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Original Research Article

Vitamin D_2 content and antioxidant properties of fruit body and mycelia of edible mushrooms by UV-B irradiation

Shih-Jeng Huang [a], Chun-Ping Lin [b], Shu-Yao Tsai [b,c,*]

[a] Department of Health Food, Chung Chou University of Science and Technology, Yuanlin, Changhua 51003, Taiwan, ROC
[b] Department of Health and Nutrition Biotechnology, Asia University, 500, Lioufeng Rd., Wufeng, Taichung 41354, Taiwan, ROC
[c] Department of Nutrition, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan, ROC

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ABSTRACT

Our objective was to study the effect of ultraviolet-B (UV-B) light irradiation on the vitamin D_2 content of several edible fruit bodies and mycelia and their antioxidant properties. Eleven species of fresh mushroom fruiting bodies, including species from each of the six genera, *Agaricus*, *Agrocybe*, *Auricularia*, *Hypsizigus*, *Lentinula* and *Pholiota*, and five species from *Pleurotus* genus, were irradiated with UV-B light for 2 h. For three species of mushroom fruiting bodies with excellent vitamin D_2 yield, their mycelia were obtained by liquid culture, and subjected to the same time as the UV-B irradiation. Vitamin D_2 content of irradiated fruit bodies significantly increased from 0–3.93 to 15.06–208.65 μg/g, of which the amount in golden oyster mushroom increased by a maximum of 204.7 μg/g. Vitamin D_2 content in irradiated mycelia of golden oyster, oyster and pink oyster mushrooms increased from 0.28–5.93 to 66.03–81.71 μg/g, respectively. The three irradiated mycelium polysaccharide contents decreased in a range from 1.3% to 24.6%. Overall, EC_{50} values of non-irradiated and irradiated fruiting bodies and mycelia were 0.92–4.94, 0.20–6.90 and 0.02–0.84 mg/mL for reducing power, scavenging ability and chelating ability, respectively. Although UV-B irradiation influenced the content of ergothioneine, flavonoids and total phenols, these irradiated samples still contained a sufficient amount of these antioxidant components.

1. Introduction

Edible and medicinal mushrooms contain many physiologically active substances, including polysaccharides, triterpenoids, ergosterol, vitamin D and ergothioneine (Kalac, 2013). Vitamin D is important in human nutrition because deficiency in infancy/childhood can result in rickets, while in adults vitamin D deficiency is associated with osteoporosis (Holick, 2007). The vitamin D receptor has been detected in a broad range of tissues; therefore, it has been suggested that vitamin D may play a role in cardiovascular and autoimmune diseases and cancer prevention (Mullin and Dobs, 2007; Kikkinen et al., 2009).

Natural food sources for vitamin D include certain fish species, egg, milk and mushrooms (Holden et al., 2008). Vitamin D exists as two distinct forms: vitamin D_3 (cholecalciferol) is oily fish, egg and red meat, and vitamin D_2 (ergocalciferol) is found in yeasts and mushrooms (Shrapnel and Truswell, 2006). Ultraviolet light can be applied to mushrooms to enhance their vitamin D_2 content (Jasinghe and Perera, 2005, 2006; Teichmann et al., 2007; Ko et al., 2008; Roberts et al., 2008; Simon et al., 2011; Koyyalamudi et al., 2009, 2011; Kalaras et al., 2012).

Most studies on mushroom fruiting bodies try to enhance their vitamin D content. Low vitamin D_2 content occurs naturally in many mushrooms, with a high potential for large increases based on the presence of ergosterol by ultraviolet light treatment. However, information about the application of ultraviolet light on mushroom mycelium is not available. Since the mycelium is easily produced as compared to fruiting body and is readily irradiated due to its larger flat surface, it has great potential for the

Abbreviations: UV-B, ultraviolet-B; EC, effective concentration; HPLC, high-performance liquid chromatograph; DPPH, 2,2-diphenyl-1-picrylhydrazyl; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; FB0, fresh fruiting bodies UV-B irradiated for 0 h; FB2, fresh fruiting bodies UV-B irradiated for 2 h; MY0, freshly harvested mycelia UV-B irradiated for 0 h; MY2, freshly harvested mycelia UV-B irradiated for 2 h.

* Corresponding author at: Department of Health and Nutrition Biotechnology, Asia University, 500, Lioufeng Rd., Wufeng, Taichung 41354, Taiwan, ROC. Tel.: +886 4 2332 3456x20028; fax: +886 4 2332 1126.
E-mail address: sytsai@asia.edu.tw (S.-Y. Tsai).

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development of health foods containing a high content of vitamin D. Using ultraviolet irradiation on mushrooms to increase vitamin D content not only provides another option for people to supplement vitamin D from non-animal sources but also helps vegetarians to maintain vitamin D status. Mushrooms are a good source of beneficial components (such as ergothioneine, total phenols, flavonoids, and polysaccharides) that may be destroyed by UV irradiation, but there are no data. Given the growing use of irradiation to enhance vitamin D, the need to demonstrate an effect on these components is valuable. Eleven species of fresh mushroom fruiting bodies are commercially available in Taiwan, including species from each of the six genera of *Agaricus*, *Agrocybe*, *Auricularia*, *Hypsizigus*, *Lentinula* and *Pholiota* and five species from *Pleurotus* genus. In addition, limited information about the vitamin D_2 content of these mushrooms is available and there have been no reports on the vitamin D_2 content of mycelia.

We irradiated fresh fruiting bodies and freshly harvested mycelia with ultraviolet light to study its effect on the vitamin D and polysaccharides content. We also studied the antioxidant properties of ethanolic extracts from irradiated fruiting bodies and mycelia, including reducing power, scavenging ability on radicals, and chelating ability on ferrous ions, and compared them to non-irradiated samples. The content of potential antioxidant components (ergothioneine, flavonoids and total phenols) was also determined.

2. Materials and methods

2.1. Fruiting bodies and mycelia

Eleven species of fresh mushrooms included Brazilian (*Agaricus blazei*), popular (*Agrocybe cylindracea*), wood ear (*Auricularia polytricha*), hon-shimeji (*Hypsizigus marmoreus*), shiitake (*Lentinula edodes*), sticky pholiota (*Pholiota nameko*), king oyster (*Pleurotus eryngii*), golden oyster (*Pleurotus citrinopileatus*), ferulae (*Pleurotus ferulae*), oyster (*Pleurotus ostreatus*) and pink oyster (*Pleurotus salmoneostramineus*) mushrooms were obtained from Q-Yo Bio-Technology Farm, Changhua County, Taiwan (121°E, 24°N, 556 m). Immediately after harvest, the whole fresh fruiting bodies (10–15 kg each) were kept at 4 °C in a cooler and transported to the laboratory for irradiation treatment within 1 h. We selected the three highest levels of vitamin D_2 of irradiated fruiting bodies, performing cultured mycelia. Three species of mycelia (golden oyster, oyster, and pink oyster mushrooms) cultures were obtained from the inner living tissues of the fruit bodies in potato dextrose agar (Difco Laboratories, Sparks, MD, USA) medium at 25 °C. For the production of mycelia, the culture was inoculated into 250-mL flask containing 90 mL of liquid medium and incubated at 25 °C and 100 rpm. The liquid medium (1 L) consisted of 20 g of glucose, 5 g of yeast extract, 0.068 g of KH_2PO_4, and 2.3 g of $MgSO_4 \cdot 7H_2O$. After 7 days of incubation, the mycelia were harvested, washed five times with deionised water, and fresh mycelia was obtained by wringing water from 400-mesh cloth. Both fruiting bodies and mycelia were irradiated in fresh and moist state on the day of harvest.

2.2. Irradiation of mushrooms

Fresh fruiting bodies (FB, 500 g) and moist mycelia (MY, 400 g) were randomly selected by being placed in a single layer in 30 cm × 30 cm stainless steel trays. For UV-B irradiation, 15 trays from each treatment were done for each species mushroom. Untreated fresh fruiting bodies and moist mycelia were controls. Photographs of the fresh fruiting bodies are displayed in Fig. 1. A tray of mushrooms was placed 19 cm from the source of irradiation, a UV-B lamp (280–360 nm, Sankyo Denki, G15T8E, Tokyo, Japan) for 2 h at ambient temperature (25 °C). The UV-B irradiation intensity was measured by using a UVX 31 radiometer (UVP, Upland, CA, USA) to be 0.36 mW/cm^2 and the irradiation doses for 2 h were 25.9 kJ/m^2. During all steps of preparation, care was taken that samples were not exposed to incidental UV light. After UV-B irradiation, all samples were stored frozen at −20 °C until freeze drying, then ground in an RT-34 pulverizing machine (Rong Tsong Precision Technology Co., Taichung, Taiwan), sequentially ground and sieved until all particles were <0.4 mm. The powdered samples were dispensed into 500-mL matte plastic bottle, surrounded with aluminium foil, and stored in darkness at 4 °C before use. The moisture content was determined by moisture loss from freeze-drying until constancy was achieved.

2.3. Vitamin D_2 assay

Vitamin D_2 was extracted and analysed according to the method of Tung et al. (2007) with some modification. Mushroom powder (5 g) was mixed with 10 mL of dimethyl sulfoxide (Merck, Darmstadt, Germany) and ultrasound-oscillated at 45 °C for 30 min. Then 10 mL of methanol and water (1:1, v/v) and 20 mL of hexane were added and the mixture was ultrasound-oscillated at 45 °C for 30 min and centrifuged at 3000 × *g* for 10 min. The residue was extracted twice with 20 mL of hexane and centrifuged.

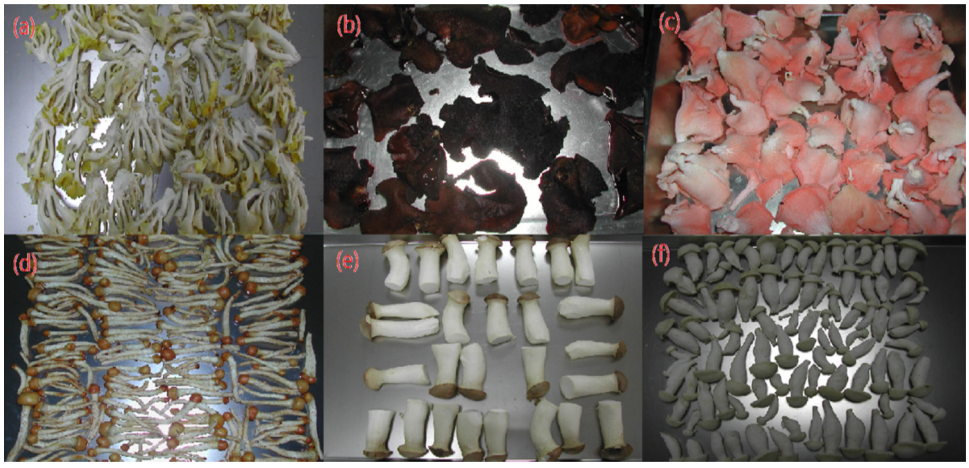

Fig. 1. Photograph of golden oyster (a), wood ear (b), pink oyster (c), sticky pholiota (d), king oyster (e) and ferulae (f) before UV-B irradiation. The top row of each quadrant represents sector mushroom; the bottom row of each quadrant represents stick mushroom.