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EFFECT OF UV-B LIGHT AND SUNLIGHT EXPOSURE ON THE VITAMIN D_2 CONTENT OF BUTTON (*AGARICUS BISPORUS*) AND OYSTER (*PLEUROTUS OSTREATUS*) MUSHROOMS

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Mushrooms are found to have vitamin D_2 in an inactive form (*ergosterol*), which when exposed to UV light can be converted to its active form (*ergocalciferol*). This study had scrutinized the vitamin D_2 content of cultivated button (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus ostreatus*), treated post-harvest using UV-B (sample B) and sunlight (sample C) on exposing them to the respective treatments for 30 min each. The untreated mushrooms (sample A) of both varieties were used as a baseline comparator to the vitamin D_2 content in mushrooms exposed to sun and UV light. The mushrooms were exposed to sunlight between 11 a.m to 3.00 p.m, as it is the time when UVB radiation is at its peak. The vitamin D_2 content of the mushrooms were estimated using the liquid chromatography mass spectrometry. The UV light treatment caused an increase in the vitamin D_2 content of button mushroom from 0.41 to 7.41 µg/100 g and from 0.34 to 8.39 µg/100 g in oyster mushrooms. On exposure to sunlight, the vitamin D_2 content of button mushrooms was found to be 2.29 µg/100 g whereas that of the oyster mushrooms was found to be below the levels of quantification. Thus, UV light provides an effective method for increasing vitamin D_2 levels in button and oyster mushrooms.

Keywords: Button mushroom, Oyster mushroom, UV-B light, Vitamin D_2 content, Sunlight

INTRODUCTION

Vitamin D deficiency is fast becoming a pandemic affecting people across age groups, irrespective of the latitude they live in Palacios and Gonzalez (2014). Vitamin D, the prohormone (Holick, 2003), also known as calciferol, has two major forms: Vitamin D_2 and Vitamin D_3. Vitamin D is taken for granted and is assumed to be plentiful in our diet. Unfortunately, very few foods naturally contain Vitamin D, and only a few foods are fortified (Holick, 2004). Vitamin D_2 (ergocalciferol) is largely human-made and added to foods or is found in sources like mushrooms, yeast and fungi, whereas Vitamin D_3 (cholecalciferol) is synthesized in the skin of humans and is also consumed in the diet through the intake of animal-based foods (Holick, 2003; and Ross *et al.*, 2011). Even though Vitamin D production can be endogenous with sufficient skin exposure to solar ultraviolet beta (UV-B) radiation, this has become problematic due to wearing sunscreen, melanin, and aging, which interfere with solar exposure and can lead to Vitamin D deficiency (Koyyalamudi *et al.*, 2009 and Moyad, 2009). Therefore, recommendations now focus on exogenous dietary sources and are thus complementary to sun-safe practices (Weiler, 2015).

Functional foods can be considered to be those whole, fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients (e.g.,

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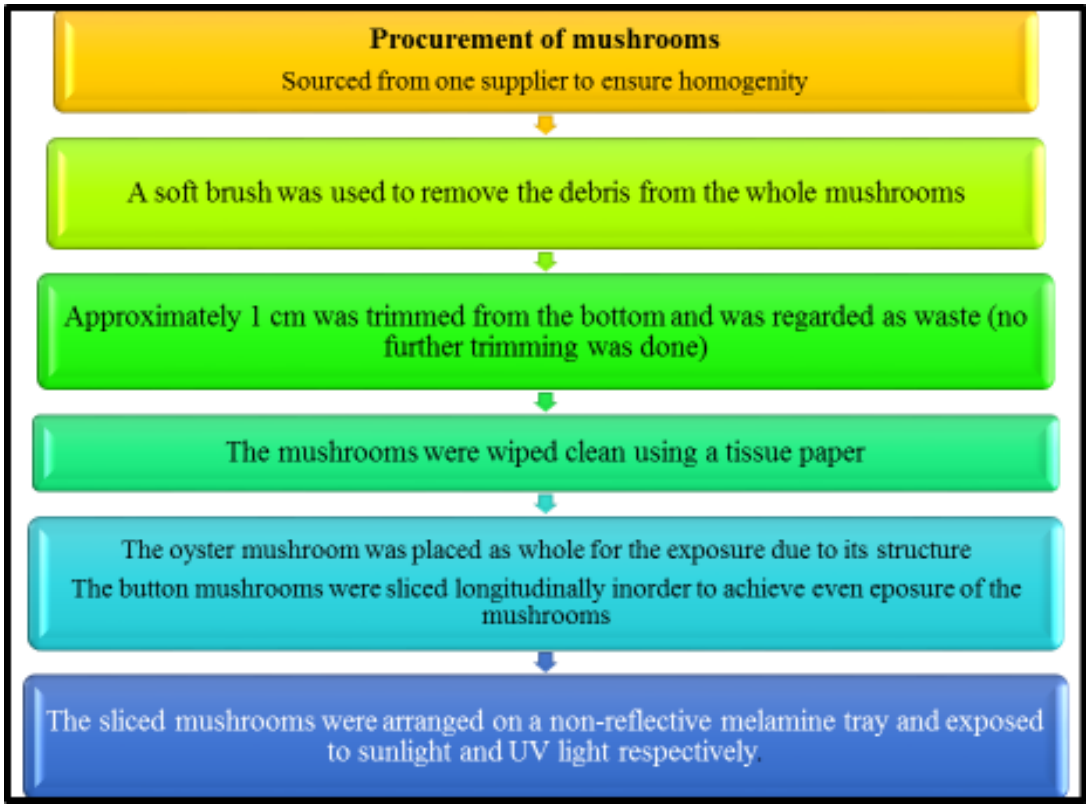
vitamins and minerals), when they are consumed at efficacious levels as part of a varied diet on a regular basis (Hasler, 2002). Mushrooms have become attractive as a functional food (Khatun *et al.*, 2012), as they are very rich in ergosterol, the principal sterol in fungi and on exposure to wavelengths <315 nm (UV-C and UV-B) from the sun (Urbain and Jakobsen, 2015) or artificial lighting (Jasinghe and Perera, 2006), this provitamin D_2 compound is converted to ergocalciferol (Vitamin D_2) in a process resembling the cutaneous synthesis of cholecalciferol (Vitamin D_3) in skin of humans (Cashman *et al.*, 2016). The nutritional value of edible mushrooms can be improved and it can be made into a more functional source of vitamin D_2 by the process of UV irradiation (Banlangsawan and Sanoamuang, 2016). Mushrooms exposed to sunlight or other sources of UV radiation are an excellent source of dietary Vitamin D_2 because they contain high concentrations of the Vitamin D precursor, provitamin D_2 (Keegan *et al.*, 2013). Therefore, the current study was aimed at identifying the most effective means of increasing the levels of vitamin D_2 present in both the mushroom varieties using UV light and sunlight.

MATERIALS AND METHODS

The mushrooms used for the study were identified and certified by a mycology specialist from the University of Madras. The mushrooms were pre-cleaned and prepared for exposure to UV light and sunlight. The procedure for this has been adapted from the work done by Phillips and Rasor (2013) as presented in Figure 1.

The mushrooms thus prepared were then subjected to exposure to UV light and sunlight. The type of UV radiation and storage of irradiated samples to prevent loss of vitamin D was based on the work by Mau *et al*. (1998) and Slawinska *et al*. (2017). The duration of exposure, was based on a study by Philips and Rasor (2013), reported an increase of 3.75 µg/70 g serving in the Vitamin D content of mushrooms on exposure to sunlight for 15 minutes. Wu and Ahn (2014), reported that the ideal duration of exposure to UV-B light to increase vitamin D content of mushrooms was from 40 to 120 minutes. Based on these studies, the current study fixed a midpoint value of 30 minutes as the duration of exposure.

Figure 1: Process for Pre-cleaning the Mushroom Varieties



The mushroom samples were exposed to UV-B light (artificial source) at a temperature of 35 °C and to sunlight (natural UV-B) at 36 °C. This was based on a study by Wu and Ahn (2014) where, mushrooms were exposed to UV B at an ambient temperature of 28° to 45 °C. A study by Harinarayan *et al*. (2013) demonstrated that on exposure to sunlight between the hours of 11 a.m. and 2 p.m. there was promotion in the levels of vitamin D production in the skin year round. On this basis the mushrooms were exposed to sunlight in the time frame of 11 a.m to 12 noon in the current study. The intensity of UV light was measured using the lux meter, UYIGAO-UA1010B, every time the experiments were carried out. The temperature of the environment was measured using a thermometer.

Artificial UV B Irradiation of Mushroom Slices

For the purpose of exposing the mushrooms to artificial UV light, a custom-made, portable, UV light cabinet was equipped with narrow band Philips UV-B light TL01, 20 W (290-320 nm). The tube used was a two feet long, single tube. The light source was placed at a distance of 30 centimetres from the mushrooms. The cabinet measured 71 x 33 x 51 cm. Before every exposure, the UV lamp was turned on and was allowed to warm for 10 minutes. The mushroom slices (button) or whole pieces (oyster) were arranged on a non-reflective melamine food tray (46.5 x 29 cm) which was positioned horizontal to the light source. After 15 minutes of exposure, the mushrooms were flipped onto the other side for 15 minutes to ensure equal exposure to UV-B light. The procedure for UV-B irradiation of the mushroom was as prescribed by Urbain *et al.* (2016). Immediately after the exposure the samples were packed into brown paper bags and refrigerated until further use.

Sunlight Exposure

The natural way of exposing mushrooms to UV-B light is by leaving it in sunlight. Mushrooms were exposed to sunlight between 11.00 am to 3.00 pm as it is the time when there was maximum amount of UV-B radiation (Balasaraswathy *et al*., 2002). The experiment was carried out in summer season (May to June, 2017). Care was taken to ensure the same location. Data regarding the intensity of UV-B light in Chennai was obtained from the Indian Meteorological Department, Pune, for sunlight exposure of the mushrooms. The mushroom slices or whole pieces were arranged onto to a non-reflective melamine food trays (46.5 x 29 cm) which was positioned horizontal to sunlight. After 15 minutes of exposure, the mushrooms were flipped onto the other side for 15 minutes to ensure equal exposure to UV-B radiation. Immediately after exposure the samples were packed into brown paper bags and refrigerated until further use.

Vitamin D Estimation

The vitamin D content in the mushrooms exposed to sunlight and UV light was evaluated using Liquid Chromatography Mass Spectrometry (LCMS) analysis in a certified laboratory.

RESULTS AND DISCUSSION

Effect of UV Light and Sunlight on the Vitamin D Content of Button and Oyster Mushrooms

Mushrooms are an unappreciated food source of vitamin D. This is because, the vitamin D content in mushrooms, are present in the form of ergosterol, which gets activated into vitamin D_2 only when it is exposed to natural (sunlight) or an artificial source of UV radiation. In this study, the vitamin D content of button and oyster mushrooms treated postharvest with UV-B light (sample B) was compared to mushrooms exposed to sunlight (sample C). The untreated mushrooms (sample A) of both varieties was used as a baseline comparator to observe the changes in the vitamin D content in the mushrooms. Details regarding the exposure of button and oyster mushrooms to UV light and sunlight have been presented in Table 1.

From Table 1, we can observe that the duration and intensity of exposure of the two varieties of mushrooms to UV light and sun light respectively were kept constant to ensure that these did not cause variability in the samples.

Studies have reported that UV-B (280 to 315 nm) was more effective in the formation of vitamin D_2 in mushrooms than exposure to UV-A or UV-C (Jasinghe and Perera, 2006; and Roberts, 2008). Besides the wavelength of UV irradiation, the efficiency of vitamin D_2 synthesis in mushrooms could be influenced by multiple parameters such as ambient temperature, exposure time, and irradiation intensity (Jasinghe *et al*., 2005; Jasinghe and Perera, 2006; Ko *et al*., 2008; and Koyyalamudi *et al*., 2009) and the effects of these factors may be either independent or interactive. Factors like time could be controlled, but the temperature of the environment and radiation intensity could not be controlled while carrying out the study, especially in case of the sunlight exposure of the mushrooms. The variations in the levels of vitamin D in the UV-B light exposed mushrooms could be because of the type of mushroom used.

Table 1: Intensity and Duration of Exposure of Mushrooms to UV Light and Sun Light

Type of Mushroom and Origin	Date of Exposure	Exposure Conditions	Time of Day	UV-B Source	Exposure Time (min.)	Temp. (°C)	Intensity of Light (lux)
Button, cultivated	31.05.2017	NA*	9.00 to 9.30 a.m.	UV B light	30	35	592x100
Oyster, cultivated	31.05.2017	NA*	2.08 to 2.41 p.m.	UV B light	30	35	592x100
Button, cultivated	23.05.2017	Clear	11.27 to 11.57 a.m.	Sunlight	30	36	1564x100 (4.23 MJm^{-2})
Oyster, cultivated	21.05.2017	Clear	11.13 to 11.43 a.m.	Sunlight	30	36	1493x100 (3.41 MJm^{-2})

Note: Not Applicable*.

Table 2: Vitamin D_2 Content of Button and Oyster Mushrooms Processed with UV Light

Parameter	Standard (No Treatment)		Treatment			
			UV-B		Sun	
	Button Mushroom	Oyster Mushroom	Button Mushroom	Oyster Mushroom	Button Mushroom	Oyster Mushroom
Vitamin D2 (µg/100 g)	0.41	0.34	7.41	8.39	2.29	BLQ
IU/84 g serving*	14	11	249	282	77	-
% of RDA**	2	2	42	47	13	-

Note: * One serving as stipulated by USFDA; ** RDA-15 µg (600 IU) for individuals aged 1-70 years; and *** BLQ - Below level of quantification.

Vitamin D Content of Mushrooms

The effect of UV light and sunlight on vitamin D content of button and oyster mushroom was as presented in Table 2. As shown in Table 2, the Vitamin D content of button and oyster mushroom on application of continuous UV-B light at an intensity of 592 x 100 lux was 7.41 µg Vitamin D_2/100 g and 8.39 µg Vitamin D_2/100 g, respectively, an increase of 18 fold and 25 fold respectively. Jasinghe *et al*. (2005) had reported that Vitamin D_2 concentration in cultivated mushroom can be enhanced up to nine fold by applying different UV irradiation methods, this study demonstrated a higher increase in the vitamin D_2 levels, which may be due to difference in cultivar and conditions of UV light exposure.

Phillips *et al*. (2011) reported a Vitamin D content ranging between 2.36 to 20.9 µg/100 g for button mushroom post UV treatment and between 0.07 to 2.59 µg/100 g for oyster mushroom. Roberts *et al*. (2008) reported a Vitamin D content of 7.98 µg/g in a lyophilized (powdered) button mushroom sample after about 10 minutes exposure to UV-B light. The variation in the levels may be attributed to the intensity, duration and orientation of the mushroom sample to the UV light source.

The button mushrooms were subjected to irradiation after slicing them, as Ko *et al*. (2008) found this to be a more efficient way of increasing the vitamin D_2 content than irradiating the gill or pileus of whole mushrooms, due to the larger exposure area. The oyster mushrooms were exposed as such (without slicing), because of its naturally large area of exposure (gills). This might be one of the reasons attributed to the increased vitamin D_2 content in oyster mushrooms. Phillips and Rasor (2013) had reported that the vitamin D_2 in all unexposed mushrooms was <30 IU/70 g.

On the basis of UV-B treatment, an 84 gram fresh serving of mushrooms (button and oyster) would provide, 249 (6.2

µg) IU and 282 (7.05 µg) IU of vitamin D_2, a quantity that is sufficient to meet half of the current RDA of 600 IU (15 µg). Several studies have concluded that exposure to UV-B offers an effective way of increasing the concentration of vitamin D_2 in mushrooms (Phillips *et al.*, 2011; Simon *et al.*, 2011; and Kristensen *et al.*, 2012).

On exposure to sunlight for 15 minutes, button mushroom was found to contain vitamin D_2 at levels of 2.29 µg/100 g compared to 0.41 in the standard (a six fold increase). The sunlight treated button mushroom was able to provide 77 IU (1.9 µg) of vitamin D_2/84 gram fresh serving. After just 15 minutes of exposure to sunlight, Urbain and Jakobsen (2015), observed that the vitamin D_2 content of button mushroom was found to increase significantly to 2.2 ± 0.5 µg/g Dry Weight (DW) from 0.1 µg/g DW, which is equivalent to 17.6 µg (704 IU) vitamin D_2 per 100 g of fresh mushrooms; comparable to levels found in fatty fish like the Atlantic salmon. The levels of vitamin D_2 in oyster mushroom was found to be below the level of quantification for the oyster mushroom. This difference in the levels of vitamin D_2 compared to our study due to the form in which the mushrooms were exposed and variation in the intensity of UV-B light. As this was a natural form of UV-B light, there could be no control over the intensity of light.

Wild sun-exposed mushrooms have a long history of safe consumption in the food supply. A comparison of the effects of sunlight on mushroom composition with those occurring in mushrooms processed using UV light found that other than the intended increase in vitamin D, no compositional changes of nutritional or toxicological significance was observed in the mushrooms (Simon *et al.*, 2011).

According to the food labelling guide FDA (2013), a food may be classified as 'rich in' or 'excellent' source of a nutrient if it contains ≥ 20 percent of the Daily Value (DV) and as a good source of nutrient if it contains 10 to 19 percent of the DV per Reference Amount Customarily Consumed (RACC). Going by this definition the UV light treated button and oyster mushroom may be considered an excellent source of vitamin D. The sunlight treated button mushroom may be considered a good source of vitamin D. However the normal, untreated button and oyster mushroom and sunlight treated oyster mushroom were not good sources of vitamin D.

CONCLUSION

Exposure to UV light and sunlight causes appreciable increase in the vitamin D_2. Vitamin D enriched button and oyster mushrooms with its array of nutrients and functional components can be incorporated into the daily diet to counteract vitamin D deficiency.

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