THE EFFECT OF UV LIGHT ON THE VITAMIN D CONTENT AND MYCELIAL GROWTH OF OYSTER MUSHROOM

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ABSTRACT

Vitamin D_2 is essential for maintaining the proper functioning of a human body, and to prevent and help cure various diseases. Mushrooms are one of the few natural sources of vitamin D. Many experiments aimed to increase the vitamin D level of cultivated mushrooms, by irradiating them with UV light to turn their ergosterol content into vitamin D. The subjects of most of these studies were post-harvest sliced or whole mushrooms. Our goal was to treat pre-harvest oyster mushroom with UV light, while the mushrooms are still growing and biologically active. UV lamps (operating on 254 and 312 nm) and 6 time periods of irradiation (15 to 90 minutes) were used. After three consecutive days of treatments the yield were measured and samples were taken for vitamin D_2 analysis. A parallel, *in vitro* experiment took place in the laboratory as well, where the same treatments (wavelengths and irradiation times) were applied on the tissue cultures of the same oyster mushroom cultivar used in the *in vivo* experiment. The mycelia growth was measured in case of all treatments. Data showed considerable increase in vitamin D_2 levels of the treated oyster mushrooms at every time period. UV irradiation caused no change in yield, but affected the growth of the *in vitro* tissue cultures significantly.

Keywords: Vitamin D, UVB light, Pleurotus, ergosterol, fungi

INTRODUCTION

Vitamin D has been acknowledged for almost a 100 years as being essential for a healthy human body. It promotes calcium and phosphorus absorption, and has an effect on cellular growth. Vitamin D supports the immune system as well and helps preventing different kind of illnesses (e.g. heart diseases, obesity, diabetes, arthritis etc.) (GRANT&HOLICK, 2005; HOLICK, 2006; HOLICK, 2008). Recent studies aimed at finding possible ways of using vitamin D in cancer prevention and treatment (MEHTA&MEHTA, 2002; BOUILLON ET AL., 2006; GARLAND ET AL., 2006). Due to studies aimed at estimating the adequacy of vitamin D of the world's population (CHAPUY ET AL., 1997; OVERSEN ET AL., 2003; GORDON ET AL; CALVO&WHITING, 2006; RODRÍGUEZ ET AL., 2008; LOOKER ET AL., 2011), expert find the European population to have more or less inadequate vitamin D level. Vitamin D deficiency has been recognized as a pandemic for a few years now (HOLICK&CHEN, 2008). Various compounds with similar biological effects are called vitamin D. Vitamins D₂ and D₃ are the ones almost exclusively responsible for providing the adequate vitamin D intake for a human body, which is 400 to 800 IU= 10 to 20 μ g (D₃ equivalent) (CHAPUY ET AL., 1997; INSTITUTE OF MEDICINE, FOOD AND NUTRITION BOARD, 2010). Ergocalciferol (vitamin D_2) can be obtained from a few natural food sources, from fortified foodstuff and from supplements, while cholecalciferol (vitamin D_3) is produced in the human skin by sunlight (OVERESEN ET AL., 2003; SHRAPNEL&TRUSWELL, 2006).

In order to prevent diseases deriving from vitamin D deficiency (rickets in children, osteopenia, osteoporosis and fractures in adults), it is essential to consume food-products with high level of vitamin D (MAU ET AL., 1998; HOLICK&CHEN, 2008). There are only a few natural sources (seafood, animal and milk products) of vitamin D₂. One serving of

these food types covers 6-80% of the daily value (U.S.D.A., AGRICULTURAL RESEARCH SERVICE, 2009).

The source of vitamin D_2 is ergosterol, which is the most abundant phytosterol in mushrooms. When exposed to UV light it undergoes photolysis and form previtamin D_2 and other photoirradiation products. Previtamin D_2 then yields vitamin D_2 by spontaneous thermal rearrangement (MATTILA ET AL., 2002). Many mushroom species contain distinct levels of vitamin D_2 and ergosterol (MATTILA ET AL., 1994).

Wild grown mushroom species have higher level of vitamin D_2 than the cultivated ones. One serving of wild grown mushrooms (80-90 g) can cover 90-500% of the daily value of vitamin D (U.S.D.A., AGRICULTURAL RESEARCH SERVICE, 2009). Although they consist less vitamin D_2 , cultivated mushrooms contain more ergosterol than wild grown mushroom species (TEICHMANN ET AL., 2007; JASINGHE ET AL., 2007).

Number of experiments and studies proved that ergosterol content of post-harvest mushrooms can be converted into vitamin D_2 by artificial UV irradiation, this way the vitamin D_2 concentration in cultivated mushroom can be enhanced up to nine folds (JASINGHE ET AL., 2005; MAU ET AL., 1998).

At the time there is no sufficient reference or published data which would indicate how ergosterol and vitamin D_2 concentration would change in cultivated mushroom, if they were treated with UV radiation not post-harvest, but before picking, still during their growing period. That is why in our study the aim was to irradiate mushrooms with UV light during cultivation, in pre-harvest stage, while they are still biologically active and still growing.

The subject of our experiment was the oyster mushroom (*Pleurotus ostreatus* (JACQ.) P. KUMM). UVB radiation in different time periods was applied to study the effect of UVB light and duration of irradiation on changes in vitamin D_2 level.

UV lamps are long time used for sterilizing surfaces and equipment in laboratories, as UV light is capable of significant inhibition of tissue growth. During the UV treatments not only the fruiting bodies got irradiated but the mycelia on the surface of the bags as well. That is why tissue cultures of the oyster mushroom were prepared for in vitro treatments in order to see, how UVB radiation affects mycelia growth. The same conditions (wavelengths, irradiation time and distance) were set up in the laboratory.

MATERIALS AND METHODS

The mushrooms studied in this experiment were cultivated in the test chamber of the Department of Vegetable and Mushroom Growing, Corvinus University of Budapest.

For the study 140 kg straw-based oyster mushroom substrate was prepared (inoculated with strain 'HK 35'). 14 bags were filled with 10 kg substrate, respectively.

Vilbert Lourmat-115M type UV lamps were set up (operating on 312 nm - in the 'B' range of UV light) above the bags, approx. 32 cm from the surface of the fruiting bodies.

During the entire growing period, in every development stage of the oyster mushroom, ideal ambient conditions were provided.

Six different irradiation time periods (5, 10, 15, 20, 25 and 30 minutes) were applied in 3 repeats (one treatment per day, for three consecutive days), this way in the end, the treated surfaces got 15 to 90 minutes irradiation.

The treatments of oyster mushroom were carried out in 2 repeats (2 bags with total 20 kg substrate). The UV irradiation went on from 4 days after the primordia appeared. 2 bags of untreated control were grown as well.

RESULTS AND DISCUSSION

The results of vitamin D_2 analysis of the oyster mushroom samples are given in $\mu g/g$ fresh weight and shown on *Figure 1*. Besides the accurate values, a curve demonstrates the trend of changes in vitamin D_2 level too (where the stated R^2 values represent the strength of correlation).

As *Figure 1*. shows, the vitamin D_2 level started increasing from 0.67 µg/g (measured in the untreated control sample) and continued rising all the way. The longest (90 min.) treatment proved to be the most efficient, as the vitamin D_2 level measured in those samples was 3.68 µg/g. Even the shortest treatment (15 min.) caused the vitamin D_2 level to grow by 14% (compared to the control), but after 90 minutes the growth was 450%.



Figure 1. Changes in vitamin D₂ content in oyster mushrooms after 0 to 90 minutes of UVB treatments

Figure 2. shows the results of the in vitro UV treatments. Due to data, UVB light causes substantial changes in mycelia growth. After three days of treatments, the treated tissue cultures grew only by an average 40-50%, while the untreated ones got almost twice as big (grew by 90%). No significant difference can be seen between each treatment, the rate of set back in growth was similar.



Figure 2. Size of the tissues cultures of oyster mushrooms after 0 to 90 minutes of UVB treatments

Besides measuring the changes of vitamin D_2 content, the appearance of the treated mushrooms was studied as well, because during *in vitro* UV treatments, a slight browning of the tissue cultures was observed. No coloration could be registered on the originally brownish-colored caps of oyster mushrooms. UVB radiation only affected the gills (turned their white into light-yellow) if they somehow were not covered by the caps and suffered direct radiation.

CONCLUSIONS

In the experiment pre-harvest oyster mushrooms were treated with UVB radiation for different time periods in order to increase the vitamin D_2 content of the fruiting bodies. Due to analysis of vitamin D_2 concentration of the mushroom samples, UV treatments of pre-harvest mushroom cultures have similar efficiency as those of post-harvest cultures. Pre-harvest UVB treatment of the oyster mushrooms resulted a maximum 450% growth in vitamin D_2 level.

The mycelial growth of the UV treated tissue cultures of the same cultivar were measured as well. Data shows, that UV light sets back the growth of the mycelia by around 40-50%.

In order to see, whether the inhibition of mycelial growth appears in the cultivation by lowering the yield of the UV treated mushroom culture, it is important to repeat the experiment with higher sample numbers, in a higher scale.

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