Ergosterol Content and Antioxidant Activity of Lion's Mane Mushroom (*Hericium erinaceus*) and Its Induction to Vitamin D_2 by UVC-Irradiation

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Abstract: Lion's Mane mushroom (*Hericium erinaceus*), LM, is a medicinal mushroom which has high protein content and contains many bioactive compounds. However, a large amount of the irregular-shape LM (Ir-LM), considered as by-products, are generated during the cultivation. The objectives of this research were to determine the ergosterol content in the LM and investigate the effect of ultraviolet (UV) irradiation on the conversion of ergosterol in the Ir-LM extract to vitamin D_2. Ir-LM extracts were investigated for its antioxidant properties before dissolved in methanol and irradiated with UVC for 120 min at 5 cm distance from the lamp. The results showed that the Ir-LM contained significantly higher ($p < 0.05$) ergosterol content (2.52 ± 0.13 mg/g dried LM) than that of regular-shape LM (Reg-LM), 2.15 ± 0.08 mg/g dried LM. Ergosterol at 1.74 ± 0.09 mg/g dried LM without vitamin D_2 was detected in the non-irradiated extract, while interestingly, the irradiated sample showed a decrease of ergosterol at 13.5% with a detection of ergocalciferol at 30.01 ± 7.09 µg/g dried LM. These obtained results exhibited a new area of post-extraction procedure aiming to enhance vitamin D_2 enriched extracts from mushroom by-products which can be value-added as a nutritional supplement in foods.

1 INTRODUCTION

Lion's Mane mushroom (*Hericium erinaceus*, LM) is an edible fungus which has been used in traditional Chinese medicine for long time (Khan et al., 2013). LM contains a significant content of bioactive compounds, ergosterol, hericenone C and hericene A (Joradon et al., 2022), that would might be responsible for several health-promoting properties (Friedman, 2015).

In general, high molecular weight substances including polysaccharides and low molecular weight substances including terpenoids can be used to categorize bioactive metabolites in the LM (Thongbai et al., 2015). The compounds with bioactivity exist in different part of the mushroom (Shen et al., 2010), for instance, low-molecular weight metabolites, erinacines were found in mycelia while hericenones were detected in fruiting bodies of LM (Thongbai et al., 2015).

There is an increasing interest in bioactive compounds from natural sources such as gamma oryzanol from rice bran oil (Rodsuwan et al., 2020), puerarin from Pueraria (Rungsardthong et al., 2021), ergosterol from LM (Joradon et al., 2022; Tachabenjarong et al., 2022), antioxidants from Sacha Inchi oil (Suwannasang et al., 2021; 2022a; 2022b), and bioactive compounds from bamboo mushroom (Binheam et al., 2022).

Ergosterol can exhibit anti-inflammatory, anti-tyrosinase and anti-cancer properties (Kang et al., 2015). According to Corrêa et al. (2017), ergosterol has the potential to lessen the negative effects from high cholesterol. Ergosterol is the most prevalent sterol presents in the membranes of fungi, and it is also a precursor of vitamin D_2. The compound is

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important for maintaining fluidity and permeability, involves with endocytosis and cytoskeletal organization inside the fungal cells (Abe & Hiraki, 2009). Mushroom ergosterol can be converted into vitamin D_2 (Jasinghe et al., 2007). With the exposure to UV irradiation, ergosterol encounters photochemical cleavage within their structure, causing the formation of the intermediate of vitamin D_2. After being heated, this intermediate then goes through thermal isomerization to create vitamin D_2. (Jasinghe et al., 2007).

One of the pro-hormones that is crucial for maintaining human health is vitamin D. Vitamin D is well known for supporting bone health and calcium homeostasis, as well as having a variety of non-skeletal effects on immunological function and cell physiology (Durrant et al., 2022). Vitamin D_2 and D_3 are found as a major forms of vitamin D (Dawson-Hughes et al., 2010). Typically, vitamin D_2 can be found in the fruiting bodies of mushrooms. Applying ultraviolet (UV) irradiation to the mushroom will lead to the conversion of ergosterol to vitamin D_2 (Jäpelt and Jakobsen, 2013). Morales et al. (2017) revealed that UVC irradiation to the ethanol extract of Shiitake mushroom (*Lentinula edodes*) at 25°C, for 1 h (4 cm away from the light source) could generate a high content of vitamin D_2 than direct irradiation of the fruiting body.

During the cultivation of LM, irregular-shape LM (Ir-LM) were found in the mushroom farm. These mushrooms are considered as by-products and sold at low prices in the mushroom market because of their inferior morphology and quality. Therefore, alternative solutions are required to increase the value of these mushroom by-products which still contain a high content of health-benefit compounds. They might be used as high nutritional food or extracts enriched with medicinal compounds for food or medicinal uses. Consequently, the objectives of this research were to determine the major bioactive compounds, ergosterol, hericenone C and hericene A contents and antioxidant properties in the extracts prepared from regular LM (Reg-LM) and Ir-LM. Further investigation on the effect of ultraviolet C (UVC) irradiation on the conversion of ergosterol in the Ir-LM extract to vitamin D_2 was carried out. Morphology of fruiting bodies and their proximate compositions were also performed. The results of this study would propose an alternative way to increase the value of the by-product by converting ergosterol in the mushroom extracts to vitamin D_2.

2 MATERIALS AND METHODS

2.1 Biological Materials and Chemicals

Fruiting bodies of Reg-LM and Ir-LM were purchased from Fresh and Friendly Farm Co., Ltd. at Thanyaburi district in Pathum Thani province, Thailand. Morphology of the LM cultivated in the farm for 4 lots were monitored and their production yield and related economic data were calculated. Ergosterol (95%) was obtained from Sigma-Aldrich Química (Madrid, Spain). Ergocalciferol (98%) (vitamin D_2) was purchased from TCI, Japan. Folin-Ciocalteu reagent and 2,2-diphenyl-1-picreylhydrazyl (DPPH) were purchased from Sisco Research Laboratories Pvt. Ltd., India while gallic acid (98%) was the product from Sigma-Aldrich, USA. Trolox reagent was obtained from M Tedia, USA. Sodium carbonate (99.5% purity) was purchased from Merck, India. Absolute ethanol (analytical grade) was bought from PanReac (Barcelona, Spain), while water and methanol (HPLC grade) were purchased from LAB-SCAN (Gliwice, Poland).

2.2 Cultivation of LM in the Mushroom Farm

Temperature in the cultivation room was controlled at 16 ± 1 °C with the photo period at 12 hours of light and 12 hours of darkness. The measurement of CO_2 in the room was measured by a sensor, and its average intensity during the cultivation was around 900 mg/L. Initial moisture content of the substrates for the mycelium growth was 70-80%. Figure 1 shows the substrate bags with LM mycelium growths in the incubation room at Fresh and Friendly Farm.

2.3 Extraction and Determination of Ergosterol

Ten grams of the freeze-dried samples from both Reg-LM and Ir-LM were soaked in 200 mL of absolute ethanol for 3 days at room temperature (25 ± 3 °C) in the dark. After filtering the suspensions, the clean supernatant was collected and all solvents were eliminated using a rotary evaporator (R114, Buchi, Switzerland), at 50 °C. The dried extract was kept at -20 °C in the dark until use. High-performance liquid chromatography (HPLC) used to determine ergosterol and vitamin D_2 in the dried extracts.

2.4 UVC-Irradiation of Ir-LM

The extract from Ir-LM was dissolved with absolute methanol in cylindrical vessels and exposed to the UVC at 254 nm with the intensity of 145 μw/cm^2 (determined by UVC meter, Solarmeter® version 8.0, Solar Light Company Inc.) at room temperature for 120 min, at 5 cm away from the lamp. HPLC was used to determine the levels of ergosterol and vitamin D_2.

2.5 Antioxidant Properties

2.5.1 Total Phenolic Content (TPC)

Total phenolic contents of the extracts from both Reg-LM and Ir-LM were evaluated using Folin-Ciocalteu reagent with the absorbance at 750 nm, measured by a 96-well microplate reader (Bio-Rad, iMark, USA). All experiments were performed in triplicates. The TPC were determined as gallic acid equivalents (GAE)/g dried LM using gallic acid as the reference (Rosa et al., 2017).

2.5.2 DPPH Scavenging Activity

The DPPH radical scavenging experiment was modified slightly from Ahmed et al. (2012) in order to assess the antioxidant ability of LM extracts. DPPH was dissolved in absolute methanol at a concentration of 0.5 mM to create DPPH radical solution. Fifty μL of DPPH solution were added to 50 μL of the extract in each well. The plate was incubated for 30 minutes in the dark at room temperature. Trolox was used to create a calibration curve, with methanol serving as the blank. Absorbance of the solution was measured by a microplate reader (Bio-Rad, iMark, USA) at 540 nm. All experiments were carried out in triplicates and scavenging ability was calculated as mg Trolox equivalent (TE)/g dried LM followed Eq. 1:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of the control, which has all reagents present minus the samples. A_{sample} is the absorbance of the mushroom extracts with reagents added.

2.5.3 ABTS Scavenging Activity

The ABTS radical scavenging assay was performed followed Ahmed et al. (2012) with minor modification. The ABTS radical was determined by reacting 200 mL of 140 mM $K_2S_2O_8$ solution with 7 mM of ABTS solution, and allowed the mixture to react for 16 hours at room temperature in the dark. Absorbance of the working ABTS solution was measured on a microplate reader (Bio-Rad, iMark) at 750 nm. ABTS solution used for the measurement was diluted with absolute methanol to gain the absorbance approximately 1.1 to 1.2 at 750 nm. In 96-well microtiter plates, 50 L of extract solution was combined with 100 L of ABTS solution. Methanol was used as a blank. The absorbance was read within 30 minutes at room temperature. All measurements were performed in triplicate. The antioxidant activity was determined as mg TE/g dried LM as detailed in Eq. 1.

2.6 Analysis of Bioactive Compounds by HPLC

Ergosterol and other compounds in the Reg-LM and Ir-LM extracts were analyzed using HPLC (Agilent Technology 1,200 series, Germany). Eclipse Zorbax XDB-C 18 (Agilent, 250 4.6 mm, 5 m) analytical column and Zorbax XDB-C 18 (Agilent, 12.5 4.6 mm, 5 m) guard column were used with the HPLC system to measure the compounds at 282 nm using the UVVIS LC detector. Methanol and water were mixed in the mobile phase at a 98:2 ratio. Ergosterol was quantified from the calibration curve of ergosterol standard. The content of hericenone C and hericene A were calculated from their peak area compared with the area of ergosterol.

The content of each bioactive compound after extraction was presented in the unit of mg/g extract, mg/g dried LM, and mg/g fresh LM. The unit mg/g extract was calculated as mg bioactive compound per gram of dried extract while mg/g dried LM and mg/g fresh LM were calculated as the mg bioactive compound per gram of dried LM powder and fresh LM fruiting body, respectively.

2.7 Statistical Data Analysis

All experiments were performed with three replications. Data were analyzed using IBM SPSS 26 for Windows (SPSS Inc.) with independent t-test to compare the means of each treatment. To compare the difference between each sample, the significance level at $p < 0.05$ was employed.

3 RESULTS AND DISCUSSIONS

3.1 Cultivation of LM in the Mushroom Farm

Production yield, percentage of Reg-LM and Ir-LM as well as the calculated economic losses were presented in Table 1. Irregular-shape LM (Ir-LM) occurred during the cultivation were sold as low price, consequently leads to economic loss for the mushroom farm. Ir-LM is considered as by-products, equals to around 20% of total LM cultivated in the farm. Total production of LM in the farm was about 3,270 Bahts/batch or 156,967 Bahts/year since approximately 48 batches were cultivated per year (Table 1). Similar information was reported by Aguayo et al. (2017) that high amounts of irregular-shape of Button mushroom (*Agaricus Bisporus*) fruiting bodies (around 20% of total production) were generated during the mushroom cultivation. They are considered as by-products since their misshaped caps or stalks did not meet the product specifications set by retailers.

Table 1: Statistical data for the production of Lion's Mane mushroom at Fresh & Friendly Farm mushroom farm*.

	Reg-LM	Ir-LM
Production yield (Kg/batch)	168 (80%)	25 (20%)
Sale amount (Bahts/batch)	109,473	13,081
Economic loss (Bahts/batch)	-	3,270
Economic loss (Bahts/year)	-	156,967

Notes: *Data were collected and averaged from 4 batches of the cultivation during February, 2022.

Irregular-shape LM was found with the covered lids, randomly in the incubation room for mycelium and primordium induction. Most of the Ir-LM was found at the top of the shelf in the incubation room (Figure 1).

Figure 1: Incubation for the mycelium growths of Lion's Mane mushroom in the substrate bags at Fresh and Friendly Farm. The temperature in the incubation room was controlled at 16 ± 1 °C.

Morphological study indicated that the fruiting bodies of Reg-LM exhibited long spore-bearing spines and intricately branches with primary (PB), secondary (SB) and tertiary (TB) branches (Figure 2, A-C), while the fruiting bodies of Ir-LM aggregated thickly in branches which presented only primary (PB) and secondary (SB) branches with short spore-bearing spines (Figure 2, D-F).

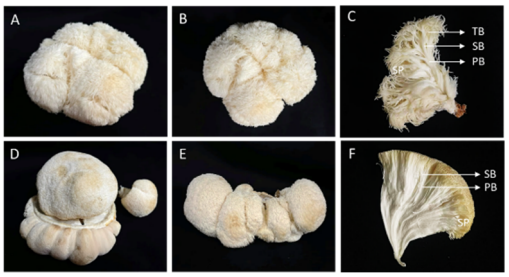

Figure 2: Macroscopic images on the fruiting bodies of Lion's Mane mushroom. (A-C): regular-shape (Reg-LM), and (D-F): irregular-shape (Ir-LM) mushroom. SP: spore-bearing spine, PB: primary branch, SB: second branch, TB: tertiary branch.

There are various environmental factors affecting the formation of primordia and fruiting bodies. The occurrence of Ir-LM might be due to both intrinsic and extrinsic factors. The spawning rate and the synthesis of volatile organic molecules like ethylene and 1-octen-3-ol may be examples of intrinsic factors. Yang et al. (2013) reported that an increase of spawning rate could reduce the time for mycelial colonization development of the fruiting bodies. According to Zhang et al. (2016), reducing the generation of ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) resulted in twice as many primordia that developed more quickly than those in the wild type strain of the button mushroom. Eastwood et al. (2013), reported that temperature and the reduced content of octenol (1-octen-3-ol) were the switches that controlled the plenary morphogenesis process, led to the production of fruiting bodies from the vegetative mycelium. Before directing the formation of undifferentiated hyphae into mature mushrooms, the volatile 1-octen-3-ol would first affect the differentiation of mycelial condenses into hyphal knots. Undifferentiated primordia would subsequently become differentiating primordia, depending on the temperature (Baars et al., 2020).

The possible extrinsic factors such as air composition and luminosity in the room might involve with the occurrence of Ir-LM since large amounts of Ir-LM were found at the top of the shelf

in the incubation room. According to Bellettini et al. (2019), mushroom fruiting body in a cultivation room might be generated by reducing the amounts of carbon dioxide and the rate of air circulation. The spawn bottles on the top shelf might expose to higher light intensity and air ventilation than the other shelf at lower levels (Figure 1). The higher light intensity in the environment caused the reduction of coloration, deformations and elongated stipe of mushroom fruiting bodies (Urben, 2004). In order to promote pin head production in button mushrooms, Visscher et al. (1979) hypothesized that an optimal concentration of ethylene and carbon dioxide are required.

3.2 Extraction and Determination of Ergosterol, Hericenone C and Hericene A

The ergosterol content shown in Table 2 reveals that Ir-LM contained significantly higher ($p < 0.05$) ergosterol content (2.52 ± 0.13 mg/g dried LM) than that of Reg-LM (2.15 ± 0.08 mg/g dried LM). Gąsecka et al. (2020) reported that the sample preparation techniques had a substantial impact on the ergosterol content of the LM mushroom. Specifically, the authors noted that ergosterol level was higher in the fresh mushroom samples (4.5 mg ergosterol/kg LM), and it declined when drying temperature was increased from 20 to 70 °C. Heleno et al. (2016) presented the use of ethanol as the extraction solvent in a soxhlet extraction of button mushrooms for 4 hours, yielding maximum concentration of ergosterol at 676 ± 3 mg/100 g. Mushroom by-products can be used for the recovery of bioactive compound from fruiting bodies or mycelium of mushrooms. Wang et al. (2015) found antichronic atrophic gastritis activity from the mycelium of LM by-products extracted with hot water (70 °C for 12 h) followed by precipitation with ethanol (80%). The antioxidant and antifungal activities in the ultrasound-assisted extract of LM mycelium with ethyl acetate were reported by Lu et al. (2014). Ergosterol was also extracted from the by-product of button mushroom fruiting bodies using ethanol and microwave-assisted at 132.8 °C and 1.6 g/L CO_2 flow rate for 19.4 min (Heleno et al., 2016).

Several chromatogram peaks were detected in both LM extracts, Reg-LM and Ir-LM and the contents of hericenone C and hericene A were compared (Figure 3, B-C). Ir-LM extract showed significantly higher ($p < 0.05$) in hericene A (1.25 ± 0.08 mg/g extract) than Reg-LM extracts (0.42 ± 0.02 mg/g extract) (Table 3). One of the bioactive substances in LM was hericene A, which had an IC_{50} value of 6.7 M and significantly reduced glucosidase activity (Lee at al., 2020). However, the content of hericenone C in both Reg-LM and Ir-LM were not significantly different. Ergosterol, hericenone C, and hericene A in the fresh Ir-LM fruiting bodies were found at 0.49 ± 0.03 mg/g fresh LM, 0.11 ± 0.01 mg/g fresh LM, and 0.02 ± 0.01 mg/g fresh LM, respectively.

Table 2: Ergosterol content in the extracts prepared from regular-shape (Reg-LM) and irregular-shape (Ir-LM) Lion's Mane mushroom.

Extract	Ergosterol		
	mg/g extract	mg/g dried LM	mg/g fresh LM
Reg-LM	19.42 ± 0.66^b	2.15 ± 0.08^b	0.41 ± 0.02^b
Ir-LM	26.77 ± 1.20^a	2.52 ± 0.13^a	0.49 ± 0.02^a

Notes: Different superscripts in the same column mean significant difference at $p < 0.05$.

Table 3: Concentration of the hericenone C and hericene A in regular-shape (Reg-LM) and irregular-shape (Ir-LM) Lion's Mane mushroom.

Extract	Hericenone C*			Hericene A*		
	mg/g extract	mg/g dried LM	mg/g fresh LM	mg/g extract	mg/g dried LM	mg/g fresh LM
Reg-LM	5.40 ± 0.18^a	0.60 ± 0.02^a	0.12 ± 0.01^a	0.42 ± 0.02^b	0.05 ± 0.01^b	0.01 ± 0.01^b
Ir-LM	5.79 ± 0.36^a	1.25 ± 0.08^a	0.11 ± 0.01^a	0.55 ± 0.04^a	0.12 ± 0.01^a	0.02 ± 0.01^a

Notes: Different superscripts in the same column mean significant difference at $p < 0.05$. *The content of hericenone C and hericene A were calculated from their peak area compared with that area of ergosterol.

3.3 Antioxidant Properties

Reg-LM and Ir-LM extracts were tested for their capacity to inhibit the DPPH radical, one of the few stable organic nitrogen radicals that presents purple color. This test relies on the determination of DPPH• loss upon sample response. Additionally, the antioxidant activity in the ABTS experiment was

calculated as the capacity of the extract to reduce color when they have direct contact with the radical $ABTS^{\bullet+}$ (Rivero-Cruz et al., 2020). DPPH and ABTS scavenging ability for Ir-LM extract were 0.27 ± 0.01 mg TE/g dried LM and 0.52 ± 0.04 mg TE/g dried LM, respectively as presented in Table 4. These antioxidant activities of Ir-LM were significantly higher ($p < 0.05$) than Reg-LM extract which presented 0.20 ± 0.01 mg TE/g dried LM and 0.46 ± 0.01 mg TE/g dried LM of DPPH and ABTS scavenging ability, respectively. The antioxidant concentration required needed to reduce the initial radical concentration by 50% is known as IC_{50}, which is a factor commonly used to evaluate antioxidant activity (Rivero-Cruz et al., 2020). The IC_{50} determined by DPPH (85.28 mg/ml), and ABTS scavenging ability (164.84 mg/ml) of Reg-LM extracts were higher than those of Ir-LM extract, 67.03 mg/ml and 151.27 mg/ml, respectively. In conclusion, Ir-LM exhibited higher antioxidant activities in terms of DPPH and ABTS than Reg-LM. The reasons that Ir-LM expressed significantly higher antioxidant capacity might be because it contained higher phenolic content (0.11 ± 0.02 mg GAE/g dried LM) than that of Reg-LM (0.06 ± 0.01mg GAE/g dried LM). Phenolic acids are important compounds contributing to antioxidant activity due to OH groups that can scavenge free radicals are present in their structures (Heleno et al., 2012).

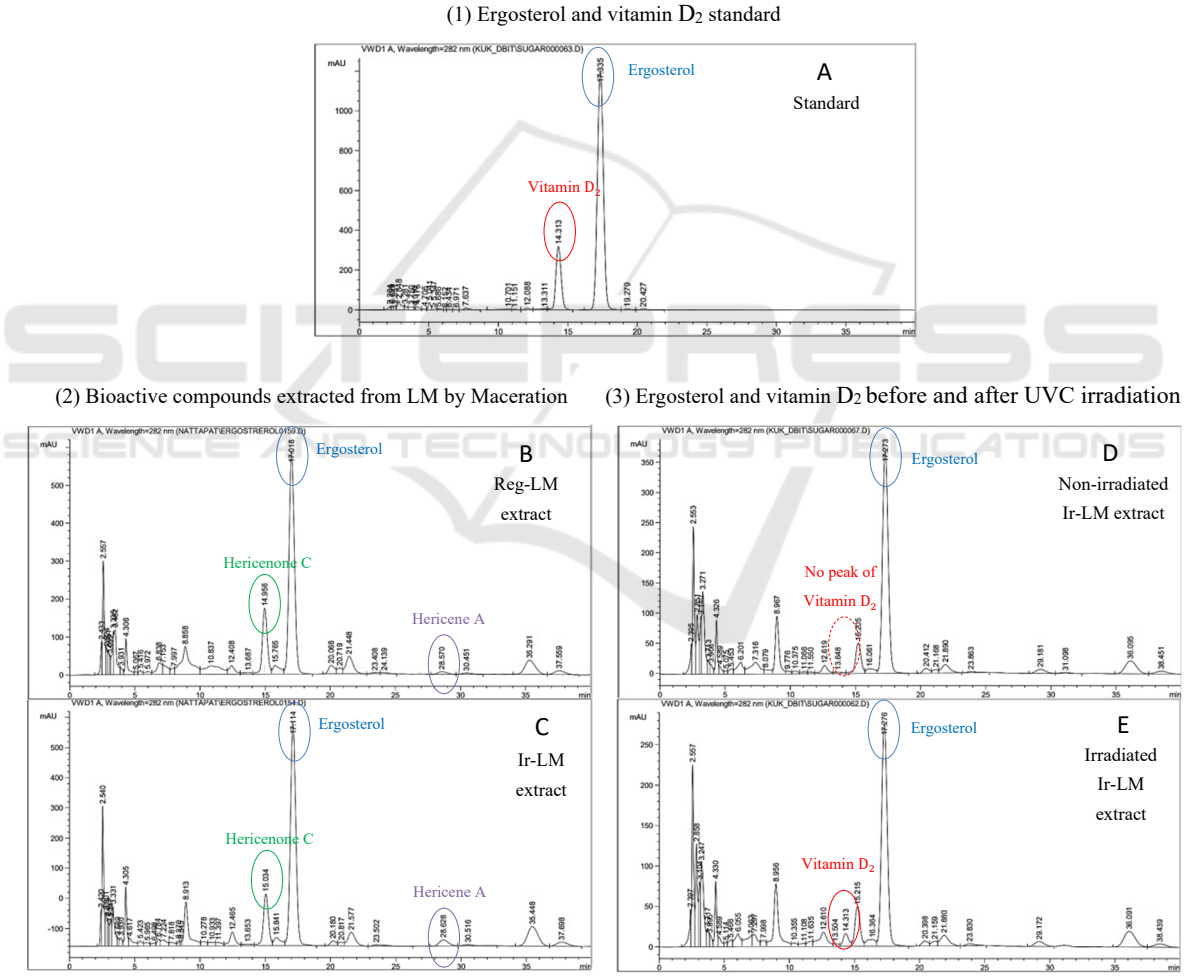

Figure 3: HPLC chromatograms of ergosterol and vitamin D_2 standard (A), crude ethanolic extract of regular-shape Lion's Mane mushroom (B), irregular-shape Lion's Mane mushroom (C), non-irradiated Ir-LM extract (D), and Ir-LM extract irradiated with UV-C (E).

Moreover, the higher ergosterol content in Ir-LM (Table 2) might involve with its antioxidant capacity. Dupont et al. (2021) described that the B-ring of ergosterol has two double bonds, which may

have antioxidant effects. Shao et al. (2010) also observed that, ergosterol was primarily responsible for the antioxidant activity in the lipophilic fraction of button mushroom. Therefore, Ir-LM was selected to be used for ultraviolet C (UVC) irradiation to convert ergosterol to vitamin D_2 in the extract.

3.4 Induction of Vitamin D_2 by UVC Irradiation

The most abundant sterol in cell membranes of fungi is ergosterol. It is critical for preserving permeability, trafficking, fluidity, and cytoskeletal structure (Abe and Hiraki, 2009). The transformation of ergosterol to vitamin D_2 can be obtained by the use of UV radiation, either artificially or naturally (Jäpelt and Jakobsen, 2013). Ergosterol undergoes photochemical cleavage at the B ring upon exposure to UV radiation, resulting in the synthesis of pre-vitamin D_2, an intermediate of vitamin D_2. After being heated, this intermediate then goes through thermal isomerization to produce vitamin D_2. The equilibrium between thermal and photochemical processes is crucial for the production of vitamin D_2 (Jasinghe et al., 2007).

The UV radiation for food processing and preservation is effective and favorable for the environment (Singh et al., 2021). Food irradiation is a technology that is secure and effective. The flavor of the product, taste, and odor, is unaffected by the radiation, and neither are the residues or poisons generated in the process (Bisht et al., 2021).

Table 4: Radical scavenging ability and total phenolic content in the extracts from the fruiting bodies of regular-shape (Reg-LM) and irregular-shape (Ir-LM) Lion's Mane mushroom.

	Reg-LM	Ir-LM
Total phenolic content (mg GAE/g dried LM)	0.06 $\pm$ 0.01^b	0.11 $\pm$ 0.02^a
DPPH radical scavenging ability (mg TE /g dried LM)	0.20 $\pm$ 0.01^b	0.27 $\pm$ 0.01^a
IC_{50} (mg/ml) by DPPH	85.28	67.03
ABTS radical scavenging ability (mg TE /g dried LM)	0.46 $\pm$ 0.01^b	0.52 $\pm$ 0.04^a
IC_{50} (mg/ml) by ABTS	164.84	151.27

Notes: Different superscripts in the same row mean significant difference at $p < 0.05$. IC_{50} is the concentration of antioxidants required to decrease the initial radical concentration by 50%; DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 3-ethylbenzthiazoline-6-sulphonic acid.

Vitamin D (D_2, or D_3, or both) ingested in the human body is incorporated into chylomicrons, which are absorbed by the lymphatic system and penetrated into venous blood. Vitamin D_2 cannot be biosynthesized by human body. Most oil-rich fish including the oil fish from salmon contains high content of vitamin D_3. Vitamin D sufficiency can enhance the absorption of calcium and phosphorus by 30-40% and 80%, respectively (Nair & Maseeh, 2012). According to the World Health Organization, low vitamin D intake, both in the form of D_2 and D_3, causes bone problems and raises the risk of other chronic diseases (Holick & Chen, 2008). Ergosterol was detected in the non-irradiated extract at 1.74 $\pm$ 0.09 mg/g dried LM, but no vitamin D_2 was found. Interestingly, a decrease of ergosterol at 13.5% with a detection of vitamin D_2 at 30.01 $\pm$ 7.09 µg/g dried LM were found in the irradiated extract (Table 5).

Table 5: Ergosterol and vitamin D_2 content in the extracts of non-irradiated and irradiated Lion's Mane mushroom with UV-C at 5 cm distance from UV-C lamp, for 2 h.

Samples	Ergosterol^{ns} (mg/g dried LM)	Vitamin D_2 (µg/g dried LM)
Non-irradiated extract (Control)	1.74 ± 0.09^a	nd
Irradiated extract	1.51 ± 0.15^a	30.01 $\pm$7.09

Notes: ns: no significant difference at $p < 0.05$.
Different superscripts in the same column mean significant difference at $p < 0.05$. nd: not detected

In addition, a decrease of ergosterol content was observed in the irradiated extract. Ergosterol might be transformed into other derivatives of vitamin D such as lumisterol or tachysterol (Morales et al., 2017). There are very few studies on the process of directly exposing mushroom extract to UVC to convert ergosterol to vitamin D_2. Morales et al. (2017) revealed that UVC irradiation to Shiitake mushroom extracts in ethanol at 25°C, for 1 h, at 4 cm distance from the lamp could generate vitamin D_2 enriched extracts higher than the irradiation of direct fruiting body. Generally, vitamin D_2 was induced by direct UV-light irradiation to fresh fruiting bodies or dried mushroom powder. Xu et al. (2020) reported that after being exposed to a high level of UVC (4 kJ/m^2) for 40 minutes, vitamin D_2 content of ground shiitake and Jew's ear powder increased from 1.38 g/g to 20.11 g/g and 4.13 g/g to 39.93 g/g, respectively.

With a UVB lamp at 25 °C, for 2 hours, and 19 cm away from the lamp, Huang et al. (2015) were able to irradiate oyster mushrooms and obtain 69 g/g vitamin D. In addition, Wittig et al. (2013) exposed

the same mushrooms to UVB radiation at 20 and 30 °C with a 10 cm distance from the light and found that after only 10 minutes of exposure, a higher vitamin D content (80 g/g) was obtained. These studies present that the mushrooms placed more closely to the UV lamp could generate a higher amount of vitamin D.

Currently, several industrial mushroom farms in the United States, Ireland, the Netherlands, and Australia have exposed their fresh mushrooms to UV light, producing at least 10 µg of vitamin D per 100 g of fresh weight. Therefore, a 100 g serving of the mushroom can satisfy 50 to 100% of a person's daily requirement for vitamin D. Additionally, UV-light-exposed dry mushrooms can also create sufficient content of vitamin D_2 for nutritional purposes (Cardwell et al., 2018).

4 CONCLUSIONS

Irregular-shape Lion's Mane (Ir-LM), mushroom considered as by-products, was used in the experiment to investigate the effect of UVC irradiation on the conversion of ergosterol in the mushroom extract to vitamin D_2. Ergosterol, hericenone C, hericene A, total phenolic content and antioxidant activities of Ir-LM were found significantly higher ($p < 0.05$) than those of Reg-LM. Irradiation with a low dose of UVC (145 µW/cm^2) for 120 minutes at 5 cm distance from the lamp caused the detection of vitamin D_2 in the irradiated extracts, but no detection in the non-irradiated sample. This research provides possible methods for a conversion to vitamin D_2 enriched extracts from mushroom by-products and use as nutritional supplement in medicinal foods. It is necessary to conduct additional research on the negative effects of UVC irradiation on the antioxidant activity, physical characteristics, and other significant nutritional parameters of irradiated mushroom extracts. The study on the effect of individual environmental factors on the induction of Ir-LM fruiting bodies are required.

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