Antioxidant activity of hydrophilic extract from straw mushroom and its effect on shrimp melanosis

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ABSTRACT: The present study was to investigate the antioxidant activity of hydrophilic extract from straw mushroom and its effect on shrimp melanosis. The antioxidant activity of the mushroom extract was determined by DPPH radical scavenging, total reducing power, hydrogen peroxide scavenging and lipid peroxidation inhibition activities. The inhibitory effect of the mushroom extract on melanosis of shrimp was analysed by immersing the shrimp into the mushroom extract. The results showed that the mushroom extract as a potent antioxidant and prevented the melanosis development effectively when compared with the controls. These observations suggested that the mushroom extract is a potential antioxidant, which has the ability to control melanosis in shrimp during ice storage.

Keywords: Mushroom, Antioxidant, Shrimp, Melanosis

Introduction

Melanosis is a natural post-mortem biochemical process in a crustacean caused by polyphenoloxidase (PPO). PPO catalyzes the oxidation of phenolic substrates to quinones, which undergo auto-oxidation and polymerization to form melanin, a high molecular weight dark pigment. It drastically reduces the product market value, leading to the considerable financial loss (Kim et al., 2000). Various techniques and mechanisms for controlling the undesirable activities of PPO in foods have been developed over the years (Whitaker, 1994). Direct application of various PPO inhibitors, such as 4-hexyl-1,3-benzenediol (4-hexylresorcinol, HR), sulphite-based compounds, and phosphates, have been shown to be very effective at preventing melanosis development in crustaceans. However, the use of synthetic compounds to inhibit melanosis in food products is limited because of strict controls by food safety legislation. For this reason, natural compounds from edible materials are generally preferred in food applications.

Mushrooms are a promising source of natural PPO inhibitors, the inhibitory effect of mushrom *(Flammulina velutipes)* extract on melanosis development in shrimp has been investigated by Jang et al. (2003). Active compounds, including certain phenolic compounds and ergothioneine (ESH), have been found in the extract of *F. velutipes* (Bao et al., 2010a). Especially, ESH has been known as a powerful scavenger (Akanmu et al., 1991). Encarnacion et al. (2011) demonstrated that the melanosis in shrimp was significantly inhibited due to antioxidative activity of ESH in the mushroom extract. Recently, the authors (Hai et al., 2013) found that ESH existed

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in the extract of *Volvariella volvacea*, and the mushroom extract significantly inhibited PPO activity. This study was, therefore, conducted to evaluate the antioxidant activity and inhibitory effect of straw mushroom (*V. volvacea*) extract on the formation of melanosis in Pacific white shrimp stored in ice.

Materials and methods

Preparation of mushroom extract

Fresh fruiting body of *V. volvacea* was donated by Hoang Van Thuan Farm (Khanh Hoa, Vietnam) after 2 months of cultivation. The mushroom extract was prepared by adapting our previously developed procedure (Bao et al., 2010a; Bao et al., 2010b). The fruiting body of the mushrooms was ground with a food processor, and 100 g of the ground material was separately extracted with 500 mL of water at 95 ± 2 °C in a 1-L glass round-bottom flask for 1 h. The supernatant was collected by centrifuging the boiled mixture at 3000 × g for 15 mins. at 4 °C and was then evaporated at 40 °C in vacuo. The residue obtained was further extracted with 50 mL of 70% (v/v) aqueous ethanol. The ethanolic solution was vortexed, left to stand at 4 °C for 2 h and subsequently centrifuged at 3000 × g for 15 mins. at 4 °C. The supernatant was collected and evaporated at 40 °C in vacuo to remove ethanol. The ethanol-free residue was dissolved in 10 mL of distilled water. Thus, 1 mL of each extract was obtained from 10 g of wet materials.

Treatment of shrimp with mushroom extract

Pacific white shrimps (*Litopenaeus vannamei*) with the size of 90-100 shrimps/kg were purchased from Tran Tien Farm (Khanh Hoa, Vietnam). The shrimps were kept alive and transported to the laboratory at Nha Trang Uiversity. The shrimps were immersed in mushroom extract solution (0.5%, 0.75% and 1.0%, v/v) using a shrimp/solution ratio of 1:2 (w/v) at 4 °C for 10 mins. The control shrimps were immersed in water at a ratio of 1:2 (w/v) for 10 mins. at 4 °C. All samples were stored in styrofoam box containing ice using a shrimp/ice ratio of 1:2 (w/w). To maintain the shrimp/ice ratio, the molten ice was removed and replaced with an equal amount of ice. Ten shrimps from each treatment were taken every 2 days up to 12 days for evaluation of melanosis development in shrimp.

Methods of analysis

- 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was analyzed following the method of Fu et al.(2002).
- Total reducing power of the mushroom extract was analyzed as described by Oyaizu (1986).
- Hydrogen peroxide scavenging activity was analyzed as described by Nabavi et al. (2009).
- Lipid peroxidation inhibition activity was analyzed following the method of Bao et al. (2014).
- Melanosis of Pacific white shrimp was measured by Color image analysis using ImageJ software of National Institute of Mental Health, Bethesda, MD (http://rsb.info.nih.gov/ij/).

Results

DPPH radical scavenging activity of the mushroom extract

DPPH radical scavenging activity of hydrophilic extract prepared from straw mushroom as shown in **Figure 1**. The amounts of the extract in the reaction mixtures ranged from 10 to 50 µL. It is evident that, in general, the extract showed an increased DPPH radical scavenging activity by their amounts in the reaction mixture. The effective volume of the extract at which DPPH radical was scavenged by 50% was 3.7µL.

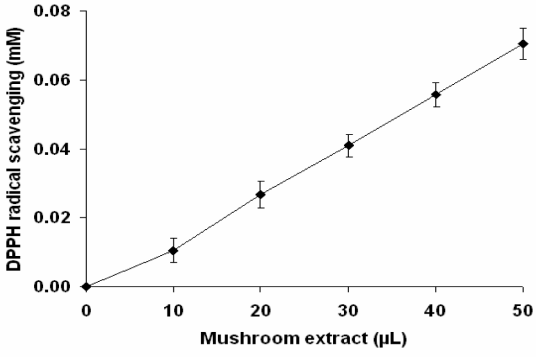

Figure 1 DPPH radical scavenging activity of hydrophilic extract prepared from straw mushroom. Data are presented as mean ± S.D. (n = 3).

Total reducing power ability of the mushroom extract

Total reducing power ability of hydrophilic extract prepared from straw mushroom as shown in **Figure 2**. The total reducing power ability of the extracts showed a similar trend in their DPPH radical scavenging activity, and exhibited dose-dependent activity between 10 and 50 µL.

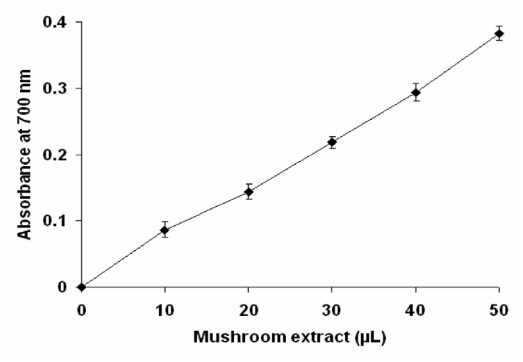

Figure 2 Total reducing power ability of hydrophilic extract prepared from straw mushroom. Data are presented as mean ± S.D. (n = 3).

Hydrogen peroxide scavenging activity of the mushroom extract

Hydrogen peroxide scavenging activity of hydrophilic extract prepared from straw mushroom as shown in **Figure 3**. These results show that the mushroom extract has an effective hydrogen peroxide scavenging activity and caused a strong dose-dependent inhibition of hydrogen peroxide.

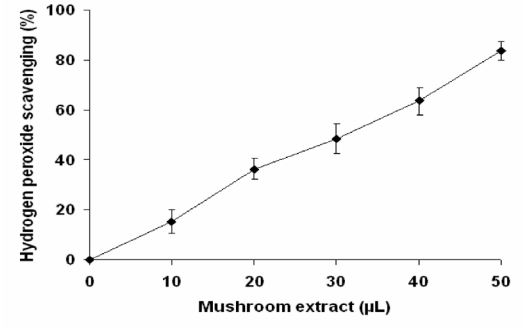

Figure 3 Hydrogen peroxide scavenging activity of hydrophilic extract prepared from straw mushroom. Data are presented as mean ± S.D. (n = 3).

Lipid peroxidation inhibition activity of the mushroom extract

Lipid peroxidation inhibition activity of hydrophilic extract prepared from straw mushroom as shown in **Figure 4**.

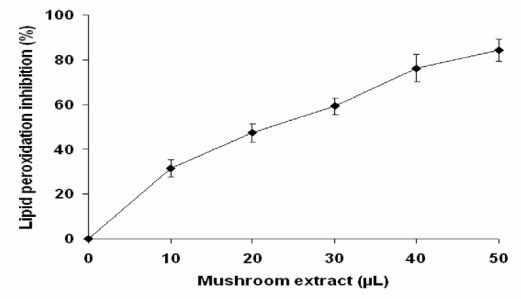

Figure 4 Lipid peroxidation inhibition activity of hydrophilic extract prepared from straw mushroom. Data are presented as mean ± S.D. (n = 3).

Previous studies reported that mushroom extracts had free radical scavenging and antioxidant activities not only *in vitro* but also *in vivo* (Bao et al., 2010a; Bao et al., 2010b). The current study showed that the mushroom *V. volvacea* extract has an antioxidant activity against lipid peroxidation. This effect is believed to be due to the radical scavenging and reduction behaviors of the mushroom extract (Bao et al., 2010a). These results strongly suggest that the mushroom *V. volvacea* is a potential source of natural antioxidants.

Inhibitory effect of the mushroom extract on melanosis of shrimp

Effect of the mushroom extract treatment on melanosis of Pacific white shrimps during iced storage as shown in **Figure 5**.

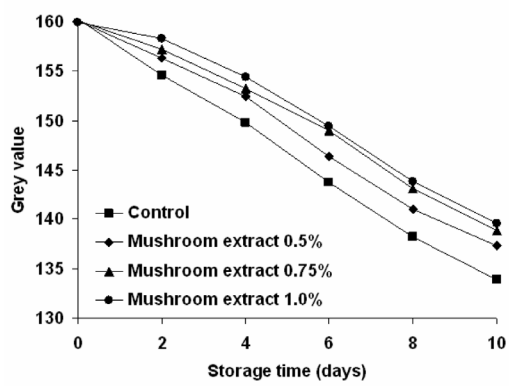

Figure 5 Effect of the mushroom extract treatment on melanosis of Pacific white shrimps during iced storage.

Inhibitory effect of *F. velutipes* extract on melanosis of shrimp was first reported by Jang et al. (2003), the effect is due to inhibition of PPO activity (Encarnacion et al., 2011). In this study, the hydrophilic extract from Straw mushroom significantly delayed melanosis in Pacific white shrimps during ice storage and the effect exhibited dose-dependent efficacy between 0.5 and 1.0% (**Figure 5**). This effect may have been due to PPO inhibitory activity of the mushroom extract (Hai et al., 2013). Mechanism of this inhibition was hypothesized to be due to interaction of the mushroom extract with Cu^{2+} at the putative binding sites of PPO (Encarnacion et al., 2011).

Conclusion

Results of the present study clearly showed that the hydrophilic extract prepared from straw mushroom is a promising source of natural antioxidants which can be used for controlling melanosis in shrimps during iced storage.

References

Akanmu, D., R. Cecchini, O. I. Aruoma, and B. Halliwell. 1991. The antioxidant action of ergothioneine. Arch. Biochem. Biophys. 288: 10-6.

Bao, H. N. D., Y. Ochiai, and T. Ohshima. 2010a. Antioxidative activities of hydrophilic extracts prepared from the fruiting body and spent culture medium of *Flammulina velutipes*. Bioresour. Technol. 101: 6248-55.

Bao, H. N. D., K. Osako, and T. Ohshima. 2010b. Value-added use of mushroom ergothioneine as a colour stabilizer in processed fish meats. J. Agric. Food. Agr. 90: 1634-41.

Bao, H. N. D., N. T. H. Duong, and P. T. Hien. 2014. Fenton reaction applied for determining antioxidative activity. J. Fish. Sci. Technol. 3: 9-15.

Encarnacion, A. B., F. Fagutao, J. Hirayama, I. Hirono, and T. Ohshima. 2011. Edible mushroom (*Flammulina velutipes*) extract inhibits melanosis in Kuruma shrimp (*Marsupenaeus japonicus*). J. Food Sci. 76(1): C52-C58.

Fu, H., D. Shieh, and C. Ho. 2002. Antioxidant and free radical scavenging activities of edible mushrooms. J. Food Lipid. 9: 35-46.

Hai, L. T., N. M. Tri, and H. N. D. Bao. 2013. Studies on extraction and antioxidant activity of extracts from straw mushroom. J. Fish. Sci. Technol. 1: 122-128.

Jang, M. S., A. Sanada, H. Ushio, M. Tanaka, and T. Ohshima. 2003. Inhibitory effect of enokitake extract on melanosis of shrimp. Fish. Sci. 69: 379-384.

Kim, J., M. R. Marshall, and C. Wei. 2000. Polyphenoloxidase. In: Haard NF, Simpson BK (ed) *Seafood enzyme utilization and influence on post harvest seafood quality*, Marcel Dekker, New York.

Nabavi, S. M., M. A. Ebrahimzadeh, S. F. Nabavi, M. Fazelian, and B. Eslami. 2009. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Phcog. Mag. 4(18): 123-7.

Oyaizu, M. 1986. Studies on products of browing reactions: antioxidative activities of productions of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307-15.

Whitaker, J. R. 1994. Enzyme inhibitors. In: Principles of Enzymology for the Food Sciences., 2nd edition. Marcel Dekker, Inc., New York. pp. 241-270.