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Effect of UV-B Exposure on the Concentration of Vitamin D_2 in Sliced Shiitake Mushroom (*Lentinus edodes*) and White Button Mushroom (*Agaricus bisporus*)

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The aim of this study was to investigate the effect of UV-B on vitamin D_2 concentration in shiitake mushrooms and in white button mushrooms. After the exposure to UV-B, at a dose of 25 kJ/m^2, the concentration of vitamin D_2 was increased to 36.7 ± 1.4, 68.6 ± 4.9, and 106.4 ± 14.7 μg/g (dry weight) for pileus, middle, and gill parts of shiitake mushroom, respectively. The gill side of whole shiitake mushrooms exposed to 0, 25, 50, and 75 kJ/m^2 increased to 2.8 ± 0.2, 13.8 ± 1.9, 40.7 ± 4.4, and 61.9 ± 10.6 μg/g (dry weight) at 25 °C, respectively. Irradiating slices of white button mushroom was a more efficient way of increasing the vitamin D_2 content than irradiating the gill or pileus of whole mushrooms, due to the larger exposure area. As the irradiation doses increased, the vitamin D_2 concentration also increased for both types of mushrooms. In conclusion, exposure to ultraviolet light offers an effective way of increasing the concentration of vitamin D_2 in mushrooms.

KEYWORDS: Vitamin D_2; shiitake mushroom; white button mushroom; UV-B

INTRODUCTION

Vitamin D plays an important role in the regulation of calcium and phosphorus in the human body and in mineralization of bones. Furthermore, it is clear that receptors for vitamin D are present in a wide variety of cells, and thereby this vitamin has biological effects that extend far beyond control of mineral metabolism (*1*). Vitamin D consists of two different compounds, vitamin D_2 from ergosterol and vitamin D_3 from 7-dehydrocholesterol (*2*). It can be produced by the action of sunlight and absorbed from the diet in the intestinal tract. Without vitamin D, the absorption rate of calcium is at best 10−15% of calcium intake. With vitamin D, it increases to 30−80% of the intake (*3*). However, the importance of vitamin D is overlooked compared with that of other nutrients for dietary foods, because it is synthesized during exposure of sunlight. However, increased use of sunscreen and decreased exposure to sunlight are reducing the synthesis of vitamin D_2 attributable to exposure to UV (ultraviolet) radiation. Therefore, we cannot rely only on the vitamin D synthesized in the skin. This is a fact that is even more pronounced for the elderly (*4*). Good food sources of vitamin D are fish, egg yolk, and milk (*5, 6*).

Vitamin D_2 has been found in some mushrooms, where it is converted from the provitamin ergosterol. Mushrooms are the only non-animal-based food containing vitamin D and ergosterol and are hence the only natural vitamin D sources for vegetarians (*7*). Among them, the two most popular mushrooms, among consumers, in the world are the shiitake mushroom (*Lentinus edodes*) and the button mushroom (*Agaricus bisporus*). The first is most popular in the East and the latter in the West, but the consumption of shiitake mushroom is gradually increasing in the West (*8*).

Previous studies showed that mushrooms have a rich ergosterol source, and the concentration of vitamin D_2 was even higher if they were exposed to sunlight or artificial ultraviolet light (*9*). According to Mau et al. (*10*) and Jasinghe et al. (*11*), mushrooms could be remarkably enriched with vitamin D_2 by UV-B irradiation. Lee et al. (*12*) reported increasing the vitamin D_2 content in the shiitake mushroom by a combination of UV-B irradiation and hot-air drying. These ways to improve the nutritional value of common mushrooms and make them more functional as a source of vitamin D are worth noting. However, only limited information is reported in the literature about vitamin D concentration in the different slices of mushrooms.

The objective of this study was to investigate the effects on the vitamin D_2 concentration of shiitake mushrooms and white

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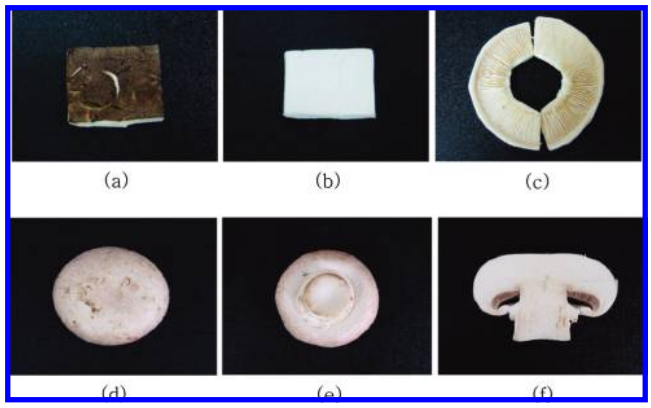

Figure 1. Sliced shiitake mushrooms (*Lentinula edodes*) in three parts: (**a**) pileus layer, (**b**) middle layer, and (**c**) gill layer. White button mushrooms (*Agaricus bisporus*) in three parts: (**d**) whole pileus, (**e**) whole gill, and (**f**) sliced.

button mushrooms by irradiation with an artificial UV-B (219−315 nm) lamp.

MATERIALS AND METHODS

Materials. Fresh shiitake mushrooms (*L. edodes*) and fresh white button mushrooms (*A. bisporus*) were purchased from a local supermarket and were used immediately in the experiments.

Preparation of Samples. After removal of the stalks, the shiitake mushrooms were used whole and in three parts (pileus, outer cap; middle layer, between cap and gill; gill, inner cap) separated with a sharp blade. The pileus and middle layer were sliced into pieces of 1.5 × 1.5 × 0.5 cm (length/width/height). White button mushroom was used whole and sliced 0.5 cm lengthwise (**Figure 1**).

Irradiation Procedure. The shiitake mushrooms were placed on shelves and exposed to the UV-B (ultraviolet-B) radiation, at doses of 25, 50, and 75 kJ/m^2 (UV Radiometer with a CX-312 sensor, Vilber Lourmat, France) in a UV chamber (Labcamp Co.) with a UV-B lamp (Vilber Lourmat, T-15M, 280–320 nm) at 25 °C. The white button mushrooms were exposed to UV-B doses of 10, 20, and 30 kJ/m^2. The irradiated samples were separately freeze-dried (IlShin Co.), homogenized immediately (Hanil Co.) before determination, and then stored at –20 °C until analysis.

Analysis of the Vitamin D_2 Concentration. The concentration of vitamin D_2 was determined by using the methods of Mattila et al. (*9, 13*) with minor modifications. Samples of freeze-dried powder (1 g) were weighed into a 250 mL flask and mixed with 1 g of L-ascorbic acid (Junsei Chemical Co.), 50 mL of ethanol (99%) (Duksan Pure Chemical Co.), and 25 mL of 50% potassium hydroxide (Junsei Chemical Co.). The mixture was shaken and subsequently saponified under reflux at 85 °C for 30 min. The mixture was then cooled to ambient temperature and poured into a separating funnel. The mixture was first extracted with 10 mL of deionized water and 30 mL of *n*-hexane (J. T. Baker), a procedure that was repeated twice. The pooled organic layers were washed three times with deionized water until neutralized. The organic layer was transferred into a flask, rotary evaporated to dryness at 50 degoC, and immediately redissolved in 2 mL of a mixed solution of eluent (methanol/acetonitrile = 75:25) and isopropyl alcohol (2:1). The samples were passed through a filter (PTFE, 13 mm, Whatman International Ltd.), with a pore size of 0.45 μm. A volume of 20 μL of filtered sample was injected into the HPLC system (Waters 1525, Waters Corp.) equipped with a 2487 dual absorbance detector (Waters Corp.) and eluted through a reverse phase C18 column (**Table 1**) (Symmetry 4.6 × 250 mm, Waters Corp.). The mobile phase was methanol/acetonitrile, 25:75, at flow rate of 1 mL/min, and UV detection was at 264 nm.

Vitamin D_2 was determined by comparing the retention times of standard (ergocalciferol, Sigma Chemicals, Steinheim, Germany) obtained, and quantification was done by using a calibration curve.

Statistical Analysis. The experimental data were subjected to an analysis of variance for a completely randomized design. The data were analyzed by Duncan's multiple-comparison method using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Significance was determined at $p > 0.05$ level for all analyses.

Table 1. Characteristics of the High-Performance Liquid Chromatograph Used

	Waters 1525
instrument	2487 dual absorbance detector 717 plus autosampler (Millipore Co.)
column	symmetry 4.6 × 250 mm
mobile phase	methanol/acetonitrile = 75:25
flow rate	1 mL/min
injection volume	20 μL
detector	UV 264 nm

RESULTS AND DISCUSSION

Vitamin D_2 Concentration in Different Parts of Shiitake Mushroom Exposed to UV-B and Effects of Irradiation Dose. The vitamin D_2 concentrations of different shiitake mushroom tissues exposed to UV-B are shown in **Figure 2**. The concentration of vitamin D_2 in control (unexposed) was 2.77 μg/g. After exposure to a UV-B dose of 25 kJ/m^2, the concentration of vitamin D_2 was increased to 36.7, 68.6, and 106.4 μg/g for pileus, middle, and gill parts, respectively. The gill did, interestingly, produce remarkably more vitamin D concentration than the pileus and middle layers did. This result was due to the varied contents of ergosterol in the different parts of shiitake mushroom and the finer morphology of the gill. Jasinghe et al. (*11*) showed that the concentration of ergosterol in the gill was the highest and twice that found in the outer layer of the cap. The gill part has the finer morphology, which allowed a larger exposure area to the UV-B irradiation, so the conversion of abundant ergosterol in gill to vitamin D increased (*10*). This is well in agreement with the results of Jasinghe et al. (*11*) and Lee et al. (*12*). They studied the effect of UV irradiation on the vitamin D_2 concentration in shiitake mushrooms and showed that contents of vitamin D_2 in gills were higher than those of caps after irradiation.

Figure 3 shows the effects of irradiation dose on the vitamin D_2 concentration in mushrooms. Vitamin D_2 concentrations in the gill of shiitake mushroom were increased from 2.77 (unexposed) to 13.8, 40.7, and 61.9 μg/g after exposures of 25, 50, and 75 kJ/m^2, respectively, at 35 °C. As the irradiation dose increased, the vitamin D_2 concentration increased, and it may

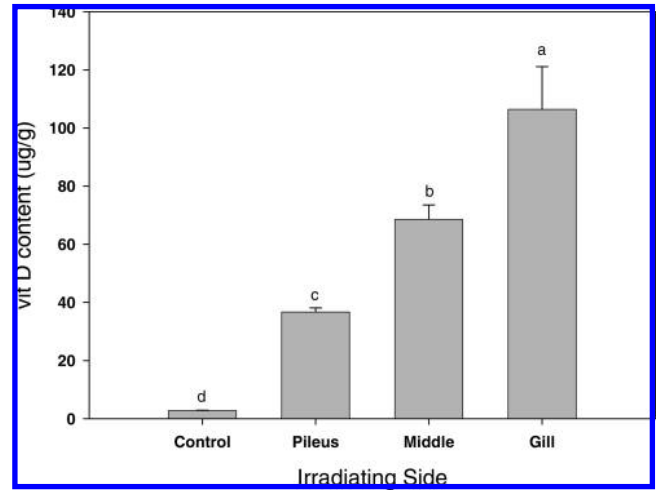

Figure 2. Vitamin D_2 contents in different parts of shiitake mushroom exposed to a UV-B dose of 25 kJ/m^2.

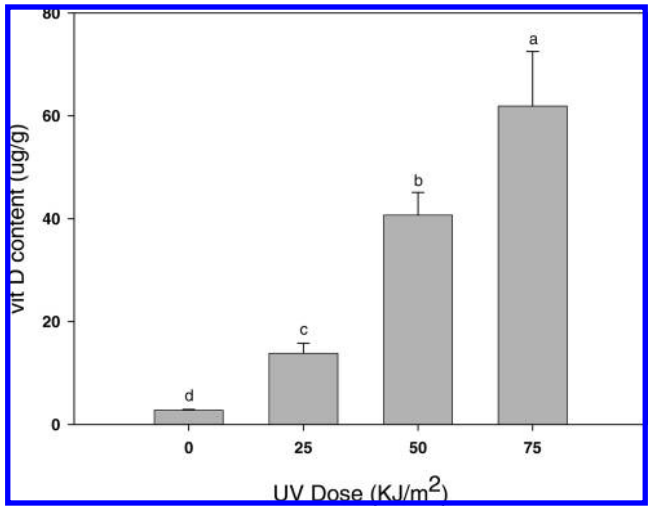

Figure 3. Effects of UV-B irradiation on the vitamin D_2 contents in gill of shiitake mushroom at 25 °C.

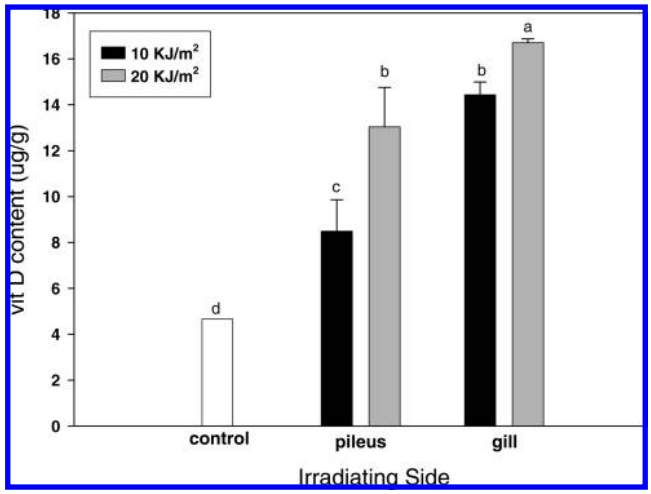

Figure 4. Vitamin D_2 contents in whole button mushroom of different sides exposed to UV-B.

result in the conversion of ergosterol into vitamin D_2 by UV-B. This result was similar to the study by Mau et al. (*10*). They reported that UV-B irradiation resulted in higher vitamin D_2 conversion for shiitake mushroom, and the increase by UV-B irradiation was time-dependent, that is, dose-dependent. However, an excessively high dosage might lead to decreased quality of mushrooms such as surface discoloration and decreased moisture content.

Vitamin D_2 Concentration in White Button Mushroom Exposed to UV-B and Effects of Irradiation Dose. Figure 4 shows the vitamin D_2 concentrations in whole white button mushroom irradiated with different sides facing UV-B. The concentrations of vitamin D_2 of the button mushrooms irradiated with pileus were 8.48 and 13.03 μg/g after 10 and 20 kJ/m^2, respectively. Those of mushroom irradiated with gill facing were 14.4 and 16.7 μg/g after 10 and 20 kJ/m^2 irradiation, respectively. The vitamin D_2 concentration of white button mushroom exposed with gill was higher than that of pileus and increased as the irradiation dose increased.

As the dose of UV irradiation increased, vitamin D_2 concentration in sliced white button mushrooms also increased (**Figure 5**). It was also shown that the conversion of ergosterol to vitamin D_2 was dependent on the irradiation dose. Dose of UV-B irradiation of white button mushrooms ranged from 10 to 30 kJ/m (*2*), lower than that of shiitake mushrooms due to surface

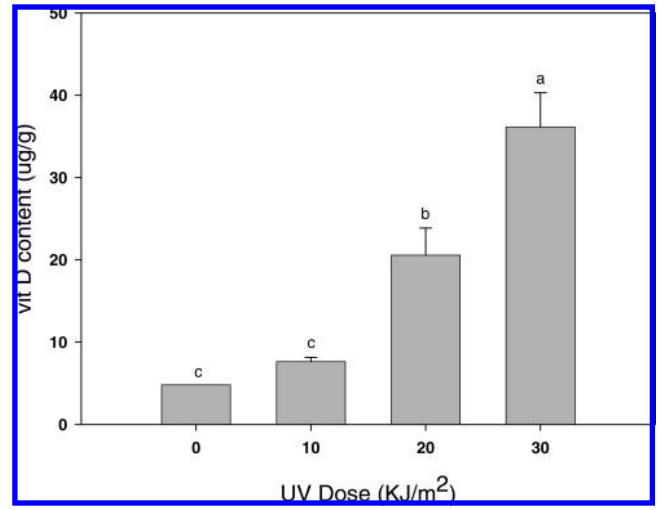

Figure 5. Effects of UV-B irradiation on the vitamin D_2 contents of sliced button mushroom at 25 °C.

discoloration. Higher dosage leads to decreased quality of mushrooms such as surface discoloration and decreased moisture content. The surface discoloration of mushroom might influence the acceptability of the product in the market.

Souci et al. (*14*) reported that ergosterol contents and the conversion rate of ergosterol to vitamin D_2 in different types of mushrooms were varied. Button mushrooms have lower vitamin D_2 content compared to other types of edible mushrooms (*14–17*). This may be due to the fact that UV-B penetration was lessened because the gill was not exposed (**Figure 1e**). As shown in the **Figure 1f**, the gill of sliced button mushroom was exposed to UV-B; therefore, vitamin D_2 concentration of sliced button mushrooms was higher than that of whole mushroom.

Conclusion. By using the method of exposing mushrooms to ultraviolet light, the levels of vitamin D_2 significantly increased. Furthermore, our results suggest that sliced mushrooms were remarkably increased in vitamin D_2 when exposed to UV-B. Because the UV irradiation acts only on the surface of the mushroom, it is important to fully expose all sides of the mushrooms to the UV-B.

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