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Kinetics of the conversion of ergosterol in edible mushrooms

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Abstract

Kinetics of conversion of ergosterol to vitamin D_2 has been investigated in cultivated edible mushrooms. It was observed that the rates of conversion of ergosterol to vitamin D_2 were varied in different types of mushrooms. Oyster mushrooms (*Pleurotus ostreatus*) showed the highest conversion rate followed by Shiitake (*Lentinula edodes*) and Abalone (*Pleurotus cystidus*) whereas the lowest conversion rate was observed in Button mushrooms (*Agaricus bisporus*). Both initial moisture content and temperature of irradiation influenced the conversion of ergosterol, and a 2×2 factorial design was used to study this influence. It was shown that the conversion of ergosterol to vitamin D_2 followed zero-order kinetics, where the rate constant varied with temperature according to the Arrhenius equation ($K_0 = 7.32\ s^{-1}$; $E_a = 51.5\ kJ\ mol^{-1}$). Vitamin D_2 yields from Shiitake mushrooms with respect to temperature of irradiation (T) and moisture content of the mushrooms (M) can be calculated from the equation:
$D_2 = -91.3 + 2.25 * T + 71 * M$.

Keywords: Arrhenius equation; Edible mushrooms; Ergosterol; Zero-order kinetics

1. Introduction

Vitamin D and calcium are well known to be vital for bone health. The deficiency of this vitamin, results in rickets in children leading to malformation of bones and osteoporosis in adults (Morgan, 2001). In addition, vitamin D has been suggested for therapeutic applications in the treatment of several diseases including hyperproliferative diseases, secondary hyperparathyroidism, post-transplant survival, and various malignancies (Peleg, 1997).

Vitamin D deficiency disorders are common all over the world (Diamond, Levy, Smith, & Day, 2000; Goswami et al., 2000; Semba, 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 since the prevalence of vitamin D deficiency is higher in people with darker skins than people with lighter skins (Harris & Hughes, 1998; Serhan, Newton, Ali, Walford, & Singh, 1999; Shanna et al., 2002). In 2001, Holick reported that vitamin D deficiency is an unrecognized epidemic among elderly and more than 50% of elderly persons, both living in their own homes and nursing homes in the USA are deficient in vitamin D (Holick, 2001, 2004).

In nature, vitamin D is found in two different forms, namely, Ergocalciferol (Vitamin D_2) and Cholecalciferol (Vitamin D_3). Vitamin D_3 is found only in animal products such as fish liver oils (high levels), eggs, butter, cheese, and milk (adequate levels). Vitamin D_2 is found mainly in plant products. Since there are only a handful of plant foodstuffs that contain vitamin D, strict vegetarians, who are not consuming even milk, are at risk of vitamin D deficiency disorders. Edible mushrooms are highly prized in the Orient for their flavour and reputed medicinal value. Cultivated mushrooms are deficient in vitamin D_2, however they are

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found to be a rich source of ergosterol, the precursor of vitamin D_2. Ergosterol in mushrooms can be converted into vitamin D_2 by UV irradiation (Jasinghe & Perera, 2005, 2006; Mau, Chen, & Yang, 1998; Perera, Jasinghe, Ng, & Mujumdar, 2003), temperature of irradiation and moisture content of mushrooms play an important role in this conversion.

Kinetics of this conversion in mushrooms and factorial effects such as temperature and moisture on the yield of vitamin D_2 are very important in prediction of the yield. Yang, Tan, and Qi (1998) reported that the conversion kinetic of ergosterol in different solvent systems were different, and have also commented that the temperature of irradiation was very important in this conversion. However, there appears to be no data on conversion kinetics of ergosterol to vitamin D_2 in natural products such as in Shiitake mushrooms. Hence the objective of our study was to investigate the conversion kinetics of ergosterol in mushrooms to vitamin D_2, and the effect of moisture content of mushrooms and temperature of irradiation in a 2×2 factorial model. This will be useful in predicting the yield of vitamin D_2 after the irradiation process.

2. Materials and methods

2.1. Materials

Fresh mushrooms of four different varieties (Shiitake, Oyster, Abalone, and Button) were obtained from the local market.

2.2. Method

Mushrooms were subjected to UV-A irradiation for different time periods. Each side of the mushrooms was irradiated with UV-A source [Mineralight UVGL – 25, San Gabriel, USA with UV-A lamp (intensity, 3.5 W/m^2 at a distance of 15 cm)]. The calculated irradiation dose was 0.21 $kJ/m^2/min$. Shiitake, Oyster, Abalone, and Button mushrooms were irradiated at a temperature of around 30 °C for different time periods in order to investigate the conversion kinetics with regards to time of irradiation. The moisture content of those mushrooms was found to be $80 \pm 2.54\%$ on a wet weight basis (w.b.). The change in moisture content of mushrooms during the irradiation process is not significant ($p > 0.05$) under the conditions described.

Fresh Shiitake mushrooms were selected to study the influence of moisture content and temperature of irradiation on the conversion of ergosterol using 2×2 factorial model. Shiitake mushrooms were irradiated with UV-A for two hours. In these experiments moisture contents of the mushrooms were adjusted to 60% and 80% (w.b.) by vacuum drying at 25 °C. The temperature of irradiation was maintained at 25 and 35 °C when they were irradiated.

In order to study the conversion kinetics of ergosterol in vitamin D_2, three different temperatures, 25, 30, and 35 °C were selected. Again a 2×2 factorial design was used in this study. The moisture content of fresh Shiitake mushrooms was measured gravimetrically by drying samples in air convection drier at 105 °C for at least 20 h.

2.3. Analysis of ergosterol and vitamin D_2

Analysis of ergosterol and vitamin D_2 was done according to a previously described method (Jasinghe & Perera, 2005). This method clearly discriminates between ergosterol and vitamin D_2. Lyophilized mushroom sample (0.500 g) was accurately weighed into 250 mL round bottom flask and mixed with 4 mL of sodium ascorbate solution (17.5 g in 100 mL of 1 M NaOH), 50 mL of ethanol (95% pure), and 10 mL of 50% potassium hydroxide. Then the mixture was saponified for 1 h under reflux at 80 °C. The mixture was cooled down to the room temperature and transferred into a separating funnel. The mixture was extracted first with 15 mL water, followed by 15 mL ethanol and then a three-stage *n*-pentane of volumes 50, 50, and 20 mL, respectively. The pooled organic layers were washed three times with 50 mL of 3% potassium hydroxide in 5% ethanol and then finally with deionized water until neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 40 °C, and immediately redissolved in 5 mL ethanol. The samples were filtered through 0.45 mm filter units. A volume of 20 μL filtered sample was injected into the 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 (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. Quantification of vitamin D_2 was done using a calibration curve.

2.4. Kinetic model of ergosterol conversion

The results of our experimental studies showed that the conversion of ergosterol to vitamin D_2 during irradiation of mushroom increased linearly with time (Fig. 2, Table 3). This trend was consistent with all three temperatures used in this study. Hence a zero order reaction equation was assumed to model kinetics of conversion of ergosterol to vitamin D_2:

$$\frac{dC}{dt} = KC^0 \tag{1}$$

where C is the concentration of ergosterol (g/g dry matter), t is the time of irradiation (s) and K is the reaction rate constant (s^{-1}). One of the most common practices to model temperature dependence of reaction rates is to use Arrhenius equation (Banga & Singh, 1994). Yang et al. (1998) also used this approach to model influence of temperature