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UV irradiated mushrooms: Potential source of Vitamin D_2 review

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Abstract
Mushroom is a fruiting body of micro-organisms called fungi. They lack chlorophyll, absorbs nutrients from soil and decaying matter for their growth. Mushrooms are valuable nutritional foods with recognized bioactive properties, such as, antioxidant (ergothionine), bone growth and mineralization (Vitamin D), anti cancer (Lectins) and immunity boosting effect. Vitamin D also called as calciferol, which is a fat soluble vitamin, plays an important role in several human metabolic processes such as calcium and phosphorus metabolism, and skeletal and neuromuscular homeostasis. Nowadays studies on vitamin D have received considerable attention over the years supported by the increasing number of reports of vitamin D deficiency. Symptoms of vitamin D deficiency includes rickets and osteomalacia arising from poor calcium and phosphorus mineralization; but other diseases such as cardiovascular disease, cancer, hypertension, stroke, diabetes rheumatoid arthritis, inflammatory bowel disease, liver diseases, and mental illness have been also reported to be associated. The fruiting body of mushrooms, either in their fresh or processed forms, is rich in sterols, mainly Ergosterol, that can be converted into vitamin D_2 by UV-radiation. The amount of vitamin D varies among mushroom species, and also within the same species. Among them, mushrooms belonging to the genera Agaricus, Lentiula and Pleurotus have been reported to contain interesting amounts of vitamin D after exposure to UV. However, there is still gap considering the knowledge of the most appropriate irradiation procedures (dose, intensity, distance between source and sample, exposure time) in order to maximize the content of vitamin D_2 in the mushrooms. This strategy will enable vitamin D_2- enhanced mushrooms to be commercially available at affordable costs.

Keywords: Vitamin D_2 (Ergocalciferol), Mushroom, Ultraviolet Irradiation, Ergosterol

Introduction

Mushroom is derived from Latin and Greek words "*Fungus*" and "*Mykes*". Mushroom is a fruiting body of micro-organisms called fungi. They lack chlorophyll, absorbs nutrients from soil for their growth. There are more than 30,000 identified types of mushrooms worldwide, 99% of these are safely edible and roughly 1% is poison, Mushrooms contain a good amount of vitamin D (Das *et al*., 2022) [6]. Table 1 indicates the botanical classification of mushrooms, they belongs to the Kingdom Fungi, Genus *Agaricus* and Species *bisporous*. The examples for edible mushrooms are white button mushrooms, paddy straw mushrooms, oyter mushrooms, shiiteke mushrooms, milky mushrooms, chanterelle mushrooms etc. And examples for poisonous mushrooms are yellow staining mushrooms, sunshine amanita, cookers amanita etc. Poisonous mushrooms looks like edible mushrooms in their morphology and life cycle. They can be identified by some unique characteristics i.e. the poisonous mushrooms have, i.e. they have bright color fruit bodies, unpleasant odour, greenish tinge and yellow green spores in their gills and they ooze milky or coloured latex from damaged portion.

Table 1: Botanical classification of mushrooms:

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agaricomycetes
Order	Agricales
Family	Agaricaceae
Genus	Agaricus
Species	A. bisporus

Mushrooms are most commonly found in dark, moist and humid temperature. Mostly they are found on the trees, beneath the trees, on soil. Wild mushrooms are commonly found in rainy seasons. Mushroom with their great variety of species, constitute a cost-effective means of both supplementing the nutrition to human kinds. 4-5 species of mushroom are of industrial significance throughout the world (Chang and Miles, 2004) [5]. In India, only 3 species, namely, *Agaricus bisporus*, *Pleurotus sajorcaju* and *Volveriell* are preferred for commercial cultivation. Mushroom is commercialized in almost all parts of the world. Production of shiitake, oyster mushroom, wood ear mushroom and Flammulina and other new mushroom species, particularly in East Asian countries like Japan, South Korea, Taiwan, China, etc. the contribution of these mushrooms to total world mushroom production has increased tremendously as compared to button mushroom which is no more the number one mushroom in terms of share in global mushroom production. Total production of mushroom in world is 42.8 million metric tons in which Japan is the largest producer of mushroom in the world followed by Korea and China (FAOSTAT). India contributes 258.81 in mushroom production in 2021-22 in which Bihar is a leading producer of mushroom (28 tonnes) followed by Maharashtra (25.60 tonnes) and Orissa (25.00 tonnes) and least producer of mushroom in India is Sikkim with 0.02 tonnes (APEDA).

Health Benefits of Mushrooms

Mushroom consumption has been reported to present interesting health promoting benefits such as antioxidant, antitumor, antimicrobial, anti-inflammatory, antityrosinase, immunomodulator, antiatherogenic and hypoglycemic activities (Alves *et al*., 2013; Carocho & Ferreira, 2013; Taofiq *et al*. 2015; Taofiq *et al*. 2016) [1, 4, 26, 28].

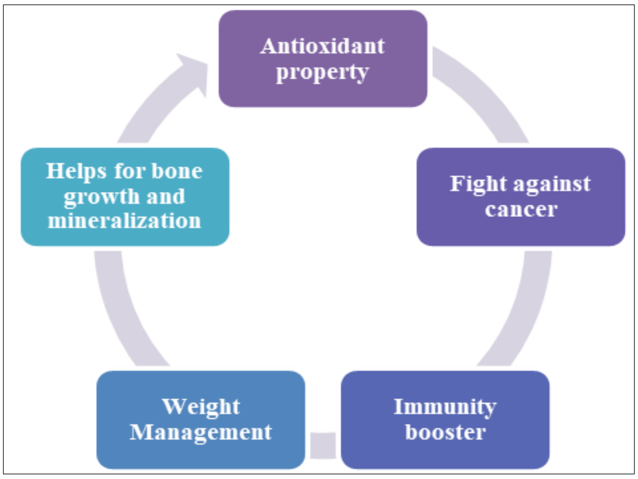

Fig 1: Health benefits of mushroom consumption

1. **Antioxidant property:** Antioxidants are the substances that can prevent or slows damage to cells caused by free radicals; mushrooms contain a powerful antioxidant called **ergothionine** which helps to lowers inflammation throughout the body.
2. **Helps for bone growth and mineralization:** Mushroom are rich source of vitamin D helpful for the bone mineralization and development.
3. **Fight against cancer:** It contains a specialized lectins that recognize cancer cells and have been found to prevent cancer cells from growing and dividing.
4. **Weight Management:** Mushrooms are helps for weight management due to the present of low fatconten Help to lower cholesterol and cardiovascular diseases- In general mushrooms are fat free and also good source of chitin (fibres) that lowers the cholesterol. Commonly present in all mushrooms and helps to reduces total cholesterol and LDL (bad cholesterol)
5. **Immunity booster:** Lentinan is a type of sugar molecule called 1, 3 beta glucan which helps for the immunity booster.

Vitamin D

Vitamin D also called as calciferol, which is a fat soluble vitamin, plays an important role in several human metabolic processes such as calcium and phosphorus metabolism, and skeletal and neuromuscular homeostasis. Vitamin D deficiency is pandemic, yet it is the most under-diagnosed and under-treated nutritional deficiency in the world (Van Schoor and Lips, 2011; Mithal *et al*., 2009; Van der Meer *et al*., 2009) [33, 18, 32]. Vitamin D deficiency is widespread in individuals irrespective of their age, gender, race and geography. Vitamin D is photosynthesized in the skin on exposure to UVB rays. Sun exposure alone ought to suffice for vitamin D sufficiency. However, vitamin D deficiency is widely prevalent despite plentiful sunshine even in tropical countries like India. There are many causes for vitamin D deficiency which includes living at high latitude, being indoors too much, using large quantities of sunscreen, overweight, kidney and liver disease, problem in digestive tract, having dark skin etc.

Vitamin D deficiency has a bearing not only on skeletal but also on extraskeletal diseases. Owing to its multifarious implications on health, the epidemic of vitamin D deficiency in India is likely to significantly contribute to the enormous burden on the healthcare system of India. Cultural and social taboos often dictate lifestyle patterns such as clothing-that may limit sun exposure and vegetarianism-which certainly limits vitamin D rich dietary options. Most Indians are vegetarians. The socioeconomically backward people constitute a large percentage of the population in India. The underprivileged generally suffer from overall poor nutrition. Vitamin D rich dietary sources are limited and unaffordable to most Indians. Vitamin D supplements are available, but most Indians are not aware that they need additional vitamin D. Additionally, the cost of these supplements is essentially prohibitive to the majority. Fortification of staple foods with vitamin D may prove to be a more viable solution towards attaining vitamin D sufficiency in India.

Types of vitamin D

- Vitamin D_2 (Ergocalciferol) – which is synthesized by plants and is not produced by the human body. The side chain of D_2 contains a double bond between carbons 22 and 23, and a methyl group on carbon 24.
- Vitamin D_3 (Cholecalciferol) – which is made in large quantities in the skin when sunlight strikes bare skin. It can also be ingested from animal sources.

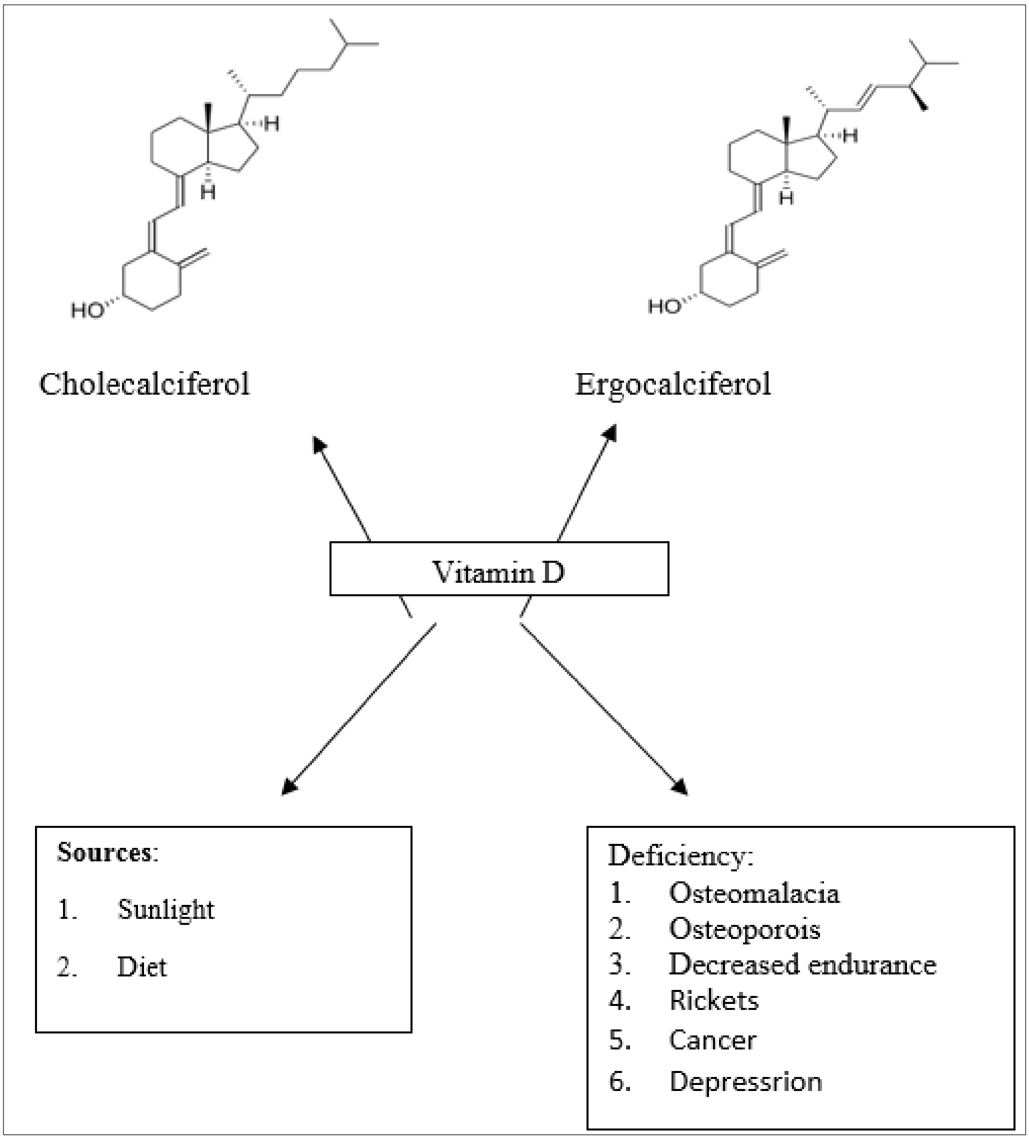

Fig 2: Variation in vitamin D, sources of vitamin D and deficiency symptoms

Metabolism of vitamin D

Both forms of vitamin D have shown to be responsible for maintaining serum levels of 25-hydroxyvitamin D in humans (Koyyalamudi *et al*., 2011) [11]. Vitamin D from sunlight, or dietary sources, is biologically inactive and undergoes two-step hydroxylationto form 25-hydroxyvitamin D (calcidiol) and metabolically active 1,25dihydroxyvitamin D (calcitriol) (Harika & Eilander, 2013) [9]. After vitamin D intake, it enters blood circulation, being transported to the liver where it becomes hydroxylated to form 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D. In the kidney, a second hydroxylation of the 25-hydroxyvitamin D occurs, resulting in the formation of 1,25-dihydroxyvitamin D, which is the most potent form of vitamin D. In fact, most of the physiological effects of vitamin D in the body are related to the activity of 1,25-dihydroxyvitamin D (Harika & Eilander, 2013) [9]. Even the contribution of food ingestion to vitamin D levels is relatively not significant, some important dietary sources of vitamin D are fish, beef liver, cod liver oil, egg yolks; additionally, mushrooms exposed to sunlight have shown to be rich in vitamin D_2 (Guan *et al*., 2016) [7].

UV radiation of Mushrooms

Electromagnetic radiation comes from the comes from different sources such as sun and transmitted in waves or particles at different wavelengths and frequencies, this broad range of wavelength is known as electromagnetic (EM) spectrum. The spectrum is generally divided into seven regions in the order of decreasing wavelength and increasing energy and frequency. Ultraviolet (UV) is an electromagnetic radiation with a wavelength ranging from 10 nm to 400 nm. This UV radiation is again sub classified as UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (180–280 nm) (Taofiq *et al*., 2017; Cardwell *et al*., 2018) [27, 3].

Numerous studies have reported the potential of using UV-treated mushrooms as an alternative source of vitamin D, with wild species presenting higher vitamin D levels when compared with cultivated ones (Kohn, 2016). Mushrooms contain very low levels of vitamin D, but following exposure to UV radiation, ergosterol undergoes series of ring rearrangement forming previtamin D and, lastly, the active form of Vitamin D_2 (Sapozhnikova *et al*., 2014; Slawinska *et al*., 2016) [23, 25]. The kinetics of the photo conversion of ergosterol into vitamin D_2 in mushrooms has not been fully understood. The factors such as temperature, moisture content, UV radiation type, different irradiation parts and irradiation dose are described to influence vitamin D yield (Jasinghe *et al*. 2007) [11].

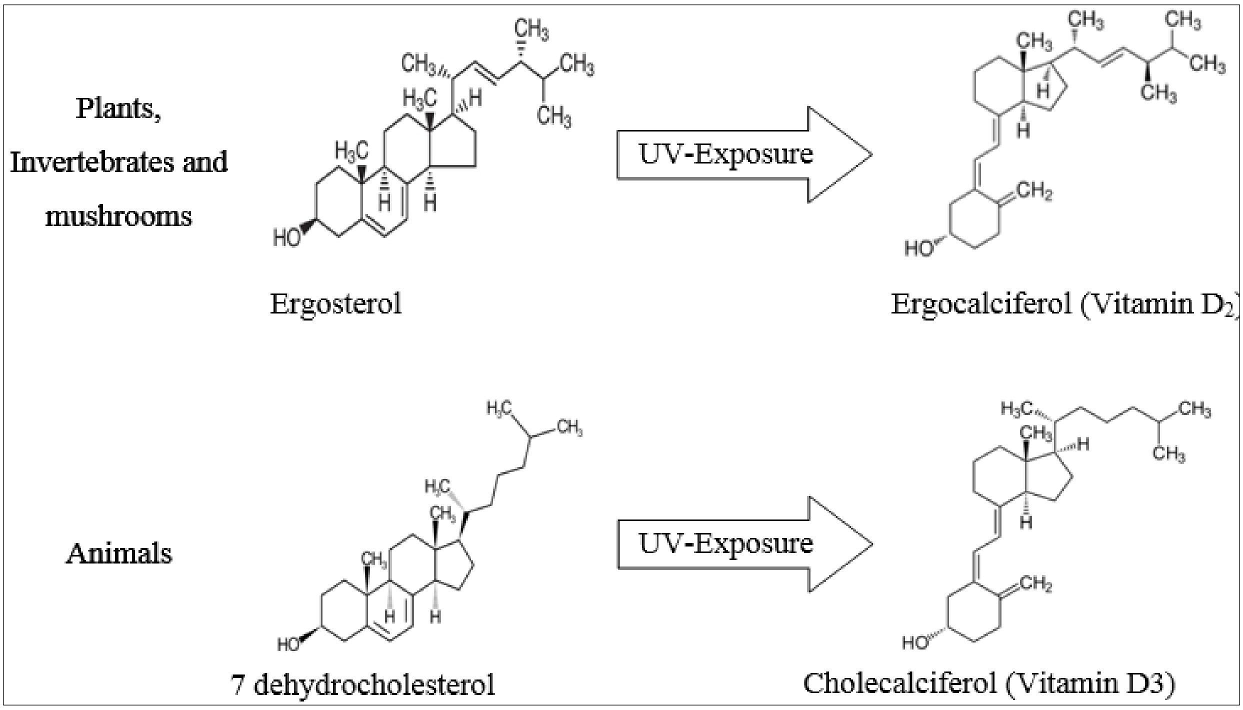

Fig 3: Synthesis of Vitamin D from different precursors present in Plants, Mushrooms and Animals

When fresh button mushrooms are deliberately exposed to midday sunlight for 15–120 min, they generate significant amounts of vitamin D_2, usually in excess of 10 µg/100 g FW (Kristensen *et al*., 2012; Simon *et al*., 2011; Phillips and Rasor 2013; Urbain and Jakobsen 2015) [16, 24, 19, 30], which approaches the daily requirement of vitamin D recommended in many countries. However, the amount of vitamin D_2 generated depends on the time of day, season, latitude, weather conditions, and exposure time. Since these mushrooms have a higher surface area to volume (hence, more ergosterol is exposed), sun-exposed sliced mushrooms produce more vitamin D_2 than whole mushrooms from the same amount of UV radiation exposure (Urbain *et al*., 2016; Phillips and Rasor 2013; Urbain and Jakobsen 2015) [31, 19, 30]. An efficient way to produce nutritionally relevant amounts of vitamin D_2 is to expose mushrooms to specific, controlled levels of UV radiation via a fluorescent UV lamp or a pulsed UV lamp. Mushrooms will generate vitamin D_2 in response to exposure to UV radiation both during growing phase and post-harvest; however, commercial growers use UV lamps post-harvest for practical reasons. Fresh mushrooms, when deliberately exposed to a UV radiation source post-harvest, will generate significant amounts of vitamin D_2 (Urbain *et al*., 2016; Phillips and Rasor 2013; Urbain and Jakobsen 2015; Mau *et al*., 1998; Koyyalamudi *et al*., 2009) [31, 19, 30, 17, 15].

Effect of UV radiation type on vitamin D yield

Jasinghe and Perera (2006) [10] studied irradiation of fresh shiitake mushrooms (*Lentinula edodes)* Oyster mushroom (*Pleurotus ostreatus*), Botton mushrooms (*Agaricus bisporus*), and Abalone mushroom (*Pleurotus cystidus*) were irradiated with Ultraviolet-A (UV-A; wavelength 315–400 nm), Ultraviolet-B (UV-B; wavelength 290–315 nm), and Ultraviolet-C (UV-C; wavelength 190–290 nm). The conversions of ergosterol to vitamin D_2 under UV-A, UV-B, and UV-C were shown to be significantly different (p< 0.01). The highest vitamin D_2 content (184 ± 5.71 µg/g DM) was observed in Oyster mushrooms irradiated with UV-B at 35 °C and around 80% moisture. Similarly, Wu and Ahn (2014) [34] reported that that UV-B was the most suitable irradiation source for vitamin D_2 synthesis in cultivated oyster mushrooms when fresh oyster mushrooms were subjected to different sources of irradiation with UV-A, UV-B, UV-C, UV-A & B, UV-A & C, UV-B & C, or UV-A & B & C lights, respectively. Simon *et al.* (2011) [24] reported that the level of vitamin D_2 present in A. biporus exposed to UVB radiation at a dose of 1.08 J/cm2 increased significantly, namely from 5.5 µg/100 g dw to 410.9 µg/ 100 g dw), which represents an increase of 747%. Other authors have reported increases after exposure to UVB of 0.1 µg/g dw to 3.98 µg/g dw (Urbain & Jakobsen, 2015) [30], 0.0003 µg/g dw to 157 µg/g dw (Bilbao-Sainz *et al*., 2017) [2] and 0.01 µg/g dw to 7.28 µg/g dw (Roberts *et al*., 2008) [20]. Whereas, Teichmann *et al*. (2005) [29] investigated irradiation of raw and processed mushroom samples including wild grown (chanterelles and king bolete) and cultivated samples (white and brown button, portabella, shiitake, oyster). They have reported that irradiation with UV light in the A region (366 nm) only slightly affected ergosterol and vitamin D_2 content. In contrast, irradiation with UV light conducted in the C region (254 nm, 0–2 h, 20 cm distance) for fresh white button mushrooms and freeze-dried chanterelles resulted in nonsignificant decrease in ergosterol content, whereas vitamin D_2 increased up to 9-fold (*Cantharellus tubaeformis*) and 14-fold (A. bisporus/white), respectively. Agaricus bisporus var. Portobello treated with UVC showed a slight increase in vitamin D_2 content i.e. from 430 µg/100 g dw to 950 µg/100 g dw (Guan *et al*., 2016) [7].

Effect of temperature

The effect of temperature on vitamin D_2 production could be demonstrated by study conducted by Wu *et al.* (2014), which showed that an increase in temperature from 15ºC to 35ºC increases the production of vitamin D_2 from 152 µg/g to 178 µg/g on dry weight basis. Likewise, findings from study carried out by Jasinghe *et al.* (2005) suggest that the optimal translation of ergosterol to vitamin D_2 in shiitake mushrooms happen at 35 °C and 78% moisture, producing 50 µg/g vitamin D_2 on dry weight basis. On the other hand, Salemi *et al*. (2021) [22] reported that the optimal temperature for the generation of 3.81 µg gr^{-1} of vitamin D per kg was determined to be 27 ºC when it is compared with varied temperatures (27 ºC, 35 ºC, and 43 ºC). An investigation done by An investigation done by Zhang *et al*. (2015) [35] the determination of the optimum conditions for the conversion of ergosterol to vitamin D_2 in shitake mushrooms (Lentinula edodes) was studied using response surface methodology (RSM). The effects of the three main variables ambient temperature (20, 30, 40, 50o C), exposure time (30, 60, 90, 120, 150 min), and irradiation intensity (0.3, 0.6, 0.9, 1.2, 1.5 W/m2) were investigated. According to the RSM ridge analysis, the optimum conditions were as follows: ambient temperature of 34.2o C, exposure time of 175.6 min, and irradiation intensity of 1.41 W/m2. Under these optimum conditions, the maximum vitamin D_2 content of 117.93 µg/g in shitake mushrooms was obtained, which agreed fairly well with the predicted value of 122.60 µg/g.

Effect of exposure time on vitamin D yield

Considering the variable irradiation time, contradicting reports have been published suggesting that longer times did not increase vitamin D_2 levels (Ruslan *et al*., 2011) [21]. Szabó and Győrfi (2012) also reported that shorter irradiation time gave higher vitamin D_2 levels in pre-harvest A. bisporus after UV treatment. According to Jasinghe *et al.* (2005) nutritionally significant concentration of vitamin D_2 (10 µg/100 g on fresh weight) in mushroom can also be efficiently attained with a pulsed UV lamp within a very small time period of 1-2 sec. In comparison, it might take several minutes for a UV fluorescent lamp to produce the same amount of vitamin D_2. Pulsed UV may thus be the most effective way to raise the levels of vitamin D_2 in mushrooms. Three pulses of UV light for 1 sec produced 11.9 and nine pulses for 3 sec produced 20 µg D_2/g DM in button mushrooms, respectively. After 12 pulses (4 sec), the maximal amount of vitamin D_2 (27 µg/g DM) was obtained (Kalaras *et al*., 2012a) [12, 13]. Furthermore, Salemi *et al*. (2020) [22] observed that irradiating mushrooms with UV-B at varied time (15 to 120 min) and temperature (27 ºC, 35 ºC, and 43 ºC) at an intensity of 3.5 W m^{-2} had an effect on production of vitamin D_2 from mushrooms. The results demonstrate that extended exposure times up to 90 min gave the highest production of vitamin D_2, while increasing the exposure time up to 120 min decreased the production from 5.10 to 3.60 µg gr^{-1} after 120 minutes of exposure. As mentioned earlier the study conducted by Zhang *et al*. (2015) [35] the exposure time varied (30, 60, 90, 120, 150 min) shown that at ambient temperature of 34.2 ºC, exposure time of 175.6 min, and irradiation intensity of 1.41 W/m2 the yield of vitamin D_2 is higher (117.93 µg/g).

Conclusion

Since mushrooms provide nutritionally relevant amounts of B group vitamins and of the minerals selenium, potassium, copper, and zinc, they are a nutritious, low energy-dense food. Currently, some larger commercial mushroom are expose fresh mushrooms to UV radiation. Exposing dried mushrooms to UV-B radiation can also generate nutritionally useful amounts of vitamin D2, although this practice is not widespread to date. It is conceivable that UV-B radiation post-harvest (for fresh mushrooms) or post-drying (for dried and powdered mushrooms) could become standard commercial practice. Sunlight, regular UV lamps, and pulsed UV lamps have the capability to raise the vitamin D2 concentrations to nutritional significance, although pulsed UV lamps may be the most cost-efficient method for commercial production of vitamin D-enhanced mushrooms, because of the low exposure time (often in 1–3 seconds) to achieve good amount of vitamin D. Vitamin D-enhanced mushrooms contain high concentrations of vitamin D2, which is bioavailable and relatively stable during storage and cooking. Therefore, consumption of vitamin D-enhanced mushrooms could substantially contribute to alleviating the global public health issue of vitamin D deficiency. The factors such as temperature, moisture content, UV radiation type, different irradiation parts and irradiation dose are also influences the yield of vitamin D2. Further researches are needed to optimize the level of UV radiation required, temperature and other conditions to produce a good amount of vitamin D2 in mushrooms, along with optimal storage conditions.

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