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Distribution of ergosterol in different tissues of mushrooms and its effect on the conversion of ergosterol to vitamin D_2 by UV irradiation

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Abstract

Analysis of ergosterol content in different tissues of Shiitake mushrooms showed a significant difference ($p < 0.01$) in its distribution. Thus, the conversion of ergosterol in whole mushrooms to vitamin D_2, by exposure to UV irradiation, was significantly affected ($p < 0.01$) by the orientation of the mushroom tissues to the UV. The highest ergosterol content was found in button mushrooms (7.80 ± 0.35 mg/g DM) while the lowest was in enoki mushrooms (0.68 ± 0.14 mg/g DM). The conversion of ergosterol to vitamin D_2 was about four times higher when gills were exposed to UV-A irradiation than when the outer caps were exposed to the same. The lowest conversion to vitamin D_2 (12.5 ± 0.28 μg/g DM) was observed for button mushrooms while the highest value (45.1 ± 3.07 μg/g DM) was observed for oyster mushrooms. The optimum moisture content of mushrooms for this conversion was around 78% on a wet basis and the temperature was around 35 °C.

Keywords: Vitamin D_2; Ergosterol; UV irradiation; Mushrooms; Lentinula edodes; Pleurotus ostreatus; Agaricus bisporus; Pleurotus cystidus; Flammulina velutipes

1. Introduction

Vitamin D, known as the "sunshine vitamin" was first discovered by Edward Mellanby during his experiments with rickets (Mellanby, 1919). It plays a vital role in calcium metabolism and bone mineralization. Vitamin D is the generic name of a closely related group of vitamins exhibiting similar biological activity of cholecalciferol (vitamin D_3). Ergocalciferol (vitamin D_2) is the synthetic form of vitamin D that can be formed from the plant steroid, ergosterol by UV irradiation (Vanden-Berg, 1997) and it is assumed to have the same biological activity as choleacalciferol. Intake of an adequate amount of vitamin D is essential to prevent rickets in children and osteomalacia in adults. Vitamin D deficiency disorders are common all over the world (Diamond, Levy, Smith, & Day, 2000; Ravinder et al., 2000; Richard, Elizabeth, Johnson, Guralnik, & Linda, 2000; Vieth, Cole, Hawker, Trang, & Rubin, 2001; Yan et al., 2000), and the probability of this happening is higher in Asian populations than the European populations. Vitamin D_3 is found in animal products such as fish liver oils (high levels), fish, eggs, butter, margarine (moderate levels) and cheese, milk (adequate levels). Therefore, strict vegetarians, who are not consuming even milk, are at risk of vitamin D deficiency disorders.

Vitamin D_2 is the form that has been generally used in food and pharmaceutical supplementation (Coultate, 2002). In nature, wild mushrooms contain very small amounts of vitamin D_2 (Mattila, Lampi, Ronkainen, Toivo, & Piironen, 2002). Even though mushrooms are deficient in vitamin D_2, earlier researchers have found them to be a rich source of ergosterol (Mattila et al.,

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2002; Mau, Chen, & Yang, 1998). Mushrooms are considered a delicacy, highly accepted by vegetarians as well as non-vegetarians. Therefore they could be used to supplement vitamin D_2 content in the diets of those populations at risk of vitamin D deficiency symptoms, if their ergosterol content can be conveniently converted to vitamin D.

Mau et al. (1998) studied the effect of UV irradiation on the conversion of ergosterol to vitamin D_2 in edible mushrooms, and found that the conversion was highest under Ultraviolet-B (UV-B; wavelength 290–315 nm) compared to Ultraviolet-C (UV-C; wavelength 190–290 nm). The effect of Ultraviolet A (UV-A; wavelength 315–400) on the conversion of ergosterol in mushrooms to vitamin D_2 was unknown. UV-A represents ≈6.3% of the incoming solar radiation and it is considered relatively harmless compared to UV-B and UV-C (Hollosy, 2002). Perera, Jasinghe, Ng, and Mujumdar (2003) reported that the conversion of ergosterol to vitamin D_2 was affected by the moisture content of mushrooms and it was concluded that the optimum moisture content for the conversion was around 70–80%. However, only limited information is reported in the literature about this conversion and the existence of different levels of ergosterol in different tissues of cultivated edible mushrooms.

Hence the objective of this research was to study the ergosterol content in different tissues of shiitake and other cultivated edible mushrooms in the region and the effect of UV-A radiation on the conversion of ergosterol to vitamin D_2 in edible mushrooms.

2. Materials and methods

2.1. Raw materials

Fresh shiitake mushrooms (*Lentinula edodes*), oyster mushrooms (*Pleurotus ostreatus*), button mushrooms (*Agaricus bisporus*), abalone mushrooms (*Pleurotus cystidus*) and enoki mushrooms (*Flammulina velutipes*) were purchased from a local supermarket for the preliminary studies and were used immediately in the experiments.

2.2. Preparation of samples for ergosterol and vitamin D_2 determinations

Mushrooms were divided into stalk or stipe, thickened cap or pileus and gills. (These parts differ structurally as well as morphologically, and so the chemical composition may also vary. Hence, determining the distribution of ergosterol and vitamin D_2 in different parts of the mushroom would be helpful in the interpretation of results for the conversion of ergosterol to vitamin D_2 in the later stages of this project).

With the help of a sharp blade, outer layers of the cap, gills and stalks were carefully separated. These three parts were separately freeze-dried, covered by aluminium foil to prevent exposure to light, and kept in a vacuum desiccator prior to analysis.

The samples were separately ground into powder with the help of a mortar and pestle before extraction and analyses, according to the procedure described later.

2.3. Irradiation of mushroom tissues

Mushrooms were divided equally into two lots: one lot was placed with their gills facing the UV source [Mineralight UVGL – 25, San Gabriel, USA] with UV-A lamp (intensity at 15 cm, 3.5 W/m^2] and the other lot was irradiated with their caps facing the UV source. The UV-A irradiation source was placed at a distance of 15 cm from the samples in an irradiation chamber and the calculated irradiation dose after a 2-h irradiation period was 25.2 kJ/m^2. No effort was made to determine whether this radiation dose was optimal for the conversion or not. The irradiated samples were separately freeze-dried, and were stored in a vacuum desiccator for further analysis. All the irradiation experiments were carried out at 27 °C, and relative humidity of 65%, unless otherwise stated.

2.4. Analyses of ergosterol and vitamin D_2

Ergosterol and vitamin D_2 were extracted and analyzed according to the method of Mau et al. (1998), modified as given below. Freeze dried mushroom sample powders (0.5 g) were accurately weighed into 250 ml round bottom flasks and mixed with 4 ml of sodium ascorbate solution (17.5 g of sodium ascorbate in 100 ml of 1 M NaOH), 50 ml of ethanol (95% pure, Riverbank Chemicals, Singapore), 10 ml of 50% potassium hydroxide (85% pure, Merck Chemicals, Darmstadt, Germany) and 50 μg of cholecalciferol (Sigma chemicals, Steinheim, Germany) as the internal standard. The mixture was saponified under reflux at 80 °C for 1 h, then, it was immediately cooled to room temperature and transferred into a separating funnel. The mixture was first extracted with 15 ml de-ionized water, followed by 15 ml ethanol and then with three-stages of *n*-pentane of volumes 50, 50 and 20 ml, respectively. The pooled organic layers were washed three times with 50 ml of 3% KOH in 5% ethanol and then finally with de-ionized water until neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 40 °C, and immediately re-dissolved in 5 ml ethanol.

The samples were passed through a 0.45 μm non-pyrogenic filter (Schleicher & Schuell, Dassel, Germany). A volume of 20 μl of filtered sample was injected into a Waters 600E HPLC system equipped with Waters

486 tunable absorbance UV detector (Waters, Milford, MA, USA) and eluted through a reverse phase C18 column (Maxsil 5 C18, 250 × 4.6 mm, Phenomenex, Torrance, CA, USA) using acetonitrile/methanol (HPLC grade Merck Chemicals, Darmstadt, Germany) (75:25) as the mobile phase at a flow rate of 2.3 ml/min. The UV detection of the eluate was performed at 282 nm.

The vitamins D_2, D_3 and ergosterol were determined by comparing the retention times of standards obtained, and quantification was done by using a calibration curve.

2.5. Statistical analysis

The results were statistically analyzed by analysis of variance [ANOVA, Vassar stats statistical computations (http://vassun.vassar.edu/~lowry/VassarStats.html)]. The data were expressed as means ± SD (standard deviation). The test results were considered significant at $p < 0.01$.

3. Results and discussion

3.1. General

Results showed that shiitake mushrooms obtained from Singapore supermarkets did not contain any vitamin D_2. Mattila et al. (2002) also reported that vitamin D_2 was almost totally absent in cultivated mushrooms. This was probably due to non-exposure of cultivated mushrooms to sunlight.

Shiitake mushrooms contained remarkably high amounts of ergosterol and its distribution varied in different parts of the mushroom tissue. Table 1 shows ergosterol contents in different parts of shiitake mushroom. Results showed that the distributions of ergosterol within the mushroom tissues were significantly different ($p < 0.01$). The highest concentration of ergosterol was found in the gills, while the lowest was present in the stalk of mushrooms. The concentration of ergosterol in gills was almost twice that found in the outer layer of the caps, which in turn had almost twice that found in the stalks. Sterols are involved in the stabilization of membranes to UV. The younger tissues, such as gills, tend to have higher concentration of sterols than the older tissues, the stalks and the caps.

Table 1
Concentration of ergosterol in different parts of shiitake mushroom

Part of the mushroom	Mean ergosterol content[a](mg/g DM [b])
Gills	10.6 ± 0.99
Outer layer of the cap	5.34 ± 0.64
Stalk	2.97 ± 0.56

[a] Means values of 27 replicates ± SD.
[b] DM, dry matter.

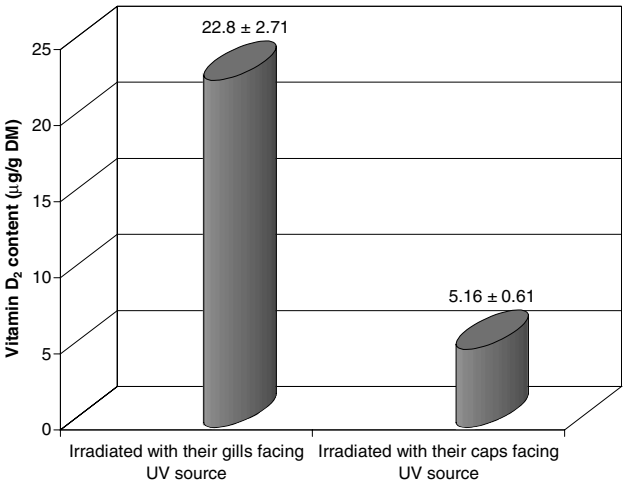

Fig. 1. Vitamin D_2 contents of Shiitake mushrooms subject to the two different ways of irradiation.

3.2. Effect of irradiation on the conversion of ergosterol to vitamin D_2

The effect of UV irradiation of different mushroom tissues exposed to the source of irradiation is shown in Fig. 1. Results showed a high rate of conversion of ergosterol to vitamin D_2 when mushrooms were irradiated with their gills facing the UV source. When the gills were facing the source of irradiation, the yield of vitamin D_2 was 22.8 ± 2.71 μg/g DM, whereas, when they were facing away from the source of irradiation (caps facing the source of irradiation), the yield of vitamin D_2 was only 5.16 ± 0.61 μg/g DM. Vitamin D_2 yield values obtained by these two different ways were significantly different ($p < 0.01$). These values were three to four times more than what was recently reported (Mau et al., 1998) for the conversion of ergosterol to vitamin D_2 in Shiitake mushrooms by UV-B and UV-C irradiation.

Even though the concentration of ergosterol in gills of shiitake mushrooms was only about twice higher than that of the outer layer of cap (Table 1), Fig. 1 clearly shows a conversion factor of ≈4. This high level of conversion of ergosterol to vitamin D_2 may be due to the fine morphology of the gills, which allows greater exposure of the surfaces to irradiation than in the case of the caps and their higher metabolic activity compared to the caps and stalks.

3.3. Ergosterol and vitamin D_2 contents in different types of edible mushrooms available in the Singapore market

The overall ergosterol contents of different types of mushrooms varied. The highest ergosterol content was found in button mushrooms (7.80 ± 0.35 mg ergosterol/g DM) while the lowest was in enoki mushrooms

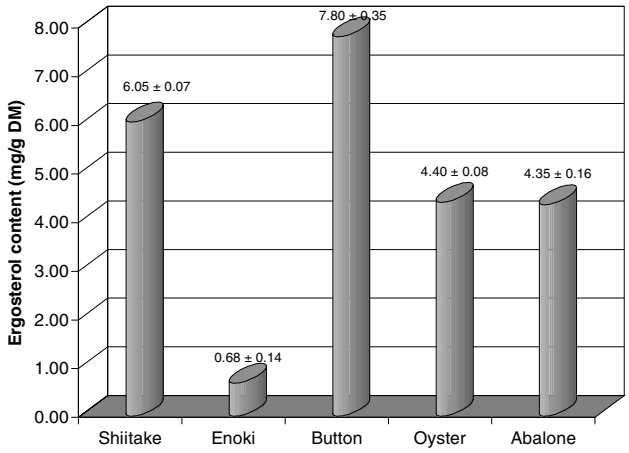

Fig. 2. Ergosterol contents of different types of mushrooms.

(0.68 ± 0.14 mg/g DM). Oyster mushrooms contained 4.40 ± 0.08 mg/g DM and the value was more or less the same for abalone mushrooms (4.35 ± 0.16 mg/g DM). Shiitake mushrooms contained 6.05 ± 0.07 mg/g DM of ergosterol. This is in agreement with the values found by Mattila et al. (2002) who observed a value of 6.79 mg/g DM of ergosterol in shiitake mushrooms. Ergosterol contents of different types of non-irradiated mushrooms are shown in Fig. 2.

3.4. Conversion of ergosterol to vitamin D_2 by UV irradiation

Vitamin D_2 content of different types of mushrooms subjected to 2 h of UV-A irradiation with their gills facing the source of irradiation is shown in Fig. 3. Button mushroom showed the lowest vitamin D_2 content (12.5 ± 0.28 μg/g DM), despite having the highest levels of ergosterol in them. This may be due to the fact that gills were not exposed in button mushrooms, thus UV radiation was unable to penetrate effectively into the gill area. Another reason for this low conversion rate of ergosterol to vitamin D_2 in button mushrooms could be the possibility of photo-degradation of vitamin D_2 on prolonged exposure to UV-A (Webb, DeCosta, & Holick, 1989). Vitamin D_2 may also be subjected to the action of tissue mono-oxygenases that transform vitamin D_2 to 25-hydroxyvitamin D_2 and 25-dihydroxyvitamin D_2, thus reducing the overall conversion of ergosterol to Vitamin D_2. Yet another possible reason for this lower than expected conversion of ergosterol to Vitamin D_2may be the lower depth of penetration of UV-A compared to the more energetic UV-B and UV-C. UV-C is known to penetrate to a depth of 35–50 μm (≈6–10 cell layers), depending on the tissue. Oyster mushrooms, on the other hand, showed the highest vitamin D_2 content (45.1 ± 3.07 μg/g DM) among the different mushrooms tested. The yield of vitamin D_2 obtained from abalone mushroom which had more or less similar ergosterol content to oyster mushroom, was only 22.6 ± 1.60 μg/g DM. Once again this may be due to their morphological differences. Vitamin D_2 content in shiitake mushrooms after 2-h UV-A irradiation was 22.8 ± 1.07 μg/g DM. Mau et al. (1998) have reported 6.58 and 12.5 μg/g DM of vitamin D_2 from shiitake mushrooms and button mushrooms respectively, after a 2-h UV-B irradiation at 12 °C. However, no indication of the orientation of the mushroom tissues to the source of UV irradiation was reported. The values we obtained for Shiitake were significantly higher than those reported by others and this may be due to the specific orientation of the mushroom to the UV source.

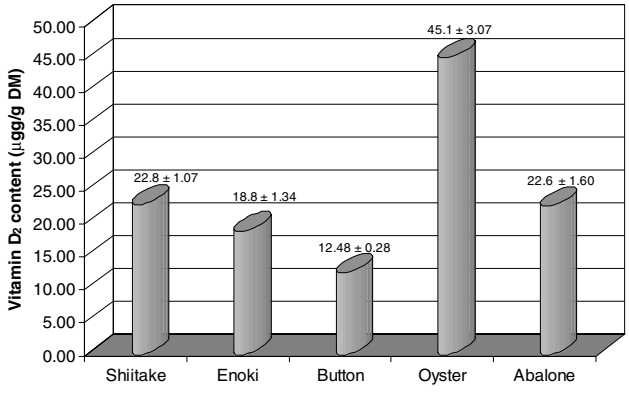

Fig. 3. Vitamin D_2 contents of the different types of mushrooms subjected to irradiation for 2 h, with their gills facing the UV source.

3.5. Effect of moisture content of mushrooms and temperature of irradiation on the conversion of ergosterol to vitamin D_2

The results obtained from irradiating shiitake mushrooms at different moisture contents with their gills facing the UV source, show that the best conversion takes place at a moisture content of around 78% on a wet basis (Fig. 4). This may be due to the dilution effect of ergosterol at very high moisture content, which is likely to bring about a lower conversion rate. At low moisture levels, the specific surface area of the tissue is increased, and, consequently, the exposure to oxygen is increased, resulting in the oxidation of vitamin D_2. Furthermore, irradiation also contributes to oxidative atmosphere (Vayalil, Elmets, & Katiyar, 2003), and photo-degradation of vitamin D_2 may occur. It can be concluded, from the results, that irradiation of mushrooms, at a moisture-content of around 70–80%, enhances the yield of vitamin D_2.

Fig. 5 shows the effect of irradiation temperature on the conversion of ergosterol to vitamin D_2 in shiitake mushrooms. The results clearly suggest that irra-

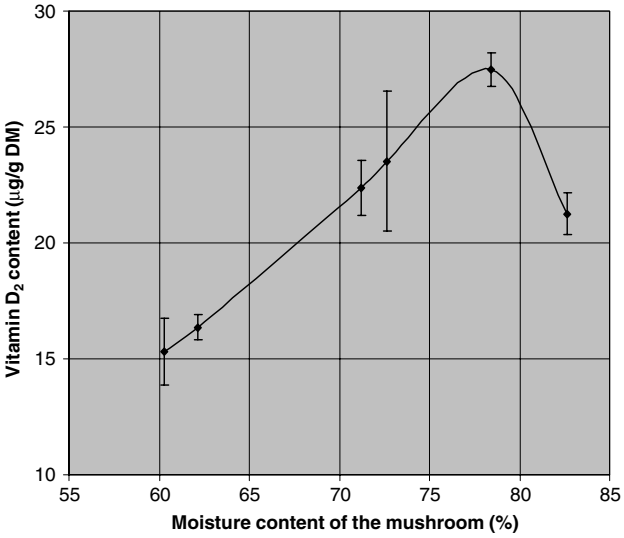

Fig. 4. Effect of moisture content of mushrooms on the conversion of ergosterol to vitamin D_2.

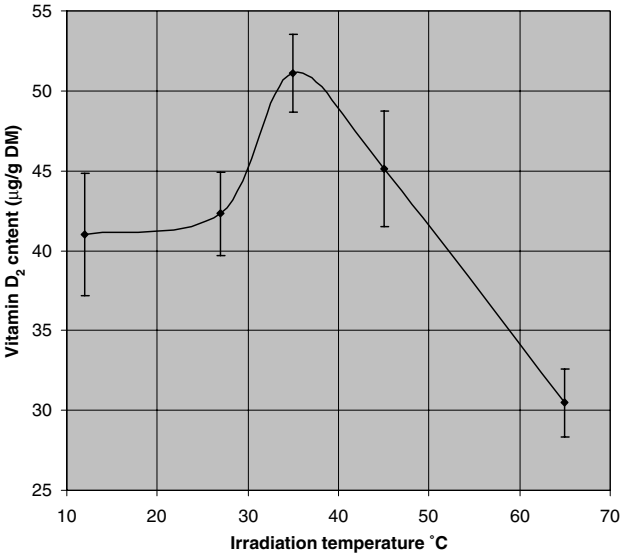

Fig. 5. Effect of temperature of irradiation on the conversion of ergosterol to vitamin D_2.

diation of mushrooms at about 35 °C, enhanced the conversion, leading to the highest yield of vitamin D_2. The decrease in conversion rate beyond 35 °C was probably due to many concurrent events: heat stress (oxidative), cell death, formation of browning pigments, further transformation of vitamin D_2 as well as photo-degradation by irradiation.

4. Conclusions

The distribution of ergosterol in different parts of shiitake mushroom varied. The conversion rate of ergosterol to vitamin D_2 was ≈4 times higher when gills were facing the UV source than when they were facing away from the source of irradiation. Mushrooms should be irradiated with their gills facing the UV source in order to maximize the conversion of ergosterol to vitamin D_2.

While the ergosterol content in different types of mushrooms varied, no detectable vitamin D_2 was observed in cultivated shiitake, oyster, abalone, button or enoki mushrooms. However, remarkably high levels of vitamin D_2 could be obtained by UV-A irradiation of mushrooms for two-hours, with their gills facing the UV source. The irradiation temperature and moisture content of mushrooms should be maintained at optimum levels, in order to maximize the yield of vitamin D_2 since they play an important role in this conversion. Under the conditions of UV irradiation, there was no apparent browning of the mushrooms; however, we found that UV-C irradiation for 2 h caused considerable browning during storage.

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