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The effect of cooking duration on radical scavenging properties of *Hypsizygus tessellatus* and *Pleurotus ostreatus*

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Abstract

Mushrooms are a popular ingredient in the human diet due to their flavour, nutritional values and functional properties, in particular, their antioxidant potential. Most mushrooms are consumed after cooking and the impact of cooking methods and durations on the antioxidant properties of various edible mushrooms have been reported. However, the reports on the effect of cooking on the antioxidant properties of *Hypsizygus tessellatus* (*shimeji*), a widely consumed mushroom in East Asia, are limited. Therefore, this study aimed to investigate the effect of cooking duration on the radical scavenging properties of *H. tessellatus*. *Pleurotus ostreatus* was included for comparison purposes. Mushroom samples were prepared raw, boiled in distilled water for 1 min, 3 mins and 5 mins, then blended and centrifuged to obtain mushroom extracts. The mushroom extracts were evaluated for their radical scavenging properties using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Results showed that 1-min cooked *H. tessellatus* extract exhibited the highest radical scavenging activity (76.6±2.14% DPPH scavenging activity) while extract boiled for 5 mins had the lowest radical scavenging activity (62.9±6.08% DPPH scavenging activity). Thus, cooking time has a significant effect on the radical scavenging activity of *H. tessellatus* extract. In conclusion, the effect of cooking on *H. tessellatus* and *P. ostreatus* can be both beneficial and disadvantageous depending on the duration.

1. Introduction

For centuries, mushrooms have been selected as one of the most popular food ingredients in the human diet, especially in Asian countries, due to their unique flavour and aroma. There are more than 2,000 species of mushrooms in nature, but only about 25 are widely accepted as food and commercialised (Valverde *et al*., 2015). The common edible mushrooms include *Agaricus bisporus* (button mushroom), *Pleurotus ostreatus* (oyster mushroom), *Pleurotus eryngii* (king oyster mushroom) and *Lentinula edodes* (shiitake mushroom). Edible mushrooms are rich in metabolites, as well as proteins, carbohydrates, dietary fibre and low lipid content. In addition, it has been determined that mushrooms have functional values such as antioxidant, i.e. radical scavenging properties (Tan *et al*., 2015) that can protect against oxidative damage. Therefore, in terms of nutritional and pharmacological properties, eating mushrooms is becoming more and more important in the human diet.

Many foods require different post-harvest processing such as drying, heating and cooking to achieve food safety and quality. During these processes, the nutritional value such as the antioxidant capacity of plants or mushrooms may be affected by the treatment methods and/or the species of plants or mushrooms (Alide *et al*., 2020; Ng, Yong and Lim, 2020; Ng *et al*., 2021). Mushrooms are commonly consumed after various cooking (heating) methods. As most bioactive compounds and naturally occurring antioxidants are susceptible to heating, the effect of heat treatment by various cooking methods on the antioxidant capacity of mushrooms has been well documented. Ng and Rosman (2019) showed that domestic cooking could yield the best antioxidant values in the extracts of various edible mushrooms including *A. bisporus*, *L. edodes* and *Pleurotus sajor-caju*. Also, Abacan *et al.* (2017) concluded that boiling mushrooms can increase its antioxidant activities. On the other hand, Ng and Tan (2017) reported that the radical scavenging capacities of *P. ostreatus* and *A. bisporus* were retained after boiling for up to 6 mins while Tan *et al*. (2015) demonstrated that boiling for 5 mins could increase, decrease or retain the antioxidant activity of selected mushroom species.

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Hypsizygus tessellatus (*shimeji*) is a widely consumed mushroom in East Asia and its phytochemical composition includes tubuloside A, 6-gingerol, isoflavan, cyclocurcumin and several other compounds with antioxidative activity (Ukaegbu *et al*., 2020). The anti-oxidant, anti-microbial and anti-cancer effects of *H. tessellatus* have also been reported (Shah *et al*., 2018; Ukaegbu *et al*., 2019). However, the reports on the effect of cooking duration on the antioxidant properties of *H. tessellatus* are limited. Therefore, this study aimed to investigate the effect of cooking, in particular the cooking duration, on the radical scavenging potential of *H. tessellatus*. *Pleurotus ostreatus* was included in the study for comparison purposes.

2. Materials and methods

2.1 Sample preparation

Fresh *H. tessellatus* (brown variant) and *P. ostreatus* samples were purchased from the local supermarket. The identification of mushroom samples was carried out by morphological examination according to Christensen (1972). Complete fruiting bodies that included the cap, gills and stipe of the mushroom samples were washed and dried with a paper towel and weighed. They were randomly divided into 100 g portions for subsequent cooking treatments.

2.2 Cooking method

All cooking experiments were done in triplicates, using 100 g of sample for each individual run according to Abacan *et al.* (2017). Mushroom samples were boiled in 100 mL of boiling water in a beaker for 1, 3, and 5 mins, respectively. The beakers were loosely covered by aluminium foil to prevent evaporation.

2.3 Preparation of mushroom extracts

The beakers containing cooked mushrooms were rapidly cooled on ice to prevent further heating. The remaining solution was discarded and 100 mL of distilled water was added to the cooked mushroom and then homogenised with an electric blender. Meanwhile, the raw mushrooms were homogenised with 100 mL distilled water and with an electric blender. The homogenate was then filtered and centrifuged at 1500×*g* for 15 mins to obtain a clear extract. The extracts of both cooked and uncooked mushrooms were kept at -20°C in the dark until analysis.

2.4 DPPH free radical scavenging assay

The radical scavenging capacity of the mushroom extract was estimated using the modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of Abacan *et al*. (2017) and Ng, Samsuri and Yong (2020). An amount of 0.5 mL of the extract was adjusted to 3 mL volume with the addition of 2 mL of distilled water and then mixed with 0.5 mL DPPH solution (0.1 mM in absolute methanol). Ascorbic acid (100 µg/mL) was included as a positive control. The reaction mixture was kept in dark for 30 mins at room temperature. The absorbance of the mixture was measured at 517 nm against a reagent blank. The result was measured as the decrease in the absorbance of DPPH radical and expressed as the percentage of DPPH quenched.

$$\text{DPPH quenched (\%)} = [(Ac - As) / Ac] \times 100\%$$

Where, Ac was absorbance of control and As was absorbance of sample.

2.5 Statistical analysis

Statistical comparison using IBM SPSS Statistics Version 26. Experiments were carried out in triplicates. All data on DPPH radical scavenging assay were the average of triplicate analyses. A significant difference in antioxidant activity of edible mushrooms at the different cooking times was analysed using one-way ANOVA. Interaction between mushroom variety and cooking duration was analysed using two-way ANOVA.

3. Results and discussion

DPPH radical-scavenging activity assay was carried out to determine the effect of boiling at different cooking duration on the free radical scavenging ability of *H. tessellatus* and *P. ostreatus*. Figure 1 shows the summary of the DPPH radical scavenging activities of the *H. tessellatus* and *P. ostreatus* extracts after different cooking duration. The scavenging activity is expressed as the percentage of DPPH quenched (%), where the higher the percentage, the better the antioxidant potential of the corresponding extract. In Figure 1, the highest DPPH radical scavenging activity was shown by *H. tessellatus* cooked for 1 min with a percentage inhibition of 76.6±2.14% which is 8.5% higher ($p < 0.05$) than the raw mushrooms. On the other hand, the lowest radical scavenging activity was shown by 5 min-cooked *H. tessellatus* extracts (62.9±6.08%) and it is 5.2% ($p < 0.05$) lower than that of the raw mushroom. For *P. ostreatus*, the % DPPH scavenging activity ranged between 64.0±3.36 and 75.8±5.64 and there are no statistically significant differences between the raw and cooked samples. The percent DPPH scavenging activity for *P. ostreatus* reported here appears to be higher than those reported in other studies in which the aqueous extracts of the raw mushroom showed between 41.13% and 50% DPPH scavenging activity (Abacan *et al.*, 2017; Kam and Ng, 2021).

The above results show that boiling the mushrooms

could either increase, decrease or cause no significant change in radical scavenging activities in the mushrooms. *H. tessellatus* boiled for 1 min shows the highest radical scavenging activity. This finding agrees with Choi *et al*. (2006) who reported that prolonged heating time enhanced the DPPH radical scavenging activity of Shiitake mushrooms. These results also support the research of Kao *et al*. (2014), in which boiling vegetables for less than 5 mins is better at retaining or increasing total antioxidant potential. However, as shown in Figure 1, extract from *H. tessellatus* cooked for 5 mins showed significantly reduced radical scavenging activity compared to its uncooked sample. This finding is also consistent with other studies that showed that boiling for 10 mins reduced the free radical scavenging activity of vegetables (Preti *et al*., 2017). Tan *et al*. (2015) also showed that the longer the boiling time, the lower the ability of biologically active compounds in various mushrooms to scavenge free radicals, supporting the findings from the present study. On the contrary, a more recent study showed that heat treatment either by boiling or microwaving for 10 mins significantly increased the antioxidant and radical scavenging potency of bitter melon (Ng and Kuppusamy, 2019). Thus, a further investigation involving cooking duration above 5 mins is warranted. This study also shows that the mushroom variety had no significant effect on the radical scavenging activity of mushrooms ($p>0.05$). The data indicate that the effect of boiling on the radical scavenging activity in different mushrooms may be different and the retention of radical scavenging activities in mushrooms depends on cooking duration.

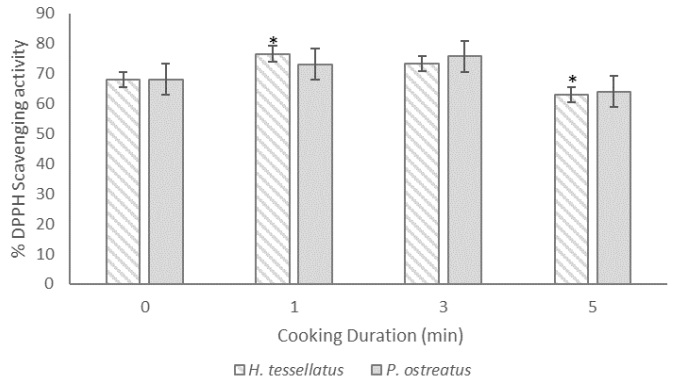

Figure 1. Effect of boiling duration on the radical scavenging activities of *H. tessellatus* and *P. ostreatus* extracts. Columns with '*' indicate significantly different ($p<0.05$) from uncooked sample.

The positive correlation between total phenolic/ flavonoid contents in plants or mushrooms and their antioxidant activities has been well reported (Abacan *et al.*, 2017; Preti *et al*., 2017; Ng, Koick and Yong , 2020; Ukaegbu *et al*., 2020). The ability of the *H. tessellatus* and *P. ostreatus* extracts to reduce DPPH free radicals demonstrated in this study indicates the presence of antioxidative compounds as free radical inhibitors in these mushrooms. The decrease in radical scavenging activity with boiling for 5 mins can be attributed to the possible leaching of soluble compounds into the cooking liquid and the breakdown of phenolic compounds during cooking (Preti *et al*., 2017). Thus, it would be important to analyse the mushroom cooking liquid in future studies. According to Tan *et al*. (2015), the decrease in antioxidant potential in boiled samples may be due to the formation of the phenol protein complex in the cooking solution. Therefore, increased duration of high-temperature cooking (100°C) may cause decomposition of the antioxidant components, which may lead to a reduction in the radical scavenging potential. On the other hand, boiling of *H. tessellatus* for 1 min showed significantly higher radical scavenging activity compared to the uncooked sample. This could be due to the upregulation of antioxidative enzymes such as quinone oxidoreductase and superoxide dismutase induced by the oxidative stress caused by the boiling process (Ng *et al*., 2014).

4. Conclusion

The present study showed that aqueous extract of 1-min boiled *H. tessellatus* contains the highest level of radical scavenging potential with free radical scavenging ability while boiling for 5 mins in distilled water had the lowest radical scavenging activity compared to the raw *H. tessellatus* extracts. The retention of radical scavenging activity in the mushroom strongly depended on the cooking duration. Experiments involving various concentrations of the mushroom extracts, thus enabling the expression of IC_{50} values would allow for a better comparison of the activities. In addition, further studies can be conducted to include cooking durations of above 5 mins. Further, the effects of other cooking methods on the antioxidant capacity of *H. tessellatus* and *P. ostreatus* should be studied to provide more information for food manufacturers, food processing companies and the catering industry to set specific methods to maintain or enhance the nutritional and pharmacological effects of these mushrooms.

Conflict of interest

The authors declare no conflict of interest.

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