbic acid in the mushrooms regardless of cooking method. To achieve the best antioxidant values, steam cooking was preferred for *F. velutipes* (1.5 min), *P. ostreatus* (4.5 min) and *L. edodes* (4.5 min) while microwave cooking for 1.5 min was a better choice for *A. bisporus*. Pressure cooked *P. eryngii* showed the best overall antioxidant value among the cooked samples. Optimised cooking method including

pressure cooking could increase the antioxidant values in the edible mushrooms.

Keywords Antioxidant activity · Ascorbic acid · Cooking method · Flavonoid · Mushroom · Water soluble phenolic content

Introduction

Mushrooms have been part of the human diet since ancient times, especially in the Eastern Asian countries. Mushroom belongs to the fungi family and 2000 species have been identified as edible (Aida et al. 2009). To date, 20 edible species are cultivated commercially on an industrial scales, including *Agaricus bisporus* (button mushroom), *Flammulina velutipes* (golden needle mushroom), *Lentinula edodes* (shiitake mushroom), *Pleurotus ostreatus* (oyster mushroom) and *Pleurotus eryngii* (king oyster mushroom) (Aida et al. 2009). The production of mushrooms has increased overtime to meet the increased demand of consumers who have raised awareness on the health benefit of mushroom. Besides the high content of proteins, carbohydrates, dietary fibre and low lipid content, edible mushroom also contains a variety of secondary metabolites such as flavonoids and phenolic compounds (Sánchez 2017). Phenolic compounds are produced by fungi in adaptation to abiotic and biotic stress conditions such as infection and low temperature (Islam et al. 2016). They are recognized as excellent antioxidants than vitamins (Podsedek 2007) and synergists to free radical scavengers (Sánchez 2017). Several types of the mushrooms have been reported to have therapeutic properties such as antidiabetic, antimicrobial, antioxidant, anticancer, lipid lowering and immune-modulating effects (Tan et al. 2015). Hence, edible mushrooms

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are increasingly being recognized as functional foods of medicinal value.

Food products generally require different processing for safety, economy and quality reasons. Processing of food by heat treatment may cause substantial changes in its chemical composition and therefore affect the nutritional and health properties (Miglio et al. 2008). The cooking process was reported to either increase, decrease or induce no significant change in the antioxidant activity of plant foods (Miglio et al. 2008; Nicoli et al. 1999; Somsu et al. 2008; Turkmen et al. 2005). For example, significant loss of antioxidant activity and compounds such as carotenoids and ascorbic acid were observed in vegetables after subjected to domestic cooking (Zhang and Hamazu 2004). In contrast, Ng et al. (2011) reported that appropriate heat treatment preserved the antioxidant capacity in vegetables mainly due to the significant retention of polyphenols. Our recent follow up study showed that customized cooking methods could induce selected heat stable proteins in the vegetables and thus preserve or improve the antioxidant activity (Ng et al. 2014).

Mushrooms are usually cooked by different methods such as boiling, microwaving, steaming, stir-frying and pressure cooking before consumption. Alteration in the total antioxidant values due to different cooking methods is of scientific importance, especially its impact on human dietary nutrition. A relatively small number of reports are available on the changes of antioxidant values in the mushrooms after heat treatment but limited to a few cultivars (Radzki et al. 2016; Sun et al. 2014; Tan et al. 2015). In addition, the effect on antioxidant values of the mushrooms subjected to domestic styles of cooking with different durations is yet to be addressed. To fill this literature gap, the present paper aims to focus on the optimum cooking system (method and duration) that results in the highest retention of the antioxidant values in five edible mushrooms commonly consumed in Malaysia.

Materials and methods

Chemicals

All chemicals used in the study were of analytical grade. Sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, 3,4,5-trihydroxybenzoic acid (gallic acid), aluminium chloride ($AlCl_3$), potassium acetate ($C_2H_3KO_2$), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin) and L-ascorbic oxidase (EC 1.10.3.3) were purchased from Sigma-Aldrich (St.

Louis, MO, USA). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), potassium peroxodisulphate ($K_2O_8S_2$), Folin-Ciocalteu's reagent, absolute ethanol (C_2H_6O), fuming hydrochloric acid (HCl), glacial acetic acid ($C_2H_4O_2$) and sodium carbonate (Na_2CO_3) were purchased from Merck (Darmstadt, Germany). 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Roche (Penzberg, Baden-Wurttemberg, Germany) respectively.

Sample preparation

Five commercially-cultivated fresh mushrooms namely the *Pleurotus ostreatus* (oyster), *Agaricus bisporus* (white button), *Flammulina velutipes* (golden needle), *Pleurotus eryngii* (king oyster) and *Lentinula edodes* (Shiitake) were purchased from a local wholesale market in Selangor, Malaysia. Each mushroom sample randomly selected from the shelf, comprised complete fruiting bodies (cap, gills, tubes, and stipe) of different sizes. The mushroom samples were morphologically identified according to the literature (Hang 1998) and authenticated by experts from the Mushroom Research Centre, University of Malaya. The mushroom samples were washed and blot-dried with paper towel prior to the weighing. They were then divided into 100 g portions for each cooking treatment.

Measurement of sample moisture content

The moisture content of each mushroom sample was estimated according to AOAC official gravimetric method 964.22 (AOAC International 1995). The samples (100 g) were sliced into smaller pieces and left for drying in a hot air oven (Mettler; Germany) at 70 °C for 24–48 h until a constant weight was achieved. The percentage moisture content was determined by using the following equation:

$$\text{Moisture content (\%)} = \frac{(\text{fresh weight} - \text{dry weight})}{\text{fresh weight}} \times 100\%$$

Cooking methods

The cooking procedures were adapted from Ng et al. (2011) with the exception of cooking duration which was determined by the researchers in their preliminary experiments. All cooking experiments were done in triplicate, using 100 g of sample for each individual run. For pressure cooked samples, the mushrooms were pressure-cooked in 100 mL of distilled water at 121 °C under 2 MPa for 15 min in a pressure cooker; for steamed samples, the mushrooms were placed on a steaming rack over 95 °C water in a closed water bath for 1.5, 3.0, 4.5 and 6.0 min;

for microwave cooked samples, the mushrooms were microwaved in 100 mL of distilled water with a commercial-800 W microwave oven at high power for 1.5, 3.0, 4.5 and 6.0 min; for boiled samples, the mushrooms were boiled in 100 mL of boiling water on a hot plate for 1.5, 3.0, 4.5 and 6.0 min. The beakers were loosely covered to prevent evaporation during the cooking.

Juice extraction

The cooked mushroom samples and the remaining liquid were cooled rapidly on ice to prevent further cooking from residual heat. They were homogenized with an electric blender. Raw (uncooked) samples were homogenized with 100 ml of distilled water. The homogenate was filtered and centrifuged at $4750\times g$ for 15 min to obtain a clear supernatant. The supernatants were kept at $-20\text{ }^{\circ}\text{C}$ in dark until analyses. All the sample analysis was performed within 1 month.

Assessment of antioxidant activity

All sample extracts were analysed in triplicates in a single run.

Ferric reducing antioxidant power (FRAP)

The total antioxidant activity of the sample was estimated as described by Benzie and Strain (1996). Iron (II) sulphate (0–2000 μM) was used as the standard. The FRAP values were expressed as μmol iron (II) sulphate equivalent per 100 g fresh weight sample ($\mu\text{mol Fe}^{2+}/100\text{ g FW}$).

Trolox equivalent antioxidant capacity (TEAC)

The ABTS radical scavenging activity of the sample was measured as described by Re et al. (1999). Trolox (0–250 $\mu\text{g}/\text{mL}$) was used as the standard. TEAC values were expressed as μmol Trolox equivalent per 100 g fresh weight sample ($\mu\text{mol TE}/100\text{ g FW}$).

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

The DPPH radical scavenging activity of the sample was measured as described by Gerhauser et al. (2003). Ascorbic acid (0–1000 μM) was used as the standard. The DPPH radical scavenging activity was expressed as percentage of DPPH quenched (%).

$$\text{DPPH quenched (\%)} = \frac{(\text{Abs}_{t=0\text{min}} - \text{Abs}_{t=60\text{min}}) / \text{Abs}_{t=0\text{min}}}{\times 100\%}$$

Water soluble phenolic content (WPC)

The WPC of the sample was determined according to the method outlined by Oki et al. (2002). Gallic acid (0–100 $\mu\text{g}/\text{mL}$) was used as the standard. The WPC value was expressed as mg gallic acid equivalent per 100 g fresh weight sample (mg GAE/100 g FW).

Total flavonoid content (TFC)

The TFC of the samples was determined according to the method outlined by Chang et al. (2002). Quercetin (0–800 $\mu\text{g}/\text{mL}$) was used as the standard. The TFC value was expressed as mg quercetin equivalent per 100 g fresh weight sample (mg Qct/100 g FW).

L-ascorbic acid (L-AA) content

The L-AA content in the extract was determined according to the method outlined by Szeto et al. (2002) with some modifications. In a paired treatment, the mushroom sample extracts (100 μl of 1 g/ml) were incubated with and without (i.e. water) L-ascorbate oxidase (EC 1.10.3.3) (5 IU/ml) for 5 min at $37\text{ }^{\circ}\text{C}$. FRAP assay was then performed on these treated extracts and L-AA (0–200 $\mu\text{g}/\text{mL}$) was used as the standard. The amount of L-AA in the extracts corresponded to the difference in FRAP values between the L-ascorbate oxidase treated sample and water treated sample. L-AA content was expressed as mg ascorbate per 100 g of fresh weight sample (mg ascorbate/100 g FW).

Statistical analysis

Data analyses were performed using GraphPad Prism[®] for Windows[®] version 5.02 (GraphPad[®] Software Inc., CA, U.S.A). All data were expressed as mean \pm standard deviation (SD) (triplicate). Statistical significance of the antioxidant activity of different raw mushroom samples was determined by using ONE-WAY ANOVA with Tukey post test. Unpaired *t* test was used to compare the changes of antioxidant values in the samples subjected to various cooking methods and duration with the respective raw (uncooked) samples. Pearson's correlation and regression test was used to determine the relationship between the biochemical assays. P-values less than 0.05 were considered statistically significant.

Result and discussion

Dry mass and moisture content of mushrooms

In the present study, fresh mushroom samples were processed and used in the measurement of antioxidant activity as compared to the dry samples used in other study (Islam et al. 2016). The dry mass and moisture content of five edible mushrooms fall in the range of 6.2 to 13.4 grams and 86.6 to 93.8% respectively (Table 1). This concurred with a previous report by Manzi et al. (1999) which reported a moisture range from 85.2 to 94.7%. Among the selected mushroom samples, *P. ostreatus* showed the highest moisture content (lowest dry mass) while *L. edodes* had the highest dry mass (lowest moisture content). The dry mass and moisture content of *A. bisporus*, *F. velutipes* and *P. eryngii* were not significantly different.

Antioxidant activity in the uncooked mushrooms

Aside from the consumption in dishes and soup, mushrooms have been used as food ingredient and seasoning for other processed food products in South East Asia countries, due to their unique texture and flavour. The five edible mushrooms are rich in antioxidant compounds such as the polyphenols, flavonoids, ascorbic acid, tocopherols, β carotene and ergosterol (Sánchez 2017). Since commercial cultivated edible mushrooms are widely consumed by local populations of lower income, the intake of antioxidants from these mushrooms is recommended to protect against chronic degenerative diseases (Kettawan et al. 2011). The interaction of various antioxidant compounds contributes to the total antioxidant activity in the mushrooms. The total antioxidant activity (reducing power and radical scavenging capacity) of the five edible mushroom samples were measured in vitro by FRAP, TEAC and DPPH radical scavenging assays. The FRAP assay has been reported to be suited for the monitoring of total antioxidant activity in the plant extracts (Benzie and Strain 1996). Besides DPPH radical scavenging assay, TEAC assay was used to complement the limitation found in DPPH assay due to spectral interference and solubility. DPPH radical scavenging assay is effective in capturing the water soluble antioxidant while the more stable ABTS radical species in TEAC assay are reactive towards both hydrophilic and hydrophobic antioxidant compounds (Re et al. 1999). Thus, the monitoring of antioxidant activity in food product with at least two procedures is strongly recommended (Brennan et al. 2011). In the present study, ascorbic acid was used as the positive control in the DPPH radical scavenging assay and it scavenged 50% of the DPPH radicals at 12.9 μM (data not shown) as compared to 8.5 μM (Gerhauser et al. 2003),

Table 1 The dry weight, moisture content, antioxidant values in the selected raw (control) edible mushrooms

Mushrooms (raw/control)	Fresh weight (g)	Dry weight (g)	Moisture content (%)	Antioxidant activity			Antioxidant compounds		
				FRAP (μmol Fe ²⁺ /100 g FW)	TEAC (μmol trolox/100 g FW)	DPPH (%)	WPC (mg GAE/100 g FW)	Total flavonoid (mg Qct/100 g FW)	Ascorbic acid (mg ascorbate/100 g FW)
<i>P. ostreatus</i>	100	6.2 ± 0.4	93.8 ± 0.4	11.0 ± 0.0	50.0 ± 4.0	32.5 ± 2.7	11.1 ± 0.2	0.7 ± 0.2	0.1 ± 0.0
<i>A. bisporus</i>	100	8.5 ± 0.5 ^a	91.5 ± 0.5 ^a	294.0 ± 6.0 ^a	91.0 ± 5.0 ^a	83.8 ± 0.7 ^a	25.0 ± 0.2 ^a	20.2 ± 2.9 ^a	1.8 ± 0.2 ^a
<i>F. velutipes</i>	100	8.9 ± 0.6 ^a	91.1 ± 0.6 ^a	51.0 ± 0.0 ^{ab}	61.0 ± 7.0 ^b	55.1 ± 5.1 ^{abcd}	17.7 ± 1.3 ^{ab}	10.9 ± 1.8 ^{ab}	1.2 ± 0.0 ^{ab}
<i>P. eryngii</i>	100	7.8 ± 0.2 ^a	92.2 ± 0.2 ^a	11.0 ± 1.0 ^{bc}	76.0 ± 11.0 ^a	40.6 ± 0.3 ^{abc}	16.6 ± 0.4 ^{ab}	0.9 ± 0.1 ^{bc}	0.2 ± 0.1 ^{bc}
<i>L. edodes</i>	100	13.4 ± 0.5 ^{abcd}	86.6 ± 0.5 ^{abcd}	208.0 ± 1.0 ^{abcd}	99.0 ± 1.0 ^{acd}	84.1 ± 1.4 ^{acd}	24.7 ± 1.1 ^{acd}	7.2 ± 1.9 ^{abd}	0.4 ± 0.3 ^{bc}

Values represent mean ± standard deviations (SD) of three independent determinations. Values within the same column followed by superscript letters 'a', 'b', 'c' and 'd' (*p* < 0.05) show statistical differences from the raw *P. ostreatus*, *A. bisporus*, *F. velutipes* and *P. eryngii* respectively

15 μM (Subramaniam et al. 2017) and 21.6 μM (Ng et al. 2011) in other studies.

This study showed that mushroom with the highest total antioxidant activity (both reducing power and radical scavenging capacity) and content (WPC, TPC and L-AA) was *A. bisporus* (Table 1). The order of the FRAP, DPPH and TEAC values for each mushroom sample remained the same, which was *A. bisporus* > *L. edodes* > *F. velutipes* > *P. eryngii* > *P. ostreatus*. This order was parallel to that of WPC, TFC and L-AA values except for *F. velutipes*, indicating that phenolic compounds and L-AA were the major antioxidant constituent in the mushroom samples (Ozyurek et al. 2014). The L-AA level in *F. velutipes* was significantly ($p < 0.05$) higher than *L. edodes*. Ferrari et al. (2012) reported that *A. bisporus* presented the highest phenolic content among the common edible mushrooms, which was in agreement with the present report. The WPC, DPPH and TEAC values for *A. bisporus* and *P. eryngii* presented in this study were comparable to the values shown in previous studies (Ferrari et al. 2012; Tan et al. 2015). The low antioxidant activity in uncooked *P. ostreatus* could be attributed to its high moisture content which exposed the sample to both external (oxidation by atmospheric oxygen) and internal enzymatic degradation. (Hossain et al. 2010).

Boiling

The cooking duration applied in all cooking methods except for pressure cooking (15 min) spanned a maximum of 6 min, with 1.5 min interval due to the negative effect reported on the antioxidant and phenolic contents in the vegetables cooked for 10 min (Wachtel-Galor et al. 2008). Table 2 shows that boiling for 1.5 min significantly ($p < 0.01$) reduced the FRAP value (27–67%) and WPC (12–29%) in all mushrooms except *P. ostreatus* when compared to the uncooked samples. Prolonged boiling (3–6 min) caused further reduction in FRAP value (45–80%) and WPC (7–61%) in the mushrooms and this concurred with the previous findings (Kettawan et al. 2011; Tan et al. 2015). The radical scavenging capacities (TEAC and DPPH) of boiled *P. ostreatus* and *A. bisporus* were retained but significant ($p < 0.05$) loss of activities (21–44%) were observed in *F. velutipes*, *P. eryngii* and *L. edodes*. Jagadish et al. (2009) reported a decrease of 8.3–53.1% DPPH radical scavenging activity in the boiled *A. bisporus*. This conflicting result may be attributed to the longer cooking duration (1 h) applied when compared to this study (1.5–6 min). Tan et al. (2015) reported that longer duration of boiling contributed to the decreased radical scavenging abilities of bioactive compounds in the varieties of mushrooms. The boiled *A. bisporus* and *F. velutipes* also showed significant ($p < 0.05$) reduction in

total flavonoid content (51% and 73% at 6 min) and L-AA (67% and 75% at 3 min) respectively. The reduction of antioxidant activities in prolonged boiled and pressure cooked samples might be partly due to the occurrence of lixiviation with the formation of phenol protein complex in the cooking water (Jimenez-Monreal et al. 2009). Mushroom with high amount of phenolic acid on the fruiting body surface (Aherne and O'Brien 2002) are extremely susceptible to this process.

This study also showed that boiling significantly ($p < 0.05$) improved the total flavonoid content in *P. ostreatus* (200–500%), *P. eryngii* (167–267%) and L-AA in *P. eryngii* (100%), *L. edodes* (175–275%). The FRAP value and total flavonoid content of *P. ostreatus* increased significantly by 45% ($p < 0.001$) and 200% ($p < 0.05$) after boiled for 4.5 min. Since the cooking water was included in the sample analysis, the increased flavonoid in the short-duration boiled mushroom samples could be attributed to the leaching of phenolic and flavonoid into the boiling water as reported in previous studies (Choi et al. 2006; Wachtel-Galor et al. 2008). The data clearly shows that the effect of boiling on the antioxidant activity in different mushrooms may be different. It is evident that the retention of antioxidant in mushroom not only depends on the duration of cooking but also the mushroom variety.

Microwave cooking

Table 3 shows a similar trend of negative effect on the total antioxidant activity (FRAP, 5–79%) and WPC (10–58%) in the microwave cooked mushrooms except for *P. ostreatus*. The 4.5 and 6 min-microwaved *L. edodes* recorded the highest loss in FRAP value (79%) and WPC (58%) when compared to the uncooked sample. Tan et al. (2015) reported significant decreased in both the antioxidant activity and total phenolic content of *Pleurotus* species after microwave cooking, which is in agreement with the present study. *P. ostreatus* recorded the highest increase in FRAP value by 91% ($p < 0.001$) after microwaved for 4.5 min. As for TEAC radical scavenging capacity, initial drop of values were observed in the microwaved *P. ostreatus* (48% at 1.5 min), *A. bisporus* (29% at 3 min) and *F. velutipes* (38% at 1.5 min) but they remain unchanged after prolonged cooking (6 min) when compared to the uncooked samples respectively. However, prolonged microwave cooking (3–6 min) significantly ($p < 0.05$) reduced the DPPH radical scavenging capacity in other mushrooms (21–42%) except for *P. ostreatus* and *A. bisporus*.

On the contrary, significant ($p < 0.05$) increased in the total flavonoid content of *P. ostreatus* (200–257%), *P. eryngii* (178–456%) and L-AA of *F. velutipes* (50–67%), *L. edodes* (550–775%) after 3 min of microwave cooking

Table 2 Effect of boiling (different durations) on the antioxidant values in five edible mushrooms

Mushrooms (duration of cooking)	Antioxidant activity					Antioxidant compounds					Δ^* (%)	
	FRAP ($\mu\text{mol Fe}^{2+}/100\text{ g FW}$)	Δ^* (%)	TEAC ($\mu\text{mol trolox}/100\text{ g FW}$)	Δ^* (%)	DPPH (%)	Δ^* (%)	WPC (mg GAE/100 g FW)	Δ^* (%)	Total flavonoid (mg QcV/100 g FW)	Δ^* (%)		Ascorbic acid (mg ascorbate/100 g FW)
<i>P. ostreatus</i>												
1.5 min	11.0 \pm 0.0		56.0 \pm 4.0		30.0 \pm 3.0		9.8 \pm 0.0 ^b	-12	4.2 \pm 0.2 ^c		500	0.2 \pm 0.1
3.0 min	10.0 \pm 0.0 ^a	-9	42.0 \pm 4.0		28.9 \pm 3.2		7.9 \pm 0.3 ^c	-29	2.5 \pm 0.4 ^c		257	0.1 \pm 0.1
4.5 min	16.0 \pm 0.0 ^c	45	48.0 \pm 6.0		30.0 \pm 3.0		8.6 \pm 0.5 ^c	-23	2.1 \pm 0.1 ^a		200	0.1 \pm 0.1
6.0 min	17.0 \pm 0.0 ^c	55	42.0 \pm 5.0		28.9 \pm 3.2		8.4 \pm 0.6 ^c	-24	1.8 \pm 0.5		0.1 \pm 0.1	
<i>A. bisporus</i>												
1.5 min	165.0 \pm 1.0 ^c	-44	98.0 \pm 0.0		82.9 \pm 0.6		22.5 \pm 0.7 ^c	-10	12.2 \pm 2.8 ^b		40	0.6 \pm 0.5 ^b
3.0 min	141.0 \pm 12.0 ^c	-52	93.0 \pm 4.0		82.6 \pm 0.9		23.2 \pm 0.5 ^b	-7	11.3 \pm 2.7 ^b		44	0.6 \pm 0.4 ^b
4.5 min	161.0 \pm 3.0 ^c	-45	94.0 \pm 4.0		80.4 \pm 3.6		23.8 \pm 0.1		10.7 \pm 0.7 ^b		47	0.9 \pm 0.2
6.0 min	154.0 \pm 3.0 ^c	-48	92.0 \pm 5.0		82.3 \pm 1.2		23.8 \pm 0.6		9.8 \pm 0.4 ^c		51	1.0 \pm 0.4
<i>F. velutipes</i>												
1.5 min	17.0 \pm 1.0 ^c	-67	55.0 \pm 4.0		36.9 \pm 3.3 ^c	-33	15.6 \pm 0.4 ^b	-12	4.9 \pm 2.2		0.1 \pm 0.1 ^c	
3.0 min	15.0 \pm 1.0 ^c	-71	44.0 \pm 6.0		30.9 \pm 3.9 ^c	-44	14.8 \pm 0.4 ^c	-16	4.0 \pm 2.4 ^a		63	0.3 \pm 0.0 ^c
4.5 min	20.0 \pm 1.0 ^c	-61	42.0 \pm 8.0		33.0 \pm 3.8 ^c	-40	12.5 \pm 0.3 ^c	-29	2.9 \pm 2.2 ^a		73	1.0 \pm 0.1
6.0 min	10.0 \pm 1.0 ^c	-80	41.0 \pm 8.0		31.2 \pm 2.1 ^c	-43	13.1 \pm 0.2 ^c	-26	2.9 \pm 2.6 ^a		73	0.4 \pm 0.1 ^c
<i>P. eryngii</i>												
1.5 min	8.0 \pm 1.0 ^c	-27	58.0 \pm 7.0 ^a		28.9 \pm 1.0 ^b	-29	11.8 \pm 0.3 ^c	-29	2.4 \pm 0.4 ^c		167	0.3 \pm 0.1
3.0 min	13.0 \pm 1.0		62.0 \pm 5.0		37.3 \pm 2.6		12.0 \pm 0.0 ^c	-28	1.7 \pm 0.5		0.3 \pm 0.1	
4.5 min	14.0 \pm 1.0 ^b	27	69.0 \pm 4.0		35.0 \pm 1.5		9.1 \pm 0.2 ^c	-45	3.3 \pm 0.4 ^c		267	0.4 \pm 0.0 ^c
6.0 min	12.0 \pm 1.0		72.0 \pm 7.0		36.2 \pm 2.9		10.9 \pm 0.5 ^c	-34	3.0 \pm 0.4 ^c		233	0.4 \pm 0.1 ^a
<i>L. edodes</i>												
1.5 min	75.0 \pm 1.0 ^c	-64	81.0 \pm 5.0		66.5 \pm 0.4 ^c	-21	17.6 \pm 0.5 ^c	-29	4.6 \pm 1.3		1.5 \pm 0.1 ^c	
3.0 min	44.0 \pm 1.0 ^c	-79	77.0 \pm 10.0		54.3 \pm 1.3 ^c	-35	9.7 \pm 0.2 ^c	-61	6.0 \pm 2.0		1.1 \pm 0.1 ^a	
4.5 min	42.0 \pm 1.0 ^c	-80	76.0 \pm 8.0 ^a		53.1 \pm 1.6 ^c	-37	10.6 \pm 0.3 ^c	-57	4.8 \pm 2.2		1.1 \pm 0.0 ^a	
6.0 min	64.0 \pm 2.0 ^c	-69	80.0 \pm 8.0		60.0 \pm 1.5 ^c	-29	14.3 \pm 0.4 ^c	-42	8.3 \pm 1.4		1.3 \pm 0.1 ^b	

Values represent mean \pm standard deviations (SD) of three independent determinations. Values within the same column followed by superscript letters 'a' ($p < 0.05$), 'b' ($p < 0.01$) and 'c' ($p < 0.001$) show statistical differences from the control group in Table 1

Δ^* Significant variation of cooked sample when compared to the respective uncooked sample

Table 3 Effect of microwave cooking (different durations) on the antioxidant values in five edible mushrooms

Mushrooms (duration of cooking)	Antioxidant activity					Antioxidant compounds						
	FRAP ($\mu\text{mol Fe}^{2+}/100\text{ g FW}$)	Δ^* (%)	TEAC ($\mu\text{mol trolox}/100\text{ g FW}$)	Δ^* (%)	DPPH (%)	Δ^* (%)	WPC (mg GAE/100 g FW)	Δ^* (%)	Total flavonoid (mg Qcvt/100 g FW)	Δ^* (%)	Ascorbic acid (mg ascorbate/100 g FW)	Δ^* (%)
<i>P. ostreatus</i>												
1.5 min	14.0 \pm 1.0 ^c	27	26.0 \pm 7.0 ^a	-48	30.4 \pm 2.9	-	11.5 \pm 0.2	-	2.5 \pm 0.5 ^c	-	257	0.2 \pm 0.0
3.0 min	18.0 \pm 0.0 ^c	64	45.0 \pm 8.0	-	26.7 \pm 4.0	-	10.1 \pm 0.3	-	2.3 \pm 0.3 ^b	-	229	0.2 \pm 0.1
4.5 min	21.0 \pm 0.0 ^c	91	34.0 \pm 6.0	-	28.1 \pm 3.2	-	8.6 \pm 0.3 ^c	-23	2.1 \pm 0.2 ^b	-	200	0.2 \pm 0.1
6.0 min	19.0 \pm 0.0 ^c	73	26.0 \pm 3.0 ^a	-48	28.1 \pm 2.9	-	7.1 \pm 0.5 ^c	-36	1.4 \pm 0.2	-	0.1 \pm 0.0	-
<i>A. bisporus</i>												
1.5 min	230.0 \pm 5.0 ^c	-22	78.0 \pm 4.0	-	82.1 \pm 1.1	-	25.3 \pm 0.0	-	14.3 \pm 2.4	-	2.3 \pm 0.2	-
3.0 min	179.0 \pm 14.0 ^c	-39	65.0 \pm 14.0 ^b	-29	82.1 \pm 1.0	-	21.1 \pm 0.6 ^c	-16	7.5 \pm 1.0 ^c	-63	2.0 \pm 0.3	-
4.5 min	161.0 \pm 7.0 ^c	-45	87.0 \pm 5.0	-	83.4 \pm 1.4	-	22.3 \pm 0.4 ^c	-11	6.1 \pm 1.1 ^c	-70	0.8 \pm 0.1	-
6.0 min	124.0 \pm 9.0 ^c	-58	83.0 \pm 3.0	-	79.3 \pm 1.9 ^a	-5	22.2 \pm 0.7 ^c	-11	4.3 \pm 0.5 ^c	-79	0.6 \pm 0.3 ^b	-67
<i>F. velutipes</i>												
1.5 min	30.0 \pm 1.0 ^c	-41	38.0 \pm 15.0 ^a	-38	48.5 \pm 1.0	-	15.9 \pm 0.1 ^a	-10	7.3 \pm 3.4	-	2.0 \pm 0.1 ^c	67
3.0 min	18.0 \pm 1.0 ^c	-65	43.0 \pm 3.0	-	41.5 \pm 2.8 ^c	-25	15.2 \pm 0.1 ^c	-14	5.1 \pm 2.3	-	1.8 \pm 0.1 ^c	50
4.5 min	19.0 \pm 1.0 ^c	-63	40.0 \pm 4.0	-	34.1 \pm 3.0 ^c	-38	17.0 \pm 0.1	-	2.2 \pm 1.7 ^b	-80	0.3 \pm 0.1 ^c	-75
6.0 min	17.0 \pm 1.0 ^c	-67	40.0 \pm 7.0	-	32.9 \pm 3.5 ^c	-40	15.3 \pm 0.3 ^c	-14	2.6 \pm 1.2 ^b	-76	0.3 \pm 0.0 ^c	-75
<i>P. eryngii</i>												
1.5 min	6.0 \pm 1.0 ^c	-45	69.0 \pm 0.0	-	32.7 \pm 3.5	-	13.9 \pm 0.1 ^c	-16	5.0 \pm 0.8 ^c	-	456	0.1 \pm 0.0
3.0 min	7.0 \pm 1.0 ^c	-36	71.0 \pm 3.0	-	29.6 \pm 3.4 ^b	-27	11.5 \pm 0.4 ^c	-31	2.5 \pm 0.5 ^a	-178	0.2 \pm 0.1	-
4.5 min	6.0 \pm 1.0 ^c	-45	38.0 \pm 10.0 ^c	-50	32.4 \pm 3.4	-	11.6 \pm 0.1 ^c	-30	1.4 \pm 0.5	-	0.3 \pm 0.0	-
6.0 min	11.0 \pm 1.0	-49	39.0 \pm 3.0 ^c	-49	32.0 \pm 2.5 ^a	-21	9.2 \pm 0.2 ^c	-45	1.4 \pm 0.4	-	0.3 \pm 0.0	-
<i>L. edodes</i>												
1.5 min	198.0 \pm 2.0 ^c	-5	96.0 \pm 3.0	-	86.2 \pm 0.5	-	18.2 \pm 0.7 ^c	-26	1.8 \pm 0.5 ^b	-75	3.5 \pm 0.3 ^c	775
3.0 min	154.0 \pm 3.0 ^c	-26	85.0 \pm 5.0	-	85.0 \pm 1.6	-	14.9 \pm 0.2 ^c	-40	1.9 \pm 0.9 ^b	-74	2.6 \pm 0.4 ^c	550
4.5 min	44.0 \pm 1.0 ^c	-79	63.0 \pm 5.0 ^c	-36	48.4 \pm 3.1 ^c	-42	10.7 \pm 0.2 ^c	-57	2.4 \pm 0.3 ^a	-67	1.0 \pm 0.0	-
6.0 min	60.0 \pm 1.0 ^c	-71	50.0 \pm 7.0 ^c	-49	52.5 \pm 2.0 ^c	-38	10.4 \pm 0.1 ^c	-58	1.9 \pm 0.6 ^b	-74	0.5 \pm 0.0	-

Values represent mean \pm standard deviations (SD) of three independent determinations. Values within the same column followed by superscript letters 'a', 'b' ($p < 0.05$), 'b' ($p < 0.01$) and 'c' ($p < 0.001$) show statistical differences from the control group in Table 1

Δ^* Significant variation of cooked sample when compared to the respective uncooked sample

indicated that the stability of water-soluble antioxidant compounds was strongly depended on cooking times. Since the extraction of bioactive compounds could be affected by texture and shape of each mushroom species (Kettawan et al. 2011), the thick and solid structure of *P. eryngii* and *P. ostreatus* may prevent the complete extraction of antioxidant compounds. Hence, short duration of intense heat treatment (i.e. microwave cooking) could promote the release of flavonoid possibly due to the disruption of mushroom cell wall by heat. In addition, microwave cooking has been suggested as a better heat treatment to retain phenolic compounds in *Boletus* mushrooms than other domestic cooking methods (Sun et al. 2014).

Although the total flavonoid content in *F. velutipes*, *P. eryngii* and *L. edodes* increased during the early 3 min-microwave cooking, the FRAP values were affected negatively, as a result of probably from the destruction of antioxidant compounds other than the phenolic compounds (Table 2). Besides, the consumption of L-AA and polyphenols as reactants in the Maillard reaction as well as the formation of pro-oxidant compounds (Nicoli et al. 1999) during heating could contribute to the loss of antioxidant value in the cooked mushrooms. The mechanism behind these processes requires further research. Overall, microwave cooking for 1.5 min is suggested as the preferred cooking method for *A. bisporus* as it retained the antioxidant values in the mushroom when compared to the uncooked sample.

Steam cooking

Similar to boiling (Table 2) and microwave cooking (Table 3), steam cooking has negative effect (7–73%, $p < 0.001$) on the total antioxidant activity (reducing power) in all the mushrooms except *P. ostreatus* (Table 4). The significant ($p < 0.001$) decreased in FRAP values of *F. velutipes* (25–45%), *P. eryngii* (55–73%) and *L. edodes* (7–33%), regardless of steaming duration, were accompanied by the reduction of WPC in the mushrooms (Table 4). However, steaming was the only cooking method that retained the WPC in *A. bisporus*, along with significant ($p < 0.001$) enhancement found in 3-min cooked *P. ostreatus* (23%). This could be attributed to the inactivation of polyphenol oxidase by heat (Cheng et al. 2013) or the increased release of individual phenolic acid from conjugated glycosides due to the breaking of glycosidic bond after short duration of steaming and thus reacted better with the Folin reagent in phenolic assay (Jimenez-Monreal et al. 2009). Wachtel-Galor et al. (2008) also reported similar finding in the cooked vegetables such as cauliflower. They suggested the lower temperature in steam cooking compared to boiling and microwaving, had less degradation effect on the phenolic content. Both the TEAC and DPPH

radical scavenging values in the mushrooms were retained after 6-min steam cooking except for *F. velutipes*, suggesting the cooking method had less impact on the antioxidants with radical scavenging properties. The radical scavenging capacity (TEAC and DPPH) of *F. velutipes* was significantly ($p < 0.05$) reduced by 18% to 54% after steam cooking.

As for total flavonoid content, significant ($p < 0.001$) increase were observed in *P. ostreatus* (400%) and *P. eryngii* (233%) after 3 min of steam cooking. Similar effect was observed earlier in boiled and microwaved mushrooms at 1.5 min of cooking (Tables 2, 3). Previous study had shown that the highest retention of flavonoids were found in steam cooked vegetables (Podsdek 2007). Interestingly, steam cooking also significantly ($p < 0.01$) increased the L-AA in *P. ostreatus* (200% at 3 min), *A. bisporus* (78% at 1.5 min), *F. velutipes* (67–117%, 1.5–6 min) and *L. edodes* (275–650%, 1.5–6 min) while the L-AA in *P. eryngii* was not affected. This could be explained by the increased extractability of L-AA as a result of the mushroom matrix softening by short duration of heating rather than the thermal degradation during the prolonged cooking process. Munyaka et al. (2010) had shown that brief thermal treatment at above 70 °C retained the reduced form of ascorbic acid in vegetable products due to the inactivation of ascorbic oxidase in the vegetables, which prevented the oxidation of L-AA to dehydroascorbic acid. The factors that contribute to thermal stability of L-AA and the mechanisms involved deserve further investigation.

The increase of total antioxidant activity (FRAP) in steamed *P. ostreatus* was accompanied by the significant ($p < 0.05$) increased of WPC, total flavonoids and L-AA content, suggesting the antioxidant activity in the mushroom was contributed mainly by water soluble antioxidant compounds. Our previous study has shown that short duration of boiling and microwave cooking could induce selected heat stable proteins such as antioxidant enzymes in the *Momordica charantia* (Ng et al. 2014). The up-regulation of superoxide dismutase and quinone oxidoreductase in the boiled and microwaved *M. charantia* counterbalance the enhanced generation of reactive oxygen species under heat stress. In addition, Cheng et al. (2012) showed that *P. ostreatus* and *A. bisporus* had high superoxide dismutase activity and the enzyme showed high thermal stability after heated at 90 °C for 10 min. Therefore, one should not rule out the possibility of the improvement in antioxidant activity of cooked mushroom may be due to the upregulation of these antioxidant enzymatic proteins during short duration of heat stress. In the present study, 4.5 min of steam cooking which showed the best antioxidant values in *P. ostreatus* and *L. edodes*, is considered the preferred cooking method while steam cooking for 1.5 min is preferred for *F. velutipes*. This concurs with Turkmen et al.

Table 4 Effect of steam cooking (different durations) on the antioxidant values in five edible mushrooms

Mushrooms (duration of cooking)	Antioxidant activity					Antioxidant compounds					Δ^* (%)	
	FRAP ($\mu\text{mol Fe}^{2+}/100\text{ g FW}$)	Δ^* (%)	TEAC ($\mu\text{mol trolox}/100\text{ g FW}$)	Δ^* (%)	DPPH (%)	Δ^* (%)	WPC (mg GAE/100 g FW)	Δ^* (%)	Total flavonoid (mg Qc/100 g FW)	Δ^* (%)		Ascorbic acid (mg ascorbate/100 g FW)
<i>P. ostreatus</i>												
1.5 min	12.0 \pm 0.0 ^a	9	53.0 \pm 6.0		33.8 \pm 2.4		12.9 \pm 0.0 ^c	16	1.4 \pm 0.4		0.1 \pm 0.1	
3.0 min	13.0 \pm 0.0 ^c	18	53.0 \pm 11.0		29.1 \pm 2.8		13.7 \pm 0.3 ^c	23	3.5 \pm 0.8 ^c	400	0.1 \pm 0.0	
4.5 min	24.0 \pm 1.0 ^c	118	50.0 \pm 9.0		29.7 \pm 2.6		11.8 \pm 0.4		3.3 \pm 0.6 ^c	371	0.3 \pm 0.0 ^b	
6.0 min	15.0 \pm 0.0 ^c	36	42.0 \pm 12.0		30.8 \pm 2.3		11.3 \pm 0.4		2.5 \pm 0.0 ^c	257	0.2 \pm 0.1	
<i>A. bisporus</i>												
1.5 min	141.0 \pm 5.0 ^c	- 52	66.0 \pm 7.0 ^a	- 27	82.6 \pm 1.1		25.3 \pm 0.4		11.2 \pm 3.4 ^b	- 45	3.2 \pm 0.3 ^b	
3.0 min	164.0 \pm 6.0 ^c	- 44	74.0 \pm 12.0		83.6 \pm 1.1		25.5 \pm 0.8		14.4 \pm 5.0		1.9 \pm 0.7	
4.5 min	175.0 \pm 4.0 ^c	- 40	86.0 \pm 7.0		83.3 \pm 0.9		25.0 \pm 0.5		12.0 \pm 1.0 ^b	- 41	1.6 \pm 0.4	
6.0 min	165.0 \pm 7.0 ^c	- 44	90.0 \pm 6.0		83.4 \pm 0.9		24.2 \pm 0.2		13.8 \pm 1.0		1.7 \pm 0.1	
<i>F. velutipes</i>												
1.5 min	48.0 \pm 2.0		44.0 \pm 8.0	- 18	45.0 \pm 3.3 ^a		16.0 \pm 0.2 ^a	- 10	6.7 \pm 3.0		2.6 \pm 0.0 ^c	
3.0 min	38.0 \pm 2.0 ^c	- 25	47.0 \pm 7.0		56.0 \pm 2.2		16.0 \pm 0.6 ^a	- 10	4.9 \pm 2.9		2.9 \pm 0.1 ^c	
4.5 min	28.0 \pm 2.0 ^c	- 45	46.0 \pm 5.0		43.9 \pm 2.5 ^b	- 20	16.0 \pm 0.8 ^a	- 10	5.7 \pm 1.6		2.2 \pm 0.1 ^c	
6.0 min	29.0 \pm 1.0 ^c	- 43	28.0 \pm 8.0 ^c	- 54	41.3 \pm 1.5 ^c	- 25	14.5 \pm 0.2 ^c	- 18	5.4 \pm 0.7		2.0 \pm 0.0 ^c	
<i>P. eryngii</i>												
1.5 min	3.0 \pm 0.0 ^c	- 73	62.0 \pm 4.0		34.2 \pm 2.3		13.9 \pm 0.4 ^c	- 16	1.1 \pm 0.0		0.1 \pm 0.1	
3.0 min	5.0 \pm 1.0 ^c	- 55	72.0 \pm 3.0		38.6 \pm 3.0		15.7 \pm 0.4		3.0 \pm 0.5 ^c	233	0.2 \pm 0.0	
4.5 min	3.0 \pm 1.0 ^c	- 73	78.0 \pm 3.0		32.7 \pm 2.6		14.1 \pm 0.3 ^c	- 15	2.4 \pm 0.3 ^a	167	0.2 \pm 0.1	
6.0 min	4.0 \pm 1.0 ^c	- 64	71.0 \pm 1.0		37.6 \pm 2.8		14.5 \pm 0.4 ^c	- 13	2.4 \pm 0.3 ^a	167	0.3 \pm 0.1	
<i>L. edodes</i>												
1.5 min	139.0 \pm 1.0 ^c	- 33	74.0 \pm 11.0 ^a	- 25	84.7 \pm 0.9		16.9 \pm 0.7 ^c	- 32	8.3 \pm 2.0		1.5 \pm 0.2 ^c	
3.0 min	161.0 \pm 3.0 ^c	- 23	84.0 \pm 9.0		84.2 \pm 1.8		15.4 \pm 0.2 ^c	- 38	8.7 \pm 1.9		3.0 \pm 0.2 ^c	
4.5 min	194.0 \pm 4.0 ^c	- 7	101.0 \pm 1.0		84.1 \pm 0.4		19.4 \pm 0.6 ^c	- 21	6.2 \pm 0.7		3.0 \pm 0.3 ^c	
6.0 min	179.0 \pm 2.0 ^c	- 14	78.0 \pm 10.0		84.6 \pm 0.4		18.8 \pm 0.3 ^c	- 24	3.2 \pm 0.5		3.0 \pm 0.2 ^c	

Values represent mean \pm standard deviations (SD) of three independent determinations. Values within the same column followed by superscript letters 'a' ($p < 0.05$), 'b' ($p < 0.01$) and 'c' ($p < 0.001$) show statistical differences from the control group in Table 1

Δ^* Significant variation of cooked sample when compared to the respective uncooked sample

(2005) who also suggested that moderate heat treatment (i.e. short duration) could be used to improve antioxidant quality of plant food products.

Pressure cooking

Pressure cooking is generally believed to critically affect the nutrient in plant products due to the high temperature, pressure and prolonged duration applied during the cooking process. Hence, it is the least favorable heating method when compared to other domestic cooking methods. This study showed that pressure cooking for 15 min exerted different effects on the antioxidant values in different type of mushrooms (Table 5). Significant ($p < 0.001$) positive impact (91%) was observed in the FRAP values of pressure cooked *P. ostreatus* and *P. eryngii* while negative effects were observed in *A. bisporus* (68%), *F. velutipes* (71%) and *L. edodes* (70%). Pressure cooking significantly ($p < 0.05$) decreased the WPC content by 17% to 45% in all mushrooms. The improvement of antioxidant activity in pressure cooked mushrooms may be due to the release of active antioxidants from the fibrous complexes during pressure cooking (low moisture level and high temperature). This is further supported by our observation that the increased of FRAP values in the pressure cooked *P. ostreatus* and *P. eryngii* were accompanied by the significant ($p < 0.05$) increased of L-AA by 200% and flavonoid content by 811% in respective mushroom (Table 5). High concentrations of flavonols are found mainly in plant foods that are characterized by a high skin to volume ratio (Ah-erne and O'Brien 2002). This study hypothesized the application of high temperature in the pressure cooked *P. eryngii* with thick and firm tissue structure could promote the release of high amounts of aglycone flavonoids from the mushroom tissues.

Apart from the reduced flavonoid content, the highest degradation of L-AA in both *A. bisporus* (95%) and *F. velutipes* (92%) contributed to the overall decreased of antioxidant activity (reducing power). Choi et al. (2006) reported that prolonged heating time and higher temperature enhanced the DPPH radical scavenging activity of *L. edodes*. On the contrary, the present study showed a significant ($p < 0.05$) decrease in the radical scavenging capacity (63% in TEAC and 33% in DPPH) of pressure cooked *L. edodes*. The decreased of radical scavenging activity and reducing power in *L. edodes* after pressure cooking for 15 min could be attributed to the prolonged exposure to heat treatment which eventually cause more WPC to breakdown (45%) (Table 5). The increase of L-AA after pressure cooking did not improve the overall antioxidant activity in *L. edodes*, as there may be destruction of other antioxidant compounds other than the polyphenols. In addition, the antioxidant activity of

phenolic compound was reported to be higher than antioxidant vitamins (Podsedek 2007). Overall, pressure cooking for 15 min is suggested as the preferred cooking method for *P. eryngii* as it was the only cooking method that significantly increased the total antioxidant activity (FRAP) and yielded the highest increased of flavonoid (811%) in the mushroom.

Correlation analysis

Table 6 depicts the linear correlation and regression analysis between different antioxidant parameters in the mushrooms. WPC was significantly ($p < 0.001$) correlated with the FRAP assay ($r = 0.8102$), TEAC ($r = 0.6634$) and DPPH radical scavenging assays ($r = 0.8219$) in the mushrooms aqueous extracts. In addition, significant ($p < 0.001$) association between the TFC level and the total antioxidant activities (FRAP, $r = 0.7210$; TEAC, $r = 0.5258$ and DPPH, $r = 0.7049$) were present in the mushroom extracts. From these correlation results, it is pertinent to suggest that the water-soluble phenolic compounds and flavonoids present in the mushroom were the main contributors to the observed antioxidant effect. This is in agreement with a previous report on the possible role of phenolic acids and flavonoids in the plant antioxidant capacity (Hossain et al. 2010). Besides, flavonoids were also shown as potent radical scavengers (Nicoli et al. 1999).

The moderate correlation of L-AA with FRAP assay ($r = 0.6128$, $p < 0.001$), TEAC ($r = 0.3023$, $p < 0.05$) and DPPH radical scavenging assays ($r = 0.6496$, $p < 0.001$) provided further evidences on the contribution of L-AA, at least partly, to the observed antioxidant activity in the cooked mushroom extracts (Tables 2, 3, 4, 5). In tandem, the results depicted in Table 6 also show that the FRAP value in the mushroom extracts positively correlated with the radical scavenging assays (TEAC, $r = 0.7174$ and DPPH, $r = 0.9321$). This indicates the bioactive compounds present in the extracts were good reductants as well as radical scavengers. As expected, both TEAC and DPPH radical scavenging assays were closely correlated ($r = 0.7700$, $p < 0.001$) as both assays use similar principle in the estimation of antioxidant activity in the mushroom extracts based on the radical scavenging activity.

Conclusion

The results clearly showed that *A. bisporus* contained the highest overall antioxidant values among the uncooked samples while *P. ostreatus* had the lowest antioxidant activity. There were differences in the antioxidant values

Table 5 Effect of pressure cooking (15 min) on the antioxidant values in five edible mushrooms

Mushrooms	Antioxidant activity					Antioxidant compounds						
	FRAP ($\mu\text{mol Fe}^{2+}/100\text{ g FW}$)	Δ^* (%)	TEAC ($\mu\text{mol trolox}/100\text{ g FW}$)	Δ^* (%)	DPPH (%)	Δ^* (%)	WPC (mg GAE/100 g FW)	Δ^* (%)	Total flavonoid (mg Qct/100 g FW)	Δ^* (%)	Ascorbic acid (mg ascorbate/100 g FW)	Δ^* (%)
<i>P. ostreatus</i>	21.0 \pm 0.0 ^c	91	26.0 \pm 3.0 ^a	– 48	31.2 \pm 2.8		7.6 \pm 0.4 ^c	– 32	1.6 \pm 0.4		0.3 \pm 0.0 ^b	200
<i>A. bisporus</i>	93.0 \pm 0.0 ^c	– 68	84.0 \pm 9.0		84.8 \pm 1.4		17.6 \pm 0.4 ^c	– 30	12.7 \pm 0.3 ^a	– 37	0.1 \pm 0.0 ^c	– 95
<i>F. velutipes</i>	15.0 \pm 0.0 ^c	– 71	51.0 \pm 2.0		34.9 \pm 2.5 ^c	– 37	14.7 \pm 0.7 ^c	– 17	3.9 \pm 2.8 ^a	– 64	0.1 \pm 0.1 ^c	– 92
<i>P. eryngii</i>	21.0 \pm 1.0 ^c	91	60.0 \pm 5.0		34.1 \pm 5.1		9.6 \pm 0.3 ^c	– 42	8.2 \pm 0.9 ^c	811	0.2 \pm 0.1	
<i>L. edodes</i>	62.0 \pm 1.0 ^c	– 70	37.0 \pm 10.0 ^c	– 63	56.2 \pm 2.7 ^c	– 33	13.5 \pm 0.4 ^c	– 45	6.5 \pm 2.0		1.9 \pm 0.1 ^c	375

Values represent mean \pm standard deviations (SD) of three independent determinations. Values within the same column followed by superscript letters 'a' ($p < 0.05$), 'b' ($p < 0.01$) and 'c' ($p < 0.001$) show statistical differences from the control group in Table 1

Δ^* Significant variation of cooked sample when compared to the respective uncooked sample

Table 6 Correlation and regression analysis of the biochemical assays

Assays	R ²	Pearson r	Significance
Between WPC and antioxidant activity			
WPC versus FRAP	0.6564	0.8102	***
WPC versus TEAC	0.4401	0.6634	***
WPC versus DPPH	0.6755	0.8219	***
Between TFC and antioxidant activity			
TFC versus FRAP	0.5199	0.7210	***
TFC versus TEAC	0.2765	0.5258	***
TFC versus DPPH	0.4969	0.7049	***
Between L-AA and antioxidant activity			
FRASC versus FRAP	0.3755	0.6128	***
FRASC versus TEAC	0.0914	0.3023	*
FRASC versus DPPH	0.4220	0.6496	***
Between reducing power and radical scavenging activity			
FRAP versus TEAC	0.5146	0.7174	***
FRAP versus DPPH	0.8689	0.9321	***
Between two radical scavenging activities			
TEAC versus DPPH	0.5929	0.7700	***
Between WPC and flavonoids			
WPC versus TFC	0.5644	0.7513	***

Seventy paired mean samples from each assay were used in the comparison

The R² value denotes the regression value and the r value denotes the Pearson's correlation value

The level of significance was expressed as * $p < 0.05$; *** $p < 0.001$

between cooked and uncooked mushrooms. The retention of antioxidant value in the mushroom strongly depended on the variety of mushroom other than the cooking method.

The duration of cooking had a stronger influence on heat labile L-AA in the mushrooms than the cooking methods. Short-duration cooking (3 min), especially steam cooking generally increased the total flavonoid and L-AA while prolonged cooking (15 min) such as pressure cooking, reduced the water soluble phenolic content in the mushrooms. In a nut shell, among the tested cooking methods, steam cooking was the better choice to obtain the best antioxidant properties of *F. velutipes* (1.5 min), *P. ostreatus* (4.5 min) and *L. edodes* (4.5 min) while microwave cooking for 1.5 min was preferred for *A. bisporus*. The pressured cooked *P. eryngii*, showed the best overall antioxidant value among the cooked samples. Optimised cooking method including pressure cooking could increase the health beneficial effects of mushrooms. The present finding would be a valuable database to the food manufacturers as it might encourage the food industry to recommend particular cooking methods to preserve or improve the nutritional quality in the edible mushrooms. This would also benefit the consumers who wish to adopt cooking techniques that could deliver the maximum health benefits.

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