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Isabel Ferreira

Ângela Fernandes

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Review

UV-irradiated mushrooms as a source of vitamin D_2: A review

Oludemi Taofiq[a,b,c], Ângela Fernandes[a,b], Lillian Barros[a], Maria Filomena Barreiro[b], Isabel C.F.R. Ferreira[a,*]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Bragança Polytechnic Institute, 5301-857 Bragança, Portugal
[c] GIP- USAL, Unidad de Nutrición y Bromatología, Faculty of Pharmacy, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain

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ABSTRACT

Background: The deficiency of vitamin D has been widely reported all over the world and linked to several chronic diseases. Mushrooms are valuable nutritional foods with recognized bioactive properties, leading the application of UV irradiation to the production of significant amounts of vitamin D_2. In this context, cultivated species such as *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* have been widely studied.

Scope and approach: 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. Considering the interest and potential of application, this review mentioned some of the physiological roles and sources of vitamin D, while the major focus was on mushroom's UV irradiation as a source of vitamin D_2. Also, topics related to its bioavailability and clinical studies evidencing the health benefits reported so far were also addressed.

Key findings and conclusions: UV-irradiated mushrooms present a high rate of conversion from ergosterol to vitamin D_2 at short treatment time and have the potential to increase serum 25-hydroxyvitamin D levels. Even though irradiated mushrooms exhibit some promising advantages, there is still a huge knowledge gap to allow for extraction, separation, recovery and purification of vitamin D_2 from irradiated mushroom at minimal process cost and high purity percentage to be utilized as bio-based ingredient to reduce vitamin D deficiency as well as present other health promoting benefits.

1. Introduction

Vitamin D, popularly referred to as ˝sunshine vitamin˝, plays an important role in several human metabolic processes such as calcium and phosphorus metabolism, and skeletal and neuromuscular homeostasis. It is mainly obtained endogenously after UV exposure, from dietary supplements and food sources (Elangovan, Chahal, & Gunton, 2017). The most well reported symptoms of vitamin D deficiency are rickets and osteomalacia arising from poor calcium and phosphorus mineralization; but other diseases such as cardiovascular disease, cancer, hypertension, stroke, diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, periodontal disease, muscular degeneration, liver diseases, mental illness, and chronic pain have been also reported to be associated with the lack of vitamin D (Kalaras, Beelman, & Elias, 2012a). In this context, studies on vitamin D have received considerable attention over the years supported by the increasing number of reports of vitamin D deficiency, now prevalent in Europe, Middle East and North America.

There are various forms of this vitamin, but the most physiologically relevant ones are vitamin D_3 or cholecalciferol, which is the most biologically active form found in animals and humans produced after skin exposure to UVB radiation, and vitamin D_2 (ergocalciferol) found in some phytoplankton, invertebrates, yeast and mushrooms in response to UV radiation (Chen et al., 2015; Malaeb, Hallit, & Salameh, 2017). Populations from countries with temperate climate ($> 30°N$ and $> 30°S$), i.e. regions where exposure to sunlight is limited especially during winter season, are subjected to little or no synthesis of vitamin D_3, and as such, dietary intake in the form of supplements or fortified foods is needed (Schoenmakers, Gousias, Jones, & Prentice, 2016).

Both forms of vitamin D have shown to be responsible for maintaining serum levels of 25-hydroxyvitamin D in humans (Koyyalamudi, Jeong, Pang, Teal, & Biggs, 2011). Vitamin D from sunlight, or dietary sources, is biologically inactive and undergoes two-step hydroxylation

* Corresponding author.
E-mail address: iferreira@ipb.pt (I.C.F.R. Ferreira).

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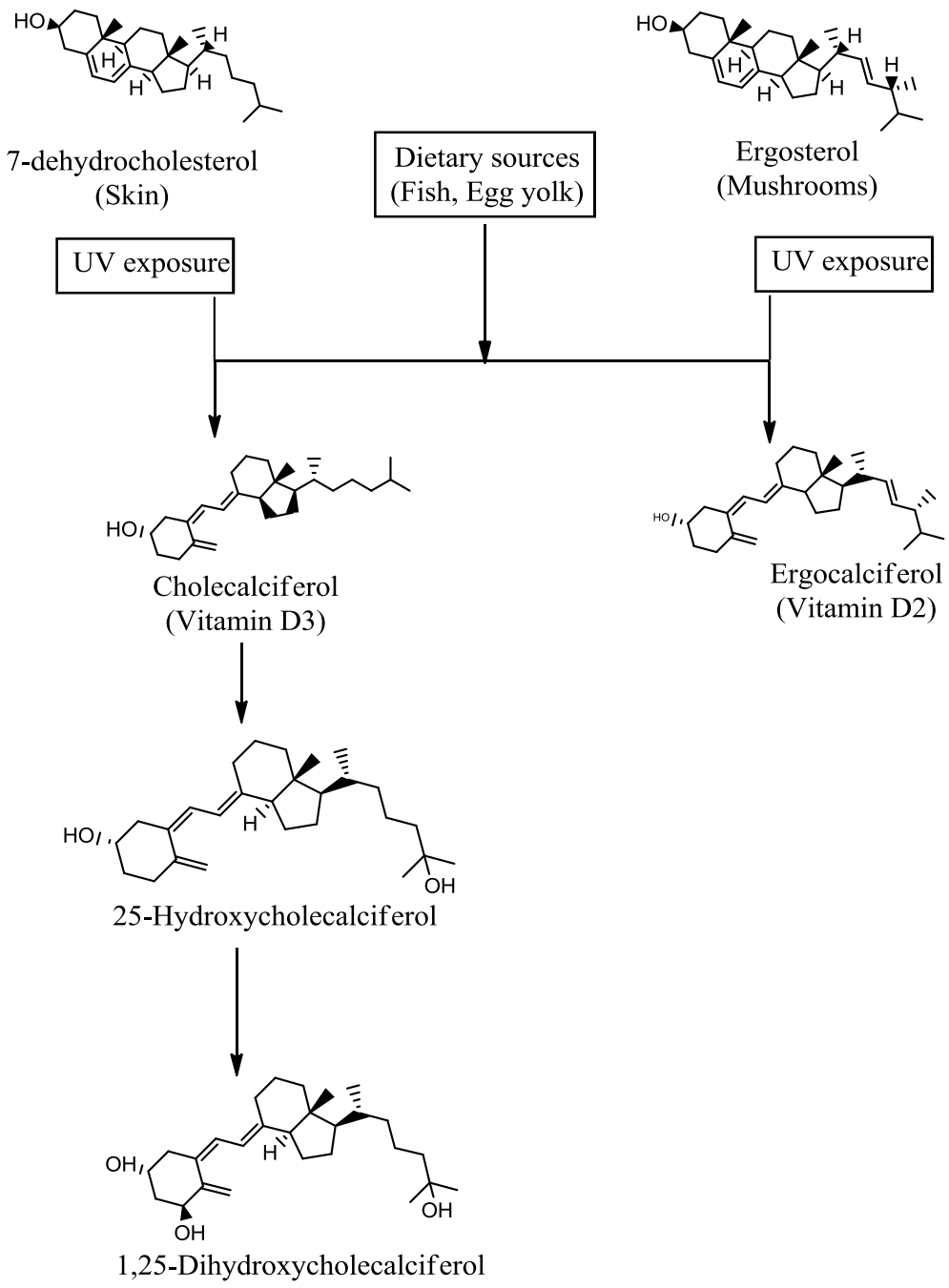

Fig. 1. Chemistry of vitamin d (adapted from Vuolo, Di Somma, Faggiano, & Colao, 2012).

to form 25-hydroxyvitamin D (calcidiol) and metabolically active 1,25-dihydroxyvitamin D (calcitriol), as shown in Fig. 1 (Harika & Eilander, 2013).

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).

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; Itkonen et al., 2016; Kim & Bae, 2016).

Facing to the described importance of vitamin D, and identified health problems related to its deficiency, the present review will address topics such as vitamin D and its main roles, main sources and strategies for their production. An emphasis will put on mushroom UV-irradiation as a promising technique for vitamin D obtainment.

2. Vitamin D and its main roles

Vitamin D deficiency is still an unrecognized epidemic, especially among the elderly people. Nevertheless, some reported data are noteworthy, namely the one concerning United States (US), where over 50 % of elderly people are lacking from vitamin D (Jasinghe, Perera, & Sablani, 2007). In general, vitamin D deficiency is mostly prevalent in the Middle East and South Asia, while low levels have also been described across the USA and Canada. Individuals living in some European countries located at higher latitudes experience low 25(OH)D

serum concentrations due to reduced UV exposure especially during winter months. In the south of Europe, severe hypovitaminosis D have been reported among elderly people, even though these countries presented lower latitudes and increased UV exposure (O'Mahony, Stepien, Gibney, Nugent, & Brennan, 2011).

Among others, vitamin D has impact on bone and muscle health, cancer disease, cardiovascular diseases, liver function, atopic dermatitis, obesity, depression and diabetes.

2.1. Bone and muscle health

The most significant role of vitamin D has been related to musculoskeletal health where deficiency is known to cause rickets in children and osteomalacia in adults, both characterized by aching muscles and bones (Tomlinson, Joseph, & Angioi, 2015). The role of vitamin D as a nutritional factor responsible for promoting bone formation and mineralization is due to its potential to stimulate osteoblasts cells and inhibit osteoclast responsible for bone resorption. Several controlled trials have shown a positive dose-response correlation between vitamin D supplementation and prevention of fracture upon fall, mainly attributed to the potential of vitamin D to provide adequate levels of calcium and phosphate (Bikle, 2014). Several signs and symptoms of bone disease, namely, growth retardation, muscle weakness, skeletal deformities, stunted growth and bowed legs have been associated with vitamin D deficiency, being the most common bone pathologies affecting Europe, the US and Nordic countries (Keegan, Lu, Bogusz, Williams, & Holick, 2013).

2.2. Cancer disease

Several studies reported in the past, have linked sun exposure and vitamin D levels to cancer disease, both prevention and prevalence. In fact, cancer mortality appeared to be significantly reduced among farmers and individuals working in agricultural businesses characterized by a constant exposure to sunlight. Even though there is a high chance to develop nonmelanoma skin cancer, a less dangerous and easy to treat disease, sun exposure also boosts the immune system against some cancer forms such as breast, colon and prostate cancers. Additionally, years of research have found a direct relationship between exposure to solar UVB radiation and premature death among Americans and British (Grant, 2009; Lim et al., 2006).

Inferior rates of prostate and ovarian cancer incidence have been linked to inhabitation at lower latitudes, while some reports have shown that cancer therapy applied in summer season resulted in better survival rates, comparatively with the ones undergone in winter, fact mainly attributed to seasonal issues, among them vitamin D levels (Tang et al., 2012).

The mechanism behind the observed antitumor properties involves activation of some specific kinases by vitamin D that tend to keep cell cycle in G1/S phase, prevent DNA synthesis and avoid growth of the malignant cells (Holick, 2014). One major limitation that hinders vitamin D and its analogues to be effectively used to treat cancer is their potential to cause calcemic toxicity (Mattila, Valkonen, & Valaja, 2011). Even though certain strategies are being utilized by these cells to produce vitamin D destructive enzymes, unconfirmed reports have suggested that improving vitamin D levels, by the intake of either vitamin D rich or fortified foods, may help to improve chemotherapy and reduce the risk of malignancy.

2.3. Cardiovascular diseases

Vitamin D has attracted recent attention due to their cardioprotective functions in several cardiovascular diseases (CVD). The mechanism behind some of its cardioprotective roles involves promoting expression of vascular endothelial growth factor (VEGF) in the endothelial cells lining the heart valves, which activates several cell signal transduction pathways responsible for endothelial cell proliferation, cell survival, migration and vascular permeability. Some studies have reported that vitamin D supplementation can serve as an adjuvant therapy to reduce serum total cholesterol levels, thereby contributing to a better cardiovascular function (Qin, Zhao, Chen, Yin, & Wang, 2015; Skaaby et al., 2012).

Vitamin D plays a major role in suppressing Rheumatic Heart Disease (RHD), the major cause of cardiac related deaths (Sarkar, Chopra, Rohit, Banerjee, & Chakraborti, 2016). The mechanism through which vitamin D helps to suppress the severity of cardiovascular diseases involves reducing the expression of genes responsible for renin production, thus down regulating the renin-angiotensin system (RAS) that primarily leads to increased blood pressure (Papandreou & Hamid, 2015).

2.4. Liver function

The role of vitamin D in preventing liver diseases is associated with its potential to down regulate several signal transduction pathways, which allows for expression of interferons, chemokines and pro-inflammatory genes such as TNF-α, IL-4, IL-6 and the toll-like receptors, reducing the risk and providing protection against hepatitis B, liver inflammation, liver cirrhosis and hepatocellular carcinoma (HCC) (Elangovan et al., 2017).

The liver has regenerative capability even when facing an acute or chronic injury; however, the mechanism of regeneration may become powerless leading to the development of acute liver failure, cirrhosis, hepatic failure and/or HCC. Vitamin D and vitamin D receptor (VDR), in liver, play a significant role ensuring that the organ is apt to perform its functions. Even though the anti-inflammatory and immune-modulatory properties of vitamin D are the mechanisms behind its hepatoprotective properties, further studies are needed to fully understand the mechanism behind vitamin D synthesis, activity and bioavailability (Kitson & Roberts, 2012).

2.5. Atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory disorder characterized, mainly, by dry and itchy skin. The physiological mechanism conducting to this status, is still not fully understood, but has been associated with environmental and dietary factors that may cause over expression of inflammatory mediators, such as nitric oxide (NO), TNF-α, interleukins and irritants, causing redness, pain and edema.

Studies have reported that deficiency of vitamin D is involved in AD development. Additionally to nutritional supplementation with to vitamin D, omega-3 fatty acids, and vitamin E are recognized as beneficial in reducing the symptoms and severity of AD (Kim & Bae, 2016). The reduction on AD severity has also been linked to the potential of vitamin D to induce expression of antimicrobial peptides like cathelicidin, filaggrin and β-defensin, which prevent skin infection and also support the barrier properties of the *Stratum corneum* (Kim & Bae, 2016; Reinholz, Ruzicka, & Schauber, 2012).

2.6. Obesity, depression and diabetes

Epidemiological studies have shown a close co-existence of vitamin D deficiency with the occurrence of obesity and diabetes. Studies have also pointed out the chance of low vitamin D levels in obese individuals due to their low appetence to participate in outdoor activities. Whether food fortification with vitamin D could help reducing the tendency to develop obesity and diabetes, this is still not fully established (Sadiya et al., 2016).

The presence of vitamin D receptors in other body tissues aside from the bone, and linked with other pathological conditions, is now being studied. Some clinical studies conducted in individuals with pre-diabetes, showed that a vitamin D supplemented diet improves and

prevents the development of full blown diabetes (Bikle, 2014).

Studies have also reported a link between some genetic factors and diabetes (Papandreou & Hamid, 2015). Nutritional and dietary products such as omega-3 fatty acids, vitamin D and vitamin B have been used as a natural supplement with antidepressant effects. Even though the mechanism behind this action is still unknown, some studies have shown that vitamin D receptors are responsible for activating some brain neurotransmitters responsible for addressing mood disorders. Thus, efforts towards more clinical studies are needed to better understand the physiological function of vitamin D in the brain and stress out more conclusive remarks (Parker, Brotchie, & Graham, 2017).

3. Sources of vitamin D

The exact concentration of serum vitamin D levels defining deficiency and sufficiency is subjected to some contradictions because of the wide range of inconsistencies reported by several bodies and governmental councils. However, the recommended daily allowance (RDA) according to the US Institutes of Medicine (IOM) has been recommended as 400 IU/d (10 μg) for infants, 600 IU/d (15 μg) for children, adolescents and adults, and 800 IU/d (20 μg) for adults aged over 70 years. The RDA values sometimes depend on clinical and environmental factors like latitude of residence, level of exposure to sun, skin pigmentation, dietary practices, clothing and cultural habits and health care system (Pludowski et al., 2017).

There are various ways in which vitamin D can be supplied to meet recommended daily intake, some of which include sunlight, and ingestion of some naturally occurring and fortifying foods. Examples include fortified milk, cheese, soy drink and fish.

3.1. Sunlight

The skin is able to synthesize vitamin D from 7-dehydrocholesterol after UV exposure from sunlight. This source becomes depleted during periods where sunlight is lacking, demanding the use of alternative sources in the form of dietary supplements, as naturally occurring or fortified foods (Jakobsen, 2007).

Exposure to the sun during the last few years has been reduced due to cultural and behavioural issues. These include advices to the general public to minimize sun exposure because of the potential danger to cause skin cancer, together with over expression of collagen and elastin associated with skin ageing. This has increased the use of sunscreen products that tend to hinder vitamin D production (Spiro & Buttriss, 2014).

3.2. Naturally occurring food sources

Very few natural occurring food sources contain vitamin D. The most common sources of vitamin D are cod liver oil, salmon, herring, kipper fillets, mackerel, sardines, tuna, anchovy, cod, trace sole, mushrooms, milk, cheese, yoghurt, meat, eggs, liver, beef, pork, cured bacon, ham, chicken and turkey. Their vitamin D levels varies from source to source; 250 μg/100 g in cod liver oil to 8–30 μg/100 g in fatty fish such as salmon, eel and mackerel and 3–9 μg/100 g in lean fish such as halibut, sole and tuna (Barnkob, Argyraki, Petersen, & Jakobsen, 2016).

Vitamin D in the form of D_3 is present in animal foods such as eggs and fish species while the one in the form of D_2 can be found in some mushrooms and phytoplankton, invertebrates, yeast and plants. In the latter case, it is formed after exposure to UV radiation that allows for photo conversion of ergosterol to ergocalciferol.

Data taken across Europe shows some variability in the dietary intake of vitamin D, with the Scandinavian countries (Sweden, Denmark, Finland and Norway) presenting higher intake when compared with Mediterranean regions such as Italy, Spain and Portugal (O'Mahony et al., 2011). Dietary sources of vitamin D vary from country to country; fish and fats in Norway, milk and dairy products in Finland, oily fish, meat and meat products, cereal and cereal products in the UK, meat, fish and spreads in Ireland, fortified milk in Canada and US, fish/shellfish and eggs in Japan, and fish in Spain (O'Mahony et al., 2011).

3.3. Fortified foods

Fortified food products are foods to which one or more essential nutrients have been added in order to restore a deficiency typical a society or specific target groups (Hashemi, Eskandari, Mesbahi, & Hanifpour, 2015). The added nutrient may be naturally absent or lost during processing. There are several different classes of fortified foods and variability exists from country to country. Food fortification has shown to be an effective alternative to surpass the lack of vitamin D in few countries such as Canada, US, Australia, New Zealand, Sweden and Finland, either in the form of D_2 or D_3.

Food fortification is a cost-effective strategy that has not only been used to restore nutrient deficiency but also to improve nutrient quality and quantity (Hashemi et al., 2015). Among the existing food types fortified with vitamin D, dairy products such as milk products, margarines and breakfast cereals are the most common (O'Mahony et al., 2011). Also, cheese, some orange juices and yoghurts are reported as vehicles for Vitamin D fortification, while due to the generalized consumption of bread, several countries have encouraged its fortification with vitamin D (Lu et al., 2007).

Considering individuals that are intolerant to lactose, and countries with low dairy product consumption, new and improved foods need to be developed as vehicles for vitamin D fortification (O'Mahony et al., 2011).

3.4. Food fortification as an alternative source of vitamin D

Because foods that are rich in vitamin D are not frequently consumed, low abundance of food sources rich in vitamin D and sometimes UV availability is low or unavailable due to environmental factors, several strategies have been proposed to reduce low levels of vitamin D. The food fortification strategy is meant to address current dietary gaps that exist among several populations as a means to increase intake and prevent vitamin D deficiency.

This strategy tends to enhance vitamin D levels across all population groups. While some countries like Canada have made fortification of milk mandatory, there are evidences that a large percentage of the population still consume less than the estimated average requirement (EAR) for vitamin D. These low levels have raised a lot of concerns over the years and several strategies are being suggested to increase the range of food vehicles to be used as well as encourage voluntary fortification practice. The US, Australia New Zealand Sweden and Finland have made fortification of some food mandatory either in the form of D_2 or D_3. Among various food types fortified with vitamin D, dairy products such as milk products, margarines and breakfast cereals, infant foods are the most common (Cashman, 2015; Wagner et al., 2008).

As very few reports are describing food fortification in processed foods due to concerns regarding the stability in terms of pH, temperature and water availability, further studies need to be conducted to examine the long-term retention and heat stability of these vitamin D fortified foods (Wagner et al., 2008). Some limitations to fortification of foods include consumer preference, cultural traditions regarding food and cost of the products especially in vegan based foods (Calvo & Whiting, 2013). A recent trend that is now routinely used involves improving the nutritional composition of animal feeds by biofortifying with vitamin D either by selective breeding, or through genetic engineering by which the vitamin D content in animals such as cultured fish, beef, pork, lamb and chicken, are increased. Mattila et al. (2011) studied the effect of vitamin D fortified chicken feed on vitamin D content of eggs and chicken meat by comparing with known commercial feeds.

The mentioned authors reported that the effect of vitamin D_3 fortified feed in the control group was effectively transferred from the hens' diet to egg yolk. An alternative biofortification strategy is to expose excised skin of animals to UV light in order to increase the conversion of 7-dehydrocholesterol (7-DHC) to vitamin D_3 (Barnkob et al., 2016). This strategy was also followed by Kühn et al. (2015) while exposing chicks to UVB radiation.

Due to the extremely high amounts of 7-DHC in the exposed skin, especially in the legs where feathers are absent, exposed chicken produced eggs that contain 5 times higher vitamin D content than non-exposed chicks that were on normal diet. Biofortification process is cost effective and sustainable, and is currently being practiced in industrialized and developing countries (Barnkob et al., 2016). Studies on developing novel approaches to vitamin D enrichment of foods have increased in recent years. Bio-addition is another popular term used to describe the process of enriching food directly with one or more nutrient. This strategy involves exposing mushrooms to UV light in order to increases vitamin D_2 (ergocalciferol) content (Calvo & Whiting, 2013).

4. Current approaches for vitamin D production

At industrial level, vitamin D can be obtained from two main sources: fish oil and lanolin, a greasy substance that can be obtained as a byproduct of the wool industry.

4.1. Fish oil

Fish oil, produced from fish and its by products, apart from vitamin D, contains high levels of omega-3s in triglycerides, diglycerides and monoglycerides. Currently, there are some concerns about fish oil supplements since they may contain unsafe and illegal levels of polychlorinated biphenyls (PCBs), mercury, lead, and other environmental toxins (Nascimento et al., 2015; Pike & Jackson, 2010).

Fish oil supplements have been used to suppress the severity of cardiovascular disease, stroke, high blood pressure, renal injury, rheumatoid arthritis and autoimmune disorders. The major draw-back of these products is the presence of environmental contaminants through atmospheric deposition, run-off and pollution from shipping. These contaminants tend to accumulate in the liver, and fish oil-rich muscles and dietary products formulated from such tissues, tend to retain very high levels. Series of refining steps such as steam stripping, cold filtration and charcoal filtration, are industrially used to remove these high molecular weight compounds but might not be completely effective (Fernandes, Rose, White, Mortimer, & Gem, 2006).

Industrial production of fish oil comprises the next steps; after fish is sourced, it is boiled and pressed to extract the raw oil. Thereafter, the obtained raw-oil is subjected to various forms of washing and molecular distillation to remove impurities such as heavy metals, pesticides, dioxins, polybrominated diphenylethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and (PCBs), before undergoing enzymatic or supercritical CO_2 extraction for concentration.

4.2. Lanolin

Industrial production of vitamin D can be also carried out from an animal based ingredient, lanolin, a greasy, waxy substance secreted by the sebaceous gland of wool bearing animals. These wools are present mainly as thermo-regulators to maintain a healthy and productive flock's life and protect them from the external environment.

Different methods are being used to shear the wool from sheep and other wool producing animals, generating lanolin and other wool products. Briefly, after the fleece has been collected from the animal, it is submerged in hot detergent to remove dirt and sweat salt, before collect crude lanolin from the solution by high-speed centrifugation. Then, the produced lanolin undergoes a series of chemical reactions to remove lanolin derivatives such as aliphatic alcohols, cholesterin, isopropyl lanolate, laneth, lanogene, lanolin alcohols, lanosterols, sterols, and triterpene alcohols (Jover, Moldovan, & Bayona, 2002). The lanolin is then subjected to a series of purification steps to form 7-dehydrocholesterol before being converted, by UV radiation, to a final dried product thereafter concentrated to form vitamin D supplement.

The whole process suggests that vitamin D supplement produced from lanolin might be cruelty-free in terms of non-animal testing but it contains an animal based ingredient, and as such, consumers that are vegans, and those that are allergic to wool products, must find alternative sources for vitamin D.

5. Mushroom's UV-irradiation for vitamin D production

Mushrooms have been consumed since earliest history because of their nutritional and medicinal properties. Their 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, 2013a, 2013b; Taofiq, González-Paramás, Martins, Barreiro, & Ferreira, 2016, 2015). They can be used as food, dietary supplements, cosmeceutical ingredients, and serve as matrices to produce novel pharmacological active compounds.

The fruiting body of mushrooms, either in their fresh or processed forms, is rich in sterols, mainly ergosterol, form 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.

5.1. Mushrooms irradiation to photoconvert ergosterol into vitamin D_2

Mushrooms' irradiation using techniques like Gamma-irradiation and electron-beam is a safe and cost effective method that has been widely used to enhance their shelf life during pre- or post-harvesting, preserving their quality, nutritional (proteins, sugars and vitamins) and bioactive (phenolics including flavonoids and flavour compounds) compositions (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Mushrooms contain very low levels of vitamin D, or are sometimes vitamin D_2 absent; 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, Byrdwell, Lobato, & Romig, 2014; Slawinska et al., 2016).

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 their cultivated counterparts (Kohn, 2016). Thus, irradiated mushrooms not only provide an alternative to animal source derived vitamin D, but also provide an attractive option for vegans or lactose intolerant individuals, taking into account that most vitamin D fortified foods are based on dairy products (Huang, Lin, Mau, Li, & Tsai, 2015).

The kinetics of the photo-conversion of ergosterol into vitamin D_2 in mushrooms has not been fully understand, but in the work of Jasinghe et al. (2007) factors such as temperature, moisture content, UV radiation type (UVB or UVC) and irradiation dose, are described to influence vitamin D yield. During photo-conversion, temperature of irradiation is significant in controlling thermal rearrangement of pre-vitamin D_2 to vitamin D_2. Also, the use of high or moderate temperatures sometimes leads to formation of by-products such as lumisterol and tachysterol diminishing the final yield, while high moisture content causes ergosterol dilution and subsequently lowering conversion rate (Jasinghe et al., 2007).

Studies assessing the effects of radiation on ergosterol conversion into vitamin D_2, in edible mushrooms, are mostly available for cultivated species, namely the ones with high production value. Examples

Table 1
Irradiated mushroom species and irradiation conditions.

Species	Origin	Sample	Radiation source	Doses	Irradiation procedure	Time of exposure	References
Auricularia auricula-judae (Bull.) J.Schröt.	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
Agaricus bisporus (J.E.Lange) Imbach	Denmark	Mycelium	UVB	0–2.400 mJ/cm	0.25 m	1 min 46 s	(Kristensen et al., 2012)
	Korea	Fresh	UVB	1.36 W/m^2	–	10 min	(Lee & Aan, 2016)
	Canada	Fresh	UVC	0.5, 1.0 and 2.0 kJ/m^2	–	50, 100 and 200 s,	(Guan et al., 2016)
	Poland	Fresh	UVB	411 mJ/cm^2	–	30 min	(Slawinska et al., 2016)
	USA	Fresh	UVB	1 J/cm^2, 492 W/m^2	–		(Bilbao-Sainz et al., 2017)
	USA	Fresh	UVB	80 − 90 mJ/cm^2, 2800–2900 mJ/cm^2	–	25 s, 10 min	(Sapozhnikova et al., 2014)
	Germany	Fresh	UVB	1.5 J/cm^2 (2.54 mW/cm^2)	–	20 min	(Nölle, Argyropoulos, Ambacher, Muller, & Biesalski, 2016)
	Australia	Fresh	UVC	1700 mW/cm^2	30 cm	12 and 30 s,	(Bennett et al., 2013)
	USA	Fresh	Pulsed UV	0.791 J/cm^2/pulse	3.18 cm	–	(Kalaras et al., 2012b)
	Sweden	Fresh	UVA, UVC	94.7, 189.5 and 379.0 J/cm^2	–	30 min, 1hr, and 2 h	(Teichmann et al., 2007)
	Singapore	Fresh	UVA, UVB and UVC	3.5, 4.9, 3.2 W/m^2 and 25.2, 35.3, and 23.0 kJ/m^2	15 cm	1 h	(Jasinghe & Perera, 2006)
	Singapore	Fresh	UVA	3.5 W/m^2, 0.21 kJ/m^2	15 cm	2 h	(Jasinghe et al., 2007)
	Germany	Fresh	UVB	1.5 J/cm^2	–	25 min	(Urbain et al., 2011)
	USA	Fresh	UVB	1.08 J/cm^2	10-15 cm	–	(Simon et al., 2011)
	USA	Fresh	Pulsed UV	0.791 J/cm^2	3.18 cm	–	(Kalaras et al., 2012a)
	Ireland	Fresh	UVA, UVB	0.13–2.80 J/cm^2	–	15 - 360 min.	(Urbain & Jakobsen, 2015)
	Ireland	Fresh	UVA, UVC	0.53 J/cm^2	–	–	(Urbain, Valverde, & Jakobsen, 2016)
		Fresh	UVC	0.125 and 0.25 J/cm^2	30, 40, and 50 cm	2.5–60 min	(Koyyalamudi, Jeong, Song, Cho, & Pang, 2009)
	Singapore	Fresh	UVA	25.2 kJ/m^2	15 cm	2 h	(Jasinghe & Perera, 2005)
	Korea	Fresh	UVB	10, 20, and 30 kJ/m^2	–	–	(Ko, Lee, Lee, and Park, 2008)
	Australia	Fresh	Pulsed UV	1.150 J/cm^2	–	–	(SKoyyalamudi et al., 2011)
	Denmark	Fresh	UVB	0–2.400 J/cm^2	0.25 m	1 min 46 s	(Kristensen et al., 2012)
	Taiwan	Fresh	UVB, UVC	0.247, 0.493, and 0.986 J/cm^2	30 cm	0.5, 1, and 2 h	(Mau, Chen, & Yang, 1998)
	Taiwan	Fresh	γ-irradiated	0.5, 2, 5 and 10 kGy	–	–	(Tsai et al., 2014)
	USA	Fresh	UVB	0.5, 1.0, and 1.5 J/cm^2	–	< 2 h	(Roberts et al., 2008)
Agaricus bisporus Portabella (J.E.Lange) Imbach	Sweden	Fresh	UVA, UVC	94.7, 189.5 and 379.0 J/cm^2	–	30 min, 1hr, and 2 h	(Teichmann et al., 2007)
Agaricus bitorquis (Quélet) Sacc.	Taiwan	Fresh	UVB, UVC	0.247, 0.493, and 0.986 J/cm^2	30 cm	0.5, 1, and 2 h	(Mau et al., 1998)
Agrocybe cylindracea, (Pers.) Fayod	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
Auricularia polytricha (Mont.) Sacc.	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
Boletus edulis Bull.	Sweden	Fresh	UVA, UVC	94.7, 189.5 and 379.0 J/cm^2	–	30 min, 1hr, and 2 h	(Teichmann et al., 2007)
Cantharellus tubaeformis.	Sweden	Fresh	UVA, UVC	94.7, 189.5 and 379.0 J/cm^2	–	30 min, 1hr, and 2 h	(Teichmann et al., 2007)
Cordyceps militaris (L.) Fr.	Taiwan	Fresh	UVB	0.36 mW/cm^2,25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, Mau, et al., 2015)
Flammulina velutipes (Curtis) Singer	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
	Singapore	Fresh	UVA	25.2 kJ/m^2	15 cm	2 h	(Jasinghe & Perera, 2005)
Hericium erinaceus (Bull.) Persoon	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
Hypsizygus marmoreus (Peck) Bigelow	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)

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Table 1 (*continued*)

Species	Origin	Sample	Radiation source	Doses	Irradiation procedure	Time of exposure	References
Lentinula edodes (Berk.) Pegler	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Poland	Fresh	UVB	411 mJ/cm^2	–	30 min	(Slawinska et al., 2016)
	USA	Fresh	UVB	80 − 90 mJ/cm^2, 2800–2900 mJ/cm^2	–	25 s, 10 min	(Sapozhnikova et al., 2014)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Sweden	Fresh	UVA, UVC	94.7, 189.5 and 379.0 J/cm^2	–	30 min, 1hr, and 2 h	(Teichmann et al., 2007)
	Singapore	Fresh	UVA, UVB and UVC	3.5, 4.9, 3.2 W/m^2 and 25.2, 35.3, and 23.0 kJ/m^2	15 cm	1 h	(Jasinghe & Perera, 2006)
	Singapore	Fresh	UVA	3.5 W/m^2, 0.21 kJ/m^2	15 cm	2 h	(Jasinghe et al., 2007)
	Singapore		UVB	35·3 kJ/m^2	15 cm	1 h	(Jasinghe, Perera, & Barlow, 2005b)
	Singapore	Fresh	UVA	25.2 kJ/m^2	15 cm	2 h	(Jasinghe & Perera, 2005)
	Korea	Fresh	UVB	25, 50, and 75 kJ/m^2	–	–	(Ko et al., 2008)
	Taiwan	Fresh	UVB, UVC	0.247, 0.493, and 0.986 J/cm^2	30 cm	0.5, 1, and 2 h	(Mau et al., 1998)
	Korea	Fresh	UVB	0.6–1.8 W/m^2	–	60-180 min	(Zhang, Wu, Song, & Ahn, 2015)
	Spain	Supercritical extracts	UVA, UVC	–	4, 14, 24 cm	0, 15, 30, 60, 120 min	(Morales et al., 2017)
Lentinus squarrosulus (Mont.)	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
Lentinus polychrous Lév.	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
Pholiota nameko (T. Itô) S. Ito & S. Imai	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
Pleurotus citrinopileatus Singer	Taiwan	Mycelium	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
Pleurotus cystidiosus O.K. Mill.	Singapore	Fresh	UVA, UVB and UVC	3.5, 4.9, 3.2 W/m^2 and 25.2, 35.3, and 23.0 kJ/m^2	15 cm	1 h	(Jasinghe & Perera, 2006)
	Singapore	Fresh	UVA	3.5 W/m^2, 0.21 kJ/m^2	15 cm	2 h	(Jasinghe et al., 2007)
	Singapore	Fresh	UVA	25.2 kJ/m^2	15 cm	2 h	(Jasinghe & Perera, 2005)
Pleurotus eryngii var. *ferulae*	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
	Taiwan	Mycelium	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Taiwan	Fresh	Pulsed UV	–	–	18 s	(Chen et al., 2015)

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Table 1 (*continued*)

Species	Origin	Sample	Radiation source	Doses	Irradiation procedure	Time of exposure	References
Pleurotus ostreatus (Jacq. ex Fr.) P.Kumm.	Budapest	Mycelium	UVB	–	32 cm	15 to 90 min	(Szabó & Győrfi, 2012)
	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
	Poland	Fresh	UVB	411 mJ/cm^2	–	30 min	(Slawinska et al., 2016)
	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
	Singapore	Fresh	UVA, UVB and UVC	3.5, 4.9, 3.2 W/m^2 and 25.2, 35.3, and 23.0 kJ/m^2	15 cm	1 h	(Jasinghe & Perera, 2006)
	Germany	Fresh	UVB	11.5 W/m^2	10–11 cm	60 min	(Maximilian Wittig, Krings, & Berger, 2013)
	Germany	Fresh	UVB	11.5 W/m^2	10 cm		(Krings & Berger, 2014)
	Singapore	Fresh	UVA	3.5 W/m^2, 0.21 kJ/m^2	15 cm	2 h	(Jasinghe et al., 2007)
	Kenya	Fresh	UVA, UVC	3.5 W/m^2, 0.0327 W/m^2 0.21 kJ/m^2, 1.96 J/m^2	–	10 - 60 min	(Edward et al., 2015)
	Korea	Fresh	UVA, UVB and UVC	0.3–1.2 W/m^2	–	2 h	(Wu & Ahn, 2014)
	Singapore	Fresh	UVA	25.2 kJ/m^2	15 cm	2 h	(Jasinghe & Perera, 2005)
	Indonesia	Fresh	UVA, UVC	–	10 cm	15, 30, 60, 90, and 120 min	(Ruslan, Reza, & Damayanti, 2011)
Pleurotus pulmonarius (Fr.) Quel.	Thailand	Fresh Freeze-dried Mycelium	UVB	45.86–550.32 J/cm^2	15 cm	15-180 min	(Banlangsawan & Sanoamuang, 2016)
Pleurotus djamor (Rumph. ex Fr.) Boedijn	Taiwan	Fresh	UVB	0.36 mW/cm^2, 25.9 kJ/m^2	19 cm	2 h	(Huang, Lin, & Tsai, 2015)
Volvariella volvacea (Bul. ex Fr.) Singer	Taiwan	Fresh	UVB, UVC	0.247, 0.493, and 0.986 J/cm^2	30 cm	0.5, 1, and 2 h	(Mau et al., 1998)

Table 2
Bioaddition of Vitamin D_2 in mushrooms after undergoing UV radiation.

Mushroom	Source of radiation	Vitamin D_2		Reference
		Before radiation	After radiation	
Agaricus bisporus	UVB	0.18 μg/100 g fw	491 μg/100 g fw	(Urbain et al., 2011)
	UVB	5.5 μg/100 g dw	410.9 μg/100 g dw	(Simon et al., 2011)
	Pulsed UV	0.005 μg/g	27 μg/g dw	(Kalaras et al., 2012a)
	UVA-UVB	0.1 μg/g dw	3.9 μg/g dw	(Urbain & Jakobsen, 2015)
	UVB	–	141.32 μg/g dw	(Wittig et al., 2013)
	Pulsed UV	–	32.2 μg/g dw	(Kalaras et al., 2012b)
	UVB	–	13.10 μg/g dw	(Slawinska et al., 2016)
	UVB	179 IU/g	6292 IU/g	(Sapozhnikova et al., 2014)
	UVB	0.0003–0.0008 μg/g dw	157 μg/g dw	(Bilbao-Sainz et al., 2017)
	UVB	–	406 μg/g dw	(Nölle et al., 2016)
	Pulsed UV	–	20.9 μg/g dw	(Koyyalamudi et al., 2011)
	UVA, UVC	0.07 μg/g dw	10.14 μg/g dw	(Teichmann et al., 2007)
	UVA, UVB and UVC	–	56.5 μg/g dw	(Jasinghe & Perera, 2006)
	UVC	0.79 mg/100 g dw	1.34 mg/100 g dw	(Guan et al., 2016)
	UVB	0.01 μg/g dw	7.28 μg/g d w	(Roberts et al., 2008)
	UVB	–	67.1 μg/g dw	(Paul Urbain et al., 2016)
	UVB	–	491 μg/100 g	(Urbain et al., 2011)
	UVC	–	30 μg/kg	(Louise Bennett et al., 2013)
	UVB	–	741.50 μg/g	(Lee & Aan, 2016)
Agaricus bisporus var. *Portobello*	UVC	0.43 mg/100 g dw	0.95 mg/100 g dw	(Guan et al., 2016)
Agaricus blazei	UVB	–	22.13 μg/g dw	(Huang, Lin, & Tsai, 2015)
Agrocybe cylindracea	UVB	0.95 μg/g dw	42.36 μg/g dw	(Huang, Lin, & Tsai, 2015)
Auricularia polytricha	UVB	–	60.29 μg/g dw	(Huang, Lin, & Tsai, 2015)
Cantharellus tubaeformis	UVA, UVC	1.55 μg/g dw	14.03 μg/g dw	(Teichmann et al., 2007)
Cordyceps militaris	UVB	0.22 mg/g dry weigh	1.11 mg/g dw	(Huang, Lin, & Tsai, 2015)
Hypsizigus marmoreus	UVB	1.62 μg/g dw	15.06 μg/g dw	(Huang, Lin, & Tsai, 2015)
Lentinula edodes	UVB	–	29.33 μg/g dw	(Slawinska et al., 2016)
	UVB	–	1036 IU/g	(Sapozhnikova et al., 2014)
	UVB	0.35 μg/g dw	15.10 μg/g dw	(Huang, Lin, & Tsai, 2015)
	UVA, UVB and UVC	–	53.9 μg/g dw	(Jasinghe & Perera, 2006)
Pholiota nameko	UVB	–	61.78 μg/g dw	(Huang, Lin, & Tsai, 2015)
Pleurotus ostreatus	UVB	1.78 μg/g dw	27.89 μg/g dw	(Banlangsawan & Sanoamuang, 2016)
Pleurotus citrinopileatus	UVB	3.93 μg/g dw	208.65 μg/g dw	(Huang, Lin, & Tsai, 2015)
Pleurotus cystidus	UVA, UVB and UVC	–	79.5 μg/g dw	(Jasinghe & Perera, 2006)
Pleurotus djamor	UVB	2.13 μg/g dw	93.29 μg/g dw	(Huang, Lin, & Tsai, 2015)
Pleurotus eryngii var. *ferulae*	UVB	1.65 μg/g dw	52.30 μg/g dw	(Huang, Lin, & Tsai, 2015)
	UVB	1.56 μg/g dw	28.71 μg/g dw	(Huang, Lin, & Tsai, 2015)
Pleurotus ostreatus	UVB	–	239.67 μg/g	(Wu & Ahn, 2014)
	UVB	–	56.60 μg/g dw	(Slawinska et al., 2016)
	UVB	–	4411 IU/g	(Sapozhnikova et al., 2014)
	UVB	0.83 μg/g dw	69.00 μg/g dw	(Huang, Lin, & Tsai, 2015)
	UVA, UVB and UVC	–	184.65 μg/g dw	(Jasinghe & Perera, 2006)
	UVB	32.07 μg/g dw	141.32 μg/g dw	(Wittig et al., 2013)
	UV	–	48.19 μg/g	(Ruslan et al., 2011)

include *Agaricus bisporus* (J.E.Lange) Imbach, *Lentinula edodes* (Berk.) Pegler and *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm. Nevertheless, other species such as *Auricularia auricula-judae* (Bull.) J.Schröt., *Agaricus bitorquis* (Quélet) Sacc., *Agrocybe cylindracea* (Pers.) Fayod, *Auricularia polytricha* (Mont.) Sacc., *Boletus edulis* Bull., *Cantharellus tubaeformis*, *Cordyceps militaris* (L.) Fr., *Flammulina velutipes* (Curtis) Singer, *Hericium erinaceus* (Bull.) Persoon, *Hypsizygus marmoreus* (Peck) Bigelow, *Lentinus squarrosulus* (Mont.), *Lentinus polychrous* Lév., *Pholiota nameko* (T. Itô) S. Ito & S. Imai, *Pleurotus citrinopileatus* Singer, *Pleurotus cystidiosus* O.K. Mill., *Pleurotus djamor* (Rumph. ex Fr.) Boedijn, *Pleurotus eryngii* var. *ferulae (Lanzi) Sacc., Pleurotus pulmonarius* (Fr.) Quel., and *Volvariella volvacea* (Bul. ex Fr.) Singer., have been also reported to present considerable amounts of vitamin D after exposure to UV radiation.

Mushroom species evaluated for photo conversion to vitamin D are from different parts of the world; they include Asia (Korea, Singapore, Taiwan, Thailand and Indonesia); North America (Canada, USA); Africa (Kenya); Europe (Poland, Germany, Sweden, Ireland, Denmark) and Australia (Australia) (Table 1 and cited references).

Different UV irradiation sources such as Ultraviolet A (UVA), Ultraviolet B (UVB), and Ultraviolet C (UVC) were applied, mostly to fresh samples (Table 1 and cited references), but also to freeze-dried ones (Banlangsawan & Sanoamuang, 2016) and mycelium samples (Banlangsawan & Sanoamuang, 2016; Huang, Lin, & Tsai, 2015; Kristensen, Rosenqvist, & Jakobsen, 2012; Szabó & Győrfi, 2012). Pulsed UV (Kalaras et al., 2012a; Kalaras, Beelman, Holick, & Elias, 2012b; Koyyalamudi et al., 2011), mushroom supercritical extracts (Morales et al., 2017) and γ-irradiation (Tsai, Mau, & Huang, 2014) were also applied to fresh samples. The applied UV doses, irradiation procedure and exposure time were variable among the documented studies (Table 1).

Agaricus is a genus of saprobic basidiomycetes comprising over 400 species distributed all over the world, with *A. bisporus* (J.E. Kange) Imbach as the most cultivated one with an annual production, in Europe, of one million metric tons (Rzymski et al., 2017). Considering the number of reports available in literature concerning *A. bisporus*, it can be considered not only a valuable source of nutrients and non-nutritional components but also, in the present context, a source of vitamin D (Kohn, 2016).

According to Table 1, *A. bisporus* has been treated with UVA, UVB,

UVC and Pulsed UV following distinct irradiation conditions and giving rise to different levels of bioadded vitamin D_2 (Table 2). Simon, Phillips, Horst, and Munro (2011) reported that the level of vitamin D_2 present in *A. biporus* exposed to UVB radiation at a dose of 1.08 J/cm^2 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), 0.0003 μg/g dw to 157 μg/g dw (Bilbao-Sainz et al., 2017) and 0.01 μg/g dw to 7.28 μg/g dw (Roberts, Teichert, & McHugh, 2008). *Agaricus bisporus* var. *Portobello* treated with UVC showed a slight increase in vitamin D_2 content at 2.0 kJ/m^2 dose, namely from 430 μg/100 g dw to 950 μg/100 g dw (Guan et al., 2016). 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). Szabó and Győrfi (2012) also reported that shorter irradiation time gave higher vitamin D_2 levels in pre-harvest *A. bisporus* after UV treatment.

The conversion of ergosterol to ergocalciferol in crude mushrooms is highly reliant on the applied energy source as sufficient energy is required (at 254 nm) to break the diene bond of the B-ring of ergosterol (occurs dimerization and ring cleavage) to photo-convert it into pre-vitamin D (Ruslan et al., 2011). The most significant reports on vitamin D_2 bioaddition in mushrooms have been carried out on the fruiting bodies of mushrooms in their fresh or freeze-dried forms. However, Huang, Lin, Mau, et al. (2015) have demonstrated the advantages of UV radiated mycelia and submerged culture, mainly due to the increased surface area that allows an amplifies the exposure to the applied UV radiation. The mentioned authors reported an increase in vitamin D_2 content in *Cordyceps militaris* from 0.22 to 1.11 mg/g, and the presence of several physiologically active substances with well reported bioactive properties (adenosine, cordycepin, ergothioneine, and polysaccharides), thereby presenting a high potential of application for healthy food development, adding to high vitamin D_2 contents.

After application of Pulsed UV, with 12 pulses, the vitamin D_2 levels in *A. bisporus* samples, reach a maximum concentration of approximately 27 μg/g dw (Kalaras et al., 2012a), whereas the unprocessed control mushrooms contained no measurable levels of vitamin D_2.

Vitamin D_2 levels approached a maximum concentration of 124 μg/g dw upon treatment with 60 pulses (20 s total exposure) (Kalaras et al., 2012b). The amounts of vitamin D_2 produced in mg/g dw using pulsed UV light were directly proportional to the number of applied pulses. The yield of vitamin D_2 using 9 pulses of UV light (161 μg/g dw) was higher comparatively other study, that using continuous UV-C light with an intensity of 0.25 J/cm^2 and an exposure time of 10 min at a distance of 30 cm (141 mg/g dw) according to Koyyalamudi et al. (2011).

Application of UVC in *A. bisporus* has also resulted in an increase of vitamin D_2 levels from 0.07 μg/g dw to 10.14 μg/g dw (Teichmann, Dutta, Staffas, & Jägerstad, 2007), 790 μg/100 g dw to 1340 μg/100 g dw (Guan et al., 2016), and a level up to 56.5 μg/g dw was also reported by Jasinghe and Perera (2006). The irradiation conditions were different in each of the above case studies supporting the dissimilarity encountered in the reached vitamin D_2 levels.

Pleurotus genus is made up of over 200 saprophytic mushrooms, widely distributed in temperate and tropical regions, and with well reported nutritional and bioactive properties. *Pleurotus ostreatus*, popularly referred to as oyster mushroom, is the most common species among the *Pleurotus* genus and significant works are available reporting their potential for photo-convert ergosterol to vitamin D by exposure to UVA (Jasinghe & Perera, 2005), UVB (Banlangsawan & Sanoamuang, 2016; Aneta Slawinska et al., 2016), UVC (Jasinghe & Perera, 2006) and pulse irradiation (Chen et al., 2015). Mushrooms irradiation aiming at increase vitamin D_2 content on *Pleurotus* genus was reported by Huang, Lin, and Tsai (2015). The vitamin D_2 content after exposure was found to be 93.29 μg/g dw, 69.00 μg/g dw, 52.30 μg/g dw and 28.71 μg/g dw for *P. salmoneostramineus*, *P. ostreatus*, *P. ferulae* and *P. eryngii*, respectively. A significant increase was observed in *P. cystidus*, namely up to 79.5 μg/g dw (Jasinghe & Perera, 2006).

Lentinula edodes, popularly called shiitake, is a saprobic, wood-colonizing mushroom with a huge commercial production, only second behind *A. bisporus*. After exposure to UVB irradiation, vitamin D_2 content in *L. edodes* reached values up to 29.33 μg/g dw (Slawinska et al., 2016) or 53.9 μg/g dw (Jasinghe & Perera, 2006), with the latter corresponding to higher irradiation doses and a longer exposure times. Other species of *Lentinus* such as *Lentinus squarrosulus* (Mont.) and *Lentinus polychrous* Lév. have also been reported to contain significant amounts of vitamin D_2 after irradiation (Banlangsawan & Sanoamuang, 2016). Several other examples of species where significant amounts of vitamin D_2 have been developed after irradiation can be found in Table 2.

To be able to apply the irradiation techniques at a productive level it is important to identify the best irradiation conditions to increase vitamin D_2 content in cultivated mushrooms. In this context, some authors have already conducting optimization studies by evaluating the effects of intensity (0.5, 0.75, and 1.0 mW/cm^2), dose (0.5, 1.0, and 1.5 J/cm^2), and postharvest time (1 and 4 days) on the vitamin D_2 formation in *Agaricus bisporus* (Roberts et al., 2008). These authors identify shorter times of exposure and high intensities to be beneficial to vitamin D_2 development. Also, the distance of the UV source to mushrooms samples need to be optimized to determine the most adequate position for irradiation. From Table 1 it can be noted that very dissimilar distances between irradiation source and sample have been used (from 3.18 up to 50 cm). Morales et al. (2017) reported that the vitamin D_2 content in *L. edodes* supercritical extracts was 5-fold higher if UV source was placed 4 cm distant from the sample, comparatively with 24 cm after 1 h exposure. In a different study, Banlangsawan and Sanoamuang (2016) reported that with UV-B irradiation the amount of vitamin D_2 in the *P. ostreatus* was increased from 1.78 μg/g dw (unexposed) to 27.89 μg/g dw after exposures of 180 min.

Because of the observed dissimilarities in the irradiation conditions needed to maximize ergosterol conversion into vitamin D_2 in mushrooms, Lee and Aan (2016) have recently carried out an optimization study using the response surface methodology (RSM) considering as independent variables the exposure time, temperature, and irradiation intensity. The authors reported that an exposure time of 10.4 min, a temperature of 26.33 °C, and a UVB irradiation intensity of 1.36 W/m^2 was ideal to achieve a content of vitamin D_2 of 741.50 μg/g. In another work, Wu and Ahn (2014) identified 28.16 °C, UVB intensity of 1.14 W/m^2, and exposure time of 94.28 min as the ideal conditions to maximizes vitamin D_2 content in *P. ostreatus* up to 239.67 μg/g dw. Also, an important variable is the contact surface area. This topic was treated by Ko et al. (2008) where different parts of shitake mushroom were exposed o UVB at 25 kJ/m^2. The authors suggested that sliced mushrooms (higher surface area) resulted in higher vitamin D_2 levels (106.4 μg/g).

Irradiation studies to improve vitamin D_2 content have been carried out mainly on *Pleurotus, Agaricus and Lentinula* genera, but Huang, Lin, and Tsai (2015) conducted studies on *Agrocybe*, *Auricularia*, *Hypsizigus*, and *Pholiota* species using a UVB source placed 19 cm far from the samples. The irradiation intensity and dose was 0.36 mW/cm^2 and 25.9 kJ/m^2, respectively, for 2 h. The vitamin D_2 content after exposure was found to be 42.36 μg/g dw, 60.29 μg/g dw, 15.06 μg/g dw and 61.78 μg/g dw in *Agrocybe cylindracea*, *Auricularia auricular*, *Hypsizigus marmoreus* and *Pholiota nameko*, respectively. The authors reported *in vitro* antioxidant properties for the irradiated species due to the contribution of ergothioneine and phenolic compounds, thus reinforcing their potential to be used as food ingredients/supplements with health promoting properties.

5.2. Bioavailability of vitamin D from UV irradiated mushrooms

Some studies comparing the bioavailability of the various forms of vitamin D by monitoring serum 25(OH)D (25-hydroxyvitamin D) levels

have been reported. Intake of supplements in the form of D_3, mushroom derived D_2, fortified foods or dietary sources have shown differences but yet effective in maintaining serum 25(OH)D levels (Jakobsen, 2007). Very few studies have been able to determine the fate of vitamin D in the human gastrointestinal tract. A randomized controlled trial (RCT) data on the lebel of serum 25-hydroxyvitamin D in healthy individuals that consumed UV light treated edible mushrooms was reported by Cashman, Kiely, Seamans, and Urbain (2016). The above authors reported an increase in serum 25(OH)D when baseline vitamin D status was low but when baseline vitamin D status is high, there was no significant effect on serum 25(OH)D levels.

Vitamin D from several sources, firstly need to be released from the food matrix in which it is embedded, before undergoing a series of fate that becomes influenced by the food composition, presence and type of digestive enzymes, and the transport mechanism across intestinal cell (Borel, Caillaud, & Cano, 2013). One of the early studies on the bioavailability of vitamin D_2 from vitamin D_2-biofortified *A. bisporus* were conducted by Urbain, Singler, Ihorst, Biesalski, and Bertz (2011). Serum levels of 25(OH)D in those ingesting treated mushrooms (3.9 nmol/l) were similar to the ones of the control group receiving vitamin D supplements (4.7 nmol/l), making the former as effective as the vitamin D supplements itself in improving vitamin D levels.

Human studies were conducted by Ozzard, Hear, Morrison, and Hoskin (2008) in order to evaluate the potential of UVB-irradiated mushrooms to increase serum 25(OH)D levels. This case study was conducted on 30 years old man by administering stir-fried UVB irradiated mushrooms. Blood samples were taken and an increase in serum 25(OH)D level up to 129 % was observed coupled with an increase in calcium serum levels.

A contrasting report on vitamin D_2 bioavailability after ingestion of UV exposed mushrooms (fresh, sliced and cooked) was reported by Mehrotra et al. (2014). Forty-three prediabetic and vitamin D deficient adults were exposed to 100 g of these diets daily for 16 weeks. The authors reported little or no significant increase in 25(OH)D levels, mainly attributed to the longer exposure of fresh UV irradiated mushroom to cooking times. Nevertheless, Stephensen et al. (2012) reported a lack of difference between cooked (11.6 ± 1.1 μg/serving) and uncooked (10.9 ± 0.70 μg/serving) mushrooms.

UVB irradiated *A. bisporus* containing 15 μg of vitamin D_2 per gram of dried mushrooms was fed on female rats, and the control was fed with a vitamin D deficient diet. After 15 weeks following the above diets, there was no effect on the registered weight; UVB exposed mushroom fed rats had 30-fold of the recommended National Research Council (NRC) vitamin D level in its serum; furthermore, plasma levels of inflammatory mediators namely TNF-α and IL-1β were greatly reduced thereby suppressing the incidence of inflammation (Babu, Balan, Garthoff, & Calvo, 2014).

Chen et al. (2015) evaluated the potential of pulsed irradiated *Pleurotus eryngii* var. *ferulae,* a mushroom commonly grown in the far east, fed to female mice to enhance vitamin D synthesis and analyzed the serum osteoblast and osteoclast metabolites as indicators for bone density using NMR spectroscopy. The mice fed with irradiated mushrooms showed an increase in bone density, when compared with the mice fed with non-irradiated, with higher osteoblast and lower osteoclast suggesting that there was an increased bone formation rather than bone resorption. Blood was collected from time to time to measure ALP, osteocalcin, procollagen I C-terminal propeptide (PICP) and pyridinoline (PYD) levels. The results reveal that pulse irradiated mushrooms can exert proper resistance against bone resorption, thus maintaining bone health. The enhanced bone metabolism is due to increased vitamin D levels, which stimulate calcium and phosphorus absorption necessary for bone mineralization.

The bioavailability of biofortified UV-treated *A. bisporus*, was analyzed in thirty-eight volunteers by Stephensen et al. (2012). They have received treated mushrooms for 6 weeks and the results showed that the ergocalciferol formed after UV exposure was absorbed and showed similar levels to the ones of the control group that have received ergocalciferol supplements.

Dietary factors contribute significantly to bone mineralization and femur density has been reported by Calvo et al. (2013) to show higher bone mineralization in rats fed with UVB irradiated mushrooms, when compared with control fed rats. These authors reported a fairly consistent average body weight among tested rats while plasma levels of creatinine were used as an indicator to analyze renal function/hypercalcemia. All tested samples showed no significant difference in plasma creatinine levels suggesting that no renal impairment was observed, even after weeks of high vitamin D mushroom diet. Histological studies were conducted on the tissues of the liver and spleen of the randomized fed rat, and the results also showed a negative tendency for the vitamin enriched mushroom diet to cause hyperparathyroidism.

The potential of UVC fortified *A. bisporus* to maintain vitamin D level was also reported by Bennett et al. (2013), and the authors exploited the *in vivo* potential of the above sample to prevent memory-impairment and the incidence of neurological disorders.

To the authors best knowledge, Monaghan Mushrooms, Ireland is the only producer of UV treated mushroom as a novel food to increase vitamin D content in the EU, in agreement to Regulation (EC) No. 258/97 Article 4.2. It was initially assessed on the 10th July 2015 by the Food Safety Authority of Ireland (FSAI) before undergoing a 60 days comment period by member states of the European commission to ascertain its safety and potency. UV light treated commercially grown *A. bisporus* became the first irradiated mushroom specie to be approved and authorized as a source of vitamin D capable of yielding ≤10 μg/100 g fresh weight.

6. Concluding remarks and future perspectives

Several studies have been conducted measuring the effects of gamma irradiation and three subtypes of UV (UVA, UVB and UVC) on vitamin D_2 generation in several mushrooms. The method by which mushrooms have been treated, mostly with UV light, diverges significantly amongst studies not only by UV type and applied dose but also other factors, namely, origin and cultivar of the mushroom, time after harvest, positioning of the mushrooms to the light source, fresh or dried, among others. Trying to identify the irradiation conditions needed to maximize ergosterol conversion to vitamin D_2 in mushrooms still needs to be optimized based on some contradicting reports that have been presented so far.

The consumption of D_2 enriched mushrooms is maybe an exclusive case since irradiated mushrooms are one of the only products that would possibly contain not only D_2 but also its photoisomers. Recent studies have shown the huge potential that irradiated mushroom present as a source of vitamin D_2. The research studies have been mainly focused on commercial species such as *Agaricus* sp., *Lentinula edodes* and *Pleurotus* sp. The data showed a high rate of conversion from ergosterol to vitamin D_2 at short treatment time, which is required by the mushroom industry. Most studies have shown significant amount of vitamin D content in UV irradiated mushrooms as well as their efficacy to increase serum 25-hydroxyvitamin D levels and as such, because these mushrooms contain high bioactive and nutritional components, they can be utilized as multifunctional bio-based ingredient to reduce vitamin D deficiency as well as present other health promoting benefits.

There is still a huge knowledge gap as regards the interaction between the food matrix in which these irradiated mushrooms are embedded and their bioavailability. This report shows that irradiated mushrooms are an alternative source of vitamin D_2 whose large-scale production is commercially viable at low cost. Further studies still need to be conducted to allow for extraction, separation, recovery and purification of vitamin D_2 from irradiated mushroom with minimal process cost and high purity percentage to be utilized as fortified food ingredient or as bio-based supplements. Also, considering vitamin D can be degraded during the process of extraction and purification, another

potential possibility is to isolate ergosterol from mushrooms and then irradiate the isolated ergosterol for conversion into vitamin D_2.

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