Effect of UV-B Irradiation on Contents of Ergosterol, Vitamin D$_2$, Vitamin B$_1$ and Vitamin B$_2$ in Thai Edible Mushrooms

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ABSTRACT

Fruiting bodies, mycelia and freeze-dried powder of nine commercial types of mushrooms in Thailand including enoki, eryngi, khonkao, kradang, jew’s ear, oyster, phoenix, shiitake and yamabushitake were irradiated with ultraviolet-B (UV-B) to investigate the conversion of ergosterol to vitamin D$_2$ and the responses of vitamin B$_1$ and vitamin B$_2$ in fresh mushrooms were also analyzed after treatment. After exposed to UV-B for 15 to 180 min, at each time point, the concentration of ergosterol in fresh mushrooms was decreased whereas the amount of vitamin D$_2$ was increased. The conversion to vitamin D$_2$ was most in oyster (27.89±0.67 μg/g dry weight) but least in eryngi (4.54±0.02 μg/g dry weight) among the nine mushrooms. Vitamin B$_1$ concentration in all fresh mushrooms remained relatively stable after UV-B irradiation but vitamin B$_2$ content decreased. Prolonged irradiation (60 to 180 min) led to discoloration in fresh enoki. Additionally, mycelia and lyophilized mushrooms powder were also subjected to the UV-B exposure for ergosterol to vitamin D$_2$ inversion.

Keywords: edible mushrooms, ergosterol, ergocalciferol, mushroom mycelium, UV-B exposure

1. INTRODUCTION

Vitamin D is vital to maintain in the regulation of the metabolism of calcium and phosphate. Vitamin D promotes absorption of extra-mineral metabolism functions and needed for general cellular bioactivities of the body [1]. Vitamin D deficiency causes a wide range of aberrations relating to bones and skeletal structures e.g. mineralization of bone matrix, defective collagen synthesis, which results in rickets in children and osteomalacia in the adults [2]. The lack of vitamin D is an increasingly recognized public health such as cardiovascular diseases, multiple sclerosis, inflammatory bowel disease, macular
degeneration, mental illness and chronic pain [3]. Vitamin D deficiency among the world population is increasing, which is particularly more serious for Asians including Thais [4, 5] compared with Europeans. Infants exclusively breastfed, elderly person, people with limited their outdoor activities and adults of all ages who have increased skin pigmentation are risky in vitamin D deficiency [6].

Vitamin D, in human body, consists of two major compounds, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Vitamin D3 is present in animal products such as fish liver oil, eggs, milk and cheese, but is mainly generated from 7-dehydrocholesterol in the epidermal layer of skin during exposure to ultraviolet light [7]. Vitamin D2 is found in plant products, including mushrooms and it can be formed from the plant steroid called ergosterol, by UV irradiation [8]. Vitamin D2 is more effective for bone mineralization than vitamin D3. In addition, vitamin D2 does not have hypercalcemic effects and it is less toxic compared with vitamin D3 [9]. According to insufficiency of vitamin D in food recommended daily intakes (5-10 μg/day), it is therefore the reason to evaluate potential food providing high amount of vitamin D2 [10].

Mushrooms are highly nutritional value and medicine resource of antioxidant, preventive and therapeutic properties [11]. Mushrooms provide a valuable source of vitamins such as vitamin B1, vitamin B2 and vitamin D. They contain a high amount of ergosterol which can be converted to vitamin D2 by UV irradiation. When mushrooms are exposed to UV light, ergosterol undergoes photolysis to yield a variety of photoproduction products, principally previtamin D2, tachysterol and lumisterol. The previtamin D2 undergoes spontaneous thermal rearrangement to vitamin D2 [12]. Previous research showed that the concentration of vitamin D2 was even higher if they were exposed to sunlight or artificial ultraviolet light. Ko et al. (2008) reported the increased vitamin D2 content in shiitake and button mushroom by UV-B irradiation and its concentration also increased with irradiation intensity.

The conversion of vitamin D2 by UV light consists of three sub-regions of wavelengths, including UV-C (190-290 nm), UV-B (290-320 nm) and UV-A (320-400 nm) [13]. Jasinghe and Perera (2006) reported the conversion of vitamin D2 in shiitake, oyster, abalone and button mushrooms under UV-A, UV-B and UV-C. The highest yields of vitamin D2 were obtained under UV-B irradiation.

Some wild edible mushrooms were found to contain a limited amount of vitamin D2. The reason why these mushrooms possess a small content of vitamin D2 is that naturally, they may be exposed to UV light which comprises only 8 - 9 % of the total solar spectrum [14]. The cultivated mushrooms also had the similar circumstance [15]. Edible cultivated mushroom is one of commercial agricultural products in Thailand. UV-B irradiation is the way to improve the nutritional value of edible mushrooms and makes them more functional as a source of vitamin D2.

The objectives of this study were to determine the ergosterol content in main types of edible mushrooms of Thailand and further to investigate the effect of UV-B irradiation on the conversion of ergosterol to vitamin D2 in fresh mushrooms, mycelia and freeze-dried mushrooms. Besides, the responses of vitamin B1 and vitamin B2 in fresh mushrooms to UV-B irradiation were also investigated.
2. MATERIALS AND METHODS

2.1 Preparation of Samples

Fruiting bodies, mycelium and freeze-dried powder of nine types of mushrooms, enoki \([Flammulina velutipes]\) (Curtis Singer), eryngi \([Pleurotus eryngii]\) (Fries.), khonkao \([Lentinus squarrosulus]\) (Mont.), kradang \([Lentinus polychrous]\) (Lev.), jew’s ear \([Auricularia auricular]\) (Hook.), oyster \([Pleurotus ostreatus]\) (Fries.), phoenix \([Pleurotus pulmonarius]\) (Fr.) Quel., shiitake \([Lentinula edodes]\) (Bull.) Singer and yamabushitake \([Hericium erinaceus]\) (Bull.) Persoon were used in this experiment. Fresh mushrooms were selectively purchased from local farms in Khon Kaen province, Thailand. The samples were refrigerated at 1-4°C before treatment. Prior to the treatment, the stored mushrooms were left at room temperature for 4 h. To prepare freeze-dried mushroom powder, fresh mushrooms were sliced into pieces and separately freeze-dried, homogenized immediately. Then obtained mushroom powder was stored at -20°C until UV-B treatment. Nine types of mushroom mycelia were produced in potato dextrose broth (PDB) using internal tissues of the fruiting bodies and kept them grew in the dark chamber at room temperature. After 14 days, they were irradiated with UV-B light.

2.2 Irradiation Procedure

The UV-B unit was used with 8 UV-B lamps \((313 \pm 12 \text{ nm}, \text{Philips TL-D } 18W)\) 604 cm in length and the total treatment area was 100x120 cm². In these experiments, the irradiation source was placed 15 cm away from the samples. Fresh mushrooms, mycelium and freeze-dried powder were treated with UV-B irradiation for 15, 30, 60, 90, 120, 150 and 180 min in an irradiation chamber. The rates of irradiation dose received by the mushrooms under UV-B light were 45.86, 91.72, 183.44, 275.16, 366.89, 458.60 and 550.32 J/cm² which calculated by multiplying intensity \((J/cm^2)\) by times. All experiments were carried out at 25 - 28°C and in triplicate. After UV-B irradiation, mushrooms powder was stores at -20°C immediately. Fresh mushrooms and mycelium were separately freeze - dried and homogenized with a blender before determination and then stored at -20°C until analysis.

2.3 Analysis of Ergosterol and Vitamin D₂

The analysis and quantification of ergosterol and vitamin D₂ were done according to the method described previously [16]. This method clearly discriminated between ergosterol and vitamin D₂. Freeze-dried mushroom of each sample powders (1 g) was weighed into 250 ml round bottom flask and mixed with 1 g of L-ascorbic acid, followed by 50 ml of 99% ethanol and 25 ml of 50% potassium hydroxide. The mixture was saponified for 30 min under reflux at 85°C. It was immediately cooled to the room temperature and transferred into a separating funnel. The mixture was extracted first with 10 ml of deionized water and 30 ml of n-hexane. The organic layers were washed three times with deionized water until neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 50°C and re-dissolved in 2 ml of a mixed solution of eluent (methanol/acetone/nitrite = 75:25 v/v) and isopropyl alcohol (2:1 v/v). The sample was filtered through a 0.45 μm non pyrogenic filter. A volume of 20 μl of filtered sample was injected into the HPLC system (LC 20A, Shimadzu, Japan) and eluted through a reversed phase C18 column. The mobile phase was methanol/acetone/nitrite (75:25 v/v), at flow rate of 1 ml/min. The UV detection
of elute was performed at 264 nm. Ergosterol and vitamin D$_2$ qualitatively analyzed by comparing the retention times of standards obtained (ergosterol and ergocalciferol, Sigma Chemicals, Steinheim, Germany) and quantification was done by using a calibration curve.

2.4 Analysis of Vitamin B$_1$ and B$_2$.

Vitamin B$_1$ and B$_2$ were extracted and analyzed according to the modified method described previously [17] as follows. Mushroom powder (1 g) was weighed and transferred into 250 ml round bottom flask. Extraction solution, 10 ml of 0.1 N hydrochloric acid and 80 ml water, was added and saponified under reflux at 100°C for 15 min. The sample was filtered with Whatman No.1 after cool down and finally the volume was adjusted made up to 100 ml with deionized water. The sample was filtered through 0.45 μm non pyrogenic filter. A volume of 20 μl of filtered sample was injected into the HPLC system. HPLC analysis, a Waters symmetry C18 column was used. The mobile phase was methanol and buffer (30:70 v/v) of 50 mM potassium dihydrogen phosphate having pH 4.2 ± 0.1, adjusted with formic acid, at a flow rate of 1 ml/min. The UV detection of elute was performed at 254 nm. Vitamin B$_1$ and B$_2$ were quantified by comparing the peak area with the internal standard (Vitamin B1 and B2, Sigma Chemicals, Steinheim, Germany).

2.5 Statistical Analyses

The data were statistically analyzed by two-way Analysis of Variance (ANOVA) and presented as means ± SD (standard deviation) in triplicate. The results were considered significant at p value < 0.05. The statistical analyses were based on ANOVA and LSD test.

3. RESULTS AND DISCUSSION

3.1 Effect of UV-B Irradiation on The Conversion of Ergosterol to Vitamin D$_2$ in Mushrooms

The ergosterol contents of different types of mushrooms were varied. The highest concentration of ergosterol in control (unexposed) was found in eryngi (4.96±0.04 mg/g dry weight) and the lowest one was in jew’s ear mushrooms (2.27±0.04 mg/g dry weight) (Figure 1). After UV-B irradiation, due to extended irradiation times, the ergosterol content in overall mushrooms decreased because some of ergosterol was partially converted to vitamin D$_2$ [18]. According to the results shown in Figure 2a, the vitamin D$_2$ concentration was linearly correlated with accumulative time of irradiation and oyster mushrooms showed the highest conversion of ergosterol to vitamin D$_2$. The amount of vitamin D$_2$ in the oyster was increased from 1.78±0.54 μg/g of dry weight (unexposed) to 27.89±0.67 μg/g of dry weight after exposures of 180 min, respectively. Eryngi had the highest level of ergosterol but it showed the lowest vitamin D$_2$ conversion (4.54±0.02 μg/g dry weight). After irradiation for 120-180 min, there were no significant difference (p > 0.05) in vitamin D$_2$ level in nine types of fresh mushrooms. The conversion of ergosterol to vitamin D$_2$ was completed within 120 min. Prolonged irradiation does not contribute much to this conversion of all selected mushrooms.

The conversion of ergosterol to vitamin D$_2$ was very low of quantity. Even through ergosterol in mushroom was found in milligrams, the yield of vitamin D$_2$ from this conversion was only in micrograms. The reason of this conversion, vitamin D$_2$ yielded lower than expected after treated with UV, could possibly be from the limitation of UV rays penetration into the mushroom.
tissues i.e. UV-B is able to travel through the epidermal layer not deeper than 50 μm approximately from the surface of the mushroom. The penetration ability of UV into different mushrooms depends on the mushroom morphologies, the presence of pigments and thickness of tissues and parts of mushrooms exposed to UV-B light [19].

Enoki mushrooms were sensitive to UV-B ray. The color change could visibly see from the superficial appearance i.e. from whiteness to brownness when they were exposed to UV-B irradiation for 60 to 180 min. UV-B irradiation for long time with high irradiation intensity leads to the decrease in quality of mushrooms such as formation of browning pigment, cell death, heat stress and moisture content [20]. This result demonstrated that exposure less than 60 min was proper for irradiating mushrooms to avoid discoloration.

Not only fresh mushrooms were found to have the conversion of ergosterol to vitamin D$_2$ but also exhibited in mycelium and freeze-dried mushroom powder. Before UV-B irradiation, no vitamin D$_2$ was detected in all nine types of mushroom mycelia as they were culture in the dark chamber so the synthesis of vitamin D$_2$ from ergosterol was unable to be initiated. Once the mycelia UV-B irradiation occurred, the conversion of ergosterol to vitamin D$_2$ was stimulated. The concentrations of vitamin D$_2$ in mycelia were increased due to the longer time of irradiation (Figure 2b). The yields of vitamin D$_2$ in mycelia of all selected mushrooms were highest after irradiation for 180 min. Vitamin D$_2$ content of in mycelia of enoki mushrooms, kradang mushrooms, Jew’s ear mushrooms, phoenix mushrooms and shitake mushrooms exposed to UV-B irradiation for 180 min were not statistically significant.

Figure 2c showed the effect of irradiation to the conversion of vitamin D$_2$ in freeze-dried mushrooms powder implying that even with scarce moisture, the conversion of ergosterol to vitamin D$_2$ could be occurred. The highest yields of vitamin D$_2$ were found in freeze-dried mushrooms powder of kradang mushrooms oyster mushrooms and phoenix mushrooms which exposed to UV-B irradiation for 180 min. Additionally, the increase in vitamin D$_2$ proportion in freeze-dried mushrooms powder was higher than that in both fruiting bodies and mycelia which is due to a larger exposure area to the UV-B irradiation of freeze-dried mushrooms powder. By the reason of this, the conversion of ergosterol to vitamin D$_2$ was then greater while only on the tissue surface of fresh mushrooms and mycelia was irradiated which accordingly brought about less content of ergosterol shifted into vitamin D$_2$.

![Figure 1](image.png)

**Figure 1.** The mean ergosterol contents of nine-type mushroom subjected to irradiation for 15 to 180 min.
3.2 Effect of UV-B Irradiation to The Concentration of Vitamin B1 and B2

The vitamin B groups were analyzed in fresh mushroom. Prior to UV-B application, the vitamin B1 or thiamine and B2 or riboflavin content were respectively $2.40 \pm 0.16$ to $185.44 \pm 0.21 \, \text{μg/g dry weight}$ and $12.78 \pm 0.54$ to $121.30 \pm 1.08 \, \text{μg/g dry weight}$. After UV-B irradiation, it was found that vitamin B1 concentration remained relatively stable (Figure 3) whereas vitamin B2 content decreased because of the prolonged irradiation time (Figure 4). UV-B irradiations for 15-180 min appeared to be a significant difference in vitamin B2 degradation in all selected mushrooms. Vitamin B2 or riboflavin is very sensitive to UV light. Riboflavin loss by light is dependent on different factors such as light intensity, exposure time and wavelength. Light at 300-450 nm could be destructive to riboflavin. When irradiated with UV light, riboflavin can produce reactive oxygen species such as superoxide anion radicals, singlet oxygen, hydroxy radical, and hydrogen peroxide in the presence of atmospheric oxygen. The distribution of reactive oxygen species in a particular riboflavin-photosensitized system depends on the availability of oxygen, the concentration of riboflavin and other oxidizable substrates, and quenchers. Reactive oxygen species can cause not only the degradation of vitamins B2 but also protein, carbohydrates, lipids and the formation of off-flavor in foods [21].

**Figure 2.** The mean vitamin D₂ content in fresh mushrooms (a), mycelium (b) and freeze-dried mushrooms powder (c) after UV-B irradiation for 15-180 min.
4. CONCLUSIONS

It can be concluded from the results that the intensity of UV light given to all selected fresh mushrooms were influential to the escalation of the vitamin D\(_2\) concentration, but on the other hand the concentration of ergosterol and riboflavin were degraded. Vitamin B\(_1\) concentration remained stable after UV-B treatment (Figure 5). Prolonged UV-B irradiation dose could degrade reduced mushroom quality. The conversion of ergosterol to vitamin D\(_2\) was not only generated in fresh mushroom but also in mycelia and freeze-dried mushrooms. This finding represents an easy way to improve the nutritional value of fresh and freeze-dried mushroom in Thailand. In this experiment, irradiation of 10g fresh mushroom for 15 min was exceedingly sufficient to obtain the recommended allowance of vitamin D\(_2\) each day for 1 to 70 years of age (15 μg/day) [22] and it also did not lead to discoloration of fresh mushrooms.

Figure 3. The mean vitamin B\(_1\) content in fresh mushrooms after UV-B irradiation.

Figure 4. The mean vitamin B\(_2\) content in fresh mushrooms after UV-B irradiation.
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REFERENCES


