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Change in Concentration of Vitamin D_2 in Oyster Mushrooms Exposed to 254nm and 365nm UV-light During Growth

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Abstract The sun emits ultraviolet radiation in form of ultraviolet-A (UV-A), ultraviolet-B (UV-B), and ultraviolet-C (UV-C) bands. Ultraviolet light has the potential to boost vitamin D_2 production in mushrooms which human bodies cannot synthesize. The ergosterol in mushrooms, a component of fungal cell membranes which serves the same function as cholesterol in animal cells, can be converted into vitamin D_2 by exposure to controlled ultraviolet light. However mushrooms are conventionally grown in the dark, necessitating artificial ultraviolet irradiation. This study investigated the effects of UV-A (365nm) and UV-C (254nm) light exposure time during mushrooms growth, on the concentration of vitamin D_2 in oyster mushrooms (*Pleurotus ostreatus species*) after harvest. Mushrooms samples exposure times were varied from 10-60 minutes per day at intervals of 10 minutes, and irradiation done for three days. UV spectroscopy was used to determine the amounts of Vitamin D_2. It was found that the absorbance of vitamin D_2 for UV-A light ranged from 0.18-0.49 for the 10-60 minutes of irradiation respectively, while for UV-C light the vitamin D_2 content absorbance was 0.38-0.81 for the 10-60 minutes of irradiation respectively. There was a linear relationship between time of irradiation and absorbance vitamin D_2 content up to 50 minutes for UV-A and 40 minutes for UV-C.

Keywords UV-A and UV-C light, Oyster mushrooms, Vitamin D_2, Growth

1. Introduction

The sun emits ultraviolet radiation in the form of ultraviolet-A (UV-A; 315-400 nm), ultraviolet-B (UV-B; 280-315 nm), and ultraviolet-C (UV-C; 100-280 nm) bands [1]. Human bodies through the skin are only able to synthesize vitamin D_3 from 7-dehydrocholesterol following exposure to ultraviolet B (UV-B) but not Vitamin D_2 [2]. Studies have shown that some wild mushrooms have naturally occurring levels of vitamin D_2 in the range of 2.91-58.7 μg/100 g fresh weight [3]. In addition, it has been shown that vitamin D_2 content of mushrooms can also be enhanced through the by UV light irradiation [4].

Vitamin D is a fat soluble vitamin required by the body which plays an important role in the regulation of calcium and phosphorus in the human body and in mineralization of bones [5]. Furthermore, it is clear that receptors for vitamin D are present in a wide variety of cells, meaning this vitamin has biological effects that extend far beyond control of mineral metabolism [6]. Vitamin D consists of two different compounds, vitamin D_2 from ergosterol and vitamin D_3 from animal products or the action of sunlight on a cholesterol-like precursor, 7-dehydrocholesterol, which is in the skin [7]. Ingested vitamin D_2 and endogenously produced D_3 are converted to the biologically active form, 1, 25-dihydroxyvitamin D (1, 25$(OH)_2$D) (calcitriol) in the human body [8].

Vitamin D deficiency is an ever increasing problem in human nutrition and health. Research has shown that it affects much more than the classic diseases of rickets in children and osteomalacia in adults resulting from inadequate bone mineralization [9]. Links of vitamin D deficiency to diseases such as cardiovascular disease [10] and cancer [11] have been documented. Other diseases with links to vitamin D deficiency include hypertension, stroke, diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, periodontal disease, mental illness, propensity to fall and chronic pain [12]. There are a limited number of natural dietary sources of vitamin D leading to a real need for alternatives to improve dietary intake. Mushrooms are the only non-animal-based food containing vitamin D_2 and ergesterol hence are the only natural vitamin D_2 sources for vegetarians [13].

A study on Agaricus *bisporus* mushrooms obtained 1 day after harvesting reported that they were exposed to UV-C irradiation at intensities of 0.403, 0.316, and 0.256 mW/cm^2 from distances of 30, 40, and 50 cm, respectively [14]. These distances were chosen to determine whether a strategic placement of a UV-C light in the growers' rooms is effective in triggering the conversion of ergosterol to vitamin D_2 in cultivated mushrooms before harvesting. The increase in vitamin D_2 concentrations in micrograms per gram of dry solids was dependent on time and intensity of exposure to UV-C irradiation, which in turn was dependent on distance from the UV source. Irradiation from a distance of 30 cm at an intensity of 0.403 mW/cm^2 produced higher concentrations of vitamin D_2 after treatment for times ranging from 5-60 minutes when compared to those produced with intensities of 0.316 and 0.256 mW/cm^2 at distances of 40 and 50 cm, respectively.

However, the conditions used in other studies to enhance vitamin D_2 formation may not be suitable for application by mushroom growers because the dose and duration of UV irradiation using a combination of irradiation intensities and treatment regimens are too complicated to replicate in the mushroom grower's facility [15]. In addition, the need for whole body mushroom irradiation including button, gill, and stalk to maximize vitamin D_2 formation, the manipulation of moisture content in mushrooms by partial drying prior to UV irradiation, and the long exposure time to UV irradiation up to two hour are conditions that would be considered impractical from the industry's point of view [16]. An attempt at standardization of treatment regimen using UV-B irradiation to produce vitamin D_2 in button mushrooms for potential application on a commercial scale has been reported [17].

The goal of this study was to irradiate growing mushrooms with UV-A:365nm and UV-C:254nm light, and vary exposure times in a situation that is achievable in a mushroom conventional growing environment, and infer the effects of these variations on the amount of vitamin D_2 in the mushrooms inferred from the UV spectrometry.

2. Materials and Method

2.1. Growing of Mushrooms

Wheat grains were prepared for grain spawn by being boiled, drained, filled in containers and sterilized. The substrate was then prepared from wheat straw and was pasteurized by hot water immersion to kill contaminants. The pasteurized substrate was then spawned after ensuring that the substrate has cooled down to 30 °C. The spawn was mixed with the substrate when filling thirteen perforated bags labeled B1 to B13. Spawn run followed where the mycelium was grown through the substrate. The bags once spawned were placed in a cage that had been prepared where mycelium colonized the substrate in two to three weeks and started to form small fruiting bodies. In darkness, controlled temperature and humidity conditions were provided. Humidity was maintained between 80-100% by spraying water several times per day and the temperature was maintained between 15-25°C.

2.2. Exposure of Mushrooms to UV-C (254nm) and UV-A (365nm) Light During Growth

UV-C light (254nm) and UV-A light (365nm) irradiation began once the mushrooms cap started opening from the stem. An 8W Ultraviolet fluorescent lamp made by UVITEC (model LF- 204.LS) was used. The lamp irradiates at the ranges (254 nm) and (365nm) with a switch that shifts between the two ranges and the measured intensity was 3.5 W/m^2 for 365nm and 0.0327W/m^2 for 254nm while the rates of irradiation doses were 0.21 kJ/m^2/min for UV-A radiation and 1.96 J/m^2/min for UV-C radiation. Bag labelled B1, the control, was not exposed to UV-C and UV-A light. Six bags labelled, B2 to B7 were exposed to UV-C light while another set of six bags labelled B8 to B13, were exposed to UV-A light. Beginning with the lowest exposure time of 10 minutes for bags B2 and B8, and subsequent 10 minutes increment for the next bag up to 60 minutes for the highest exposed bags (B7 and B13) was done, for UV-C and UV-A respectively. This irradiation procedure was repeated for three days. Once the caps were fully opened and separated from the stem, the mushrooms were ready for harvesting. Harvesting was done by holding the mushrooms by their stalks and breaking them off carefully from the substrate. Samples were picked from each bag, freeze dried and ground into a powder. The powder was then used for quantification of vitamin D_2 using UV spectrophotometry.

2.3. Analysis of vitamin D_2

The samples for spectrophotometric analysis were prepared by method previously described by [18] where 0.5 g of each mushroom sample powder was 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 and 10 ml of 50% potassium hydroxide. The mixture was saponified under reflux at 80^{0}C for one hour 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 a three-stage n-pentane extraction 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 deionized water until neutralized. The organic layer was transferred into a round bottom flask rotary and was evaporated to dryness at 40^{0}C and immediately re-dissolved in 5 ml ethanol. The sample was passed through a 0.45 µm non-pyrogenic filter. UV spectroscopy, which is based on measurement of the intrinsic absorption of calciferols, plays a very modest role in quantification of vitamin D_2. In this study, spectrophotometric determination of vitamin D_2 were

then determined by method previously described by [19] where calciferol reacts with 11N hydrochloric acid in the presence of symmetrical tetrachloroethane to develop a greenish yellow colour with maximum absorption at 440-460 nm. Aliquot of 2 ml of the prepared samples of B1 - B13 were evaporated to dryness on a boiling water bath. Then 1 ml of 11 N hydrochloric acid and 1ml of symmetrical tetrachloroethane were added and the tube was warmed for 10 minutes on the water bath with occasional shaking. After cooling the volume was completed to 7 ml with acetone and the absorbance was measured using a spectrophotometer by putting a reference blank solution (the solution of the sample that had not been irradiated during growth) in a cuvette and placed in the spectrophotometer. The absorbance of the reference blank was determined at 450 nm. The blank was removed and the cuvette containing sample solution for B2 was put in the spectrophotometer and the absorbance was determined at 450 nm.

3. Results

Impact of variation of irradiation time on the conversion of ergosterol to vitamin D_2 and formation of vitamin D_2 by UV-C and UV-A light irradiation

Figure 1 shows the absorbance of Vitamin D_2 versus time of irradiation during growth. The exposure of UV-C light during growth for 60 minutes resulted in the highest absorbance of 0.81 compared to 0.49 for UV-A light exposure (Table 1).

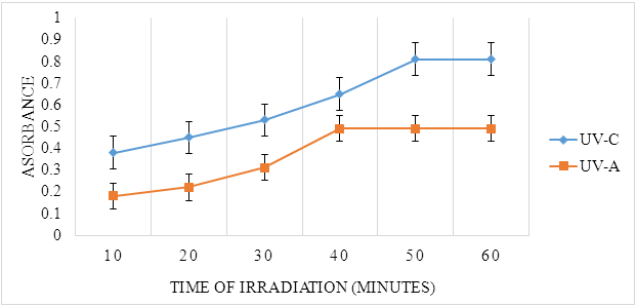

Figure 1. Absorbance of Vitamin D2 versus time of irradiation (minutes)

Table 1. Absorbance values of solutions of samples irradiated at different times by UVA and UV-C light during growth.

Time of irradiation (minutes)	10	20	30	40	50	60
UV-A absorbance	0.18	0.22	0.31	0.49	0.49	0.49
UV-C absorbance	0.38	0.45	0.53	0.65	0.81	0.81

The samples of the mushrooms that were grown without exposure to UV-C and UV-A light were found to have lowest absorbance values indicative of low Vitamin D_2 present. The absorbance of vitamin D_2 under both UV light bands increase gradually as time of exposure increases up to 40 minute for UV-C and remains constant up to 60 minutes of irradiation (Figure 2).

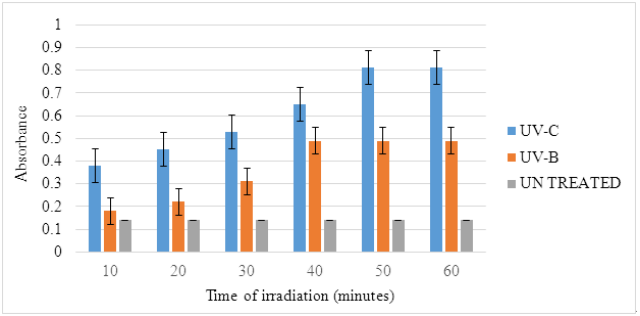

Figure 2. Comparison of absorbance of vitamin D_2 for oyster mushrooms irradiated by UV-C and UV-A light during growth and untreated mushrooms at different time of exposure.

4. Discussions

The high values of absorbance imply high concentration of vitamin D_2. The conversion of ergosterol to vitamin D2 under UV-C and UV-A were shown to be significantly different ($p < 0.05$) as shown with the standard error bars in Figure 1. The difference between the UV-C and UV-A absorbance values can be ascribed to a higher efficiency of vitamin D_2 conversion at exposure to UV-C during the growth than UV-A light. As mushrooms grow, there's increase in the amount of ergosterol, thus an increase in absorbance after repeated exposure in the subsequent days may be explained by the carryover of ergosterol formed in the mushrooms left growing for the next day. The absorbance was much lower in mushrooms exposed for 10 minutes under both UV light bands. Prolonged irradiation produces irreversible over irradiation products by dimerization and ring cleavage [20]. In addition, prolonged exposure to a close of two hours subjects the vitamin D_2 formed to the UV radiation and this may result in photo degradation of vitamin D_2 [21].

In the case of UV-A irradiation, the absorbance of vitamin D_2 increases up to 50 minutes and remains constant at 60 minutes of irradiation. The increase in the absorbance of vitamin D_2 is as a result of conversion of ergosterol in the mushroom to vitamin D_2. The absorbance of vitamin D_2 for the untreated samples ranged between (0.14 ± 0.02). This absorbance was compared with the absorbance of the irradiated sample and the comparison was represented in figure 2. Change in concentration of vitamin D_2 under both UV- bands indicated linear relationship with respect to time of exposure. The linearity of the relationship found in these two UV band was similar to what was previously reported by Roberts *et al*. 2008 following post-harvest exposure of white button mushrooms UV-B band after overnight cooling. The vitamin D_2 level of the biologically active treated mushrooms increased substantially on both wavelengths. According to published data from previous studies on post-harvest material showed the same results. In the case of UV-C treatments, even the shortest time period

(10 minutes) was enough to cause twice as high vitamin D_2 level in the mushrooms as in control. UV-A irradiation did not cause as intensive change in vitamin D_2 concentration as experienced in case of UV-C radiation. This study established that irradiation during growth leads to adequate conversation of the ergosterol that is present in the mushroom and any carry over that results from overnight growth. It was also noted that the measure of the energy required for chewing the mushroom that had been irradiated during growth until it is ready for swallowing (chewiness), was low since the exposed mushrooms had low storage modulus compared with untreated samples where the storage modulus of the samples was determined by a dynamic mechanical analyzer-2980 [22]. This implies that postharvest treatment can increase vitamin D_2 concentration but it may have little impact on chewiness which is a very vital consumer test.

5. Conclusions

a) The close to linear relationship between UV-C and UV-A dose and formation of vitamin D_2 at exposure during growth makes it possible to produce mushrooms with a well- defined content of vitamin D_2.
b) Therefore exposing mushrooms to UV-A (365nm) and UV-C (254nm) light during growth causes measurable increases in the vitamin D_2 content and as a result, mushroom can provide appreciable amounts of vitamin D_2 to the diet.
c) The concentration of vitamin D_2 depends on the wavelength of UV light and duration of exposure.
d) Consumer quality such as chewiness of the mushrooms irradiated during growth especially with UV-C light is high. That is, UV-C irradiated mushrooms required low chewing force.

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