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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