JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2015. Vol. **9**(1), p. 109-117

Optimization of UV-B irradiation on Oyster Mushroom for the Production of Vitamin D_2, Chemical Composition, Antioxidant Activity and Vitamin D_2 Stability during Storage

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(Received: 10 December 2014; accepted: 06 January 2014)

Oyster mushrooms (*Pleurotus ostreatus* Fries.), were irradiated with Ultraviolet-B (UV-B) to convert ergosterol to vitamin D_2. The vitamin D_2 concentration was increased linearly from 1.78 μg/g DM to 9.01-28.07 μg/g DM, which was correlated with increase in time of irradiation, from 15 to 180 min while the ergosterol content in mushrooms was decreased. After irradiation for 120-180 min, oyster mushrooms had the greatest level of vitamin D_2 concentration. The conversion of ergosterol to vitamin D_2 was completed within 120 min. At every irradiation time point, there were no deleterious effects on proximate, amino acid and fatty acid compositions and antioxidant properties of oyster mushrooms. UV-B irradiation for 60-180 min could lead to degradation of vitamin D_2 in oyster mushroom after 6 days under refrigerated condition. This result suggested that UV-B irradiation for 15 min with low energy was the optimum treatment for the production of vitamin D_2 recommended per day and also vitamin D_2 concentration remained relatively stable in oyster mushrooms during storage. In addition, the high level of vitamin D_2 in mushrooms after UV-B treatment for 120-180 min may be advantageous to develop into consumable food or feed products for humans or animals.

Key words: *Pleurotus ostreatus*, UV-B exposure, ergocalciferol, duration, proximate, amino acid, fatty acid, antioxidant properties.

Pleurotus ostreatus Fries., commonly known as oyster mushroom, is a white-rot fungus. This fungus can be naturally found in tropical and subtropical rainforests and cultivated in many parts of the world including Thailand[1]. Oyster mushroom is delicious food and has a great nutritional value e.g. quite rich in protein with high content of essential amino acids and fiber and poor in fat. It also has high quantities of vitamins, minerals, unsaturated fatty acids and phenolic compounds[2].

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Oyster mushroom is recognized as a good source of vitamin D. Mushrooms contain a high amount of ergosterol which can be converted to vitamin D_2.When mushrooms are exposed to UV light, ergosterol undergoes photolysis to yield a variety of photo-irradiation products, principally previtamin D_2, tachysterol and lumisterol. The previtamin $D_2$is spontaneously rearranged into vitamin D_2[3]. In nature, some wild edible mushrooms were found to contain a limited amount of vitamin D_2[4]. The reason why these mushrooms possess a small content of vitamin D_2 is that naturally, they may be exposed to UV light which comprises only 8–9 % of the total solar spectrum. The cultivated mushrooms also had deficient in vitamin D_2. They

may not be exposed to the sunlight, which is essential in the natural production of vitamin D_2[5, 6].Previous research showed that the concentration of vitamin D_2 in wild and cultivated mushrooms could be higher if they were exposed to sunlight, artificial light such as UV light and Pulsed UV light (PUV)[7, 8]. The conversion of vitamin D_2 by UV light consists of three sub-regions of wavelengths, including UV-C (190-290 nm), UV-B (290-320 nm) and UV-A (320-400 nm)[9]. Some studies of the effect of UV irradiation on the conversion of ergosterol to vitamin D_2 in edible mushrooms reported that the conversion to vitamin $D_2$was highest under UV-B compared to those under UV-A or UV-C. However, the comparison is compromised, since the UV intensities were different but the same amount of irradiation time. Hence, the actual doses of UV were different[10, 11]. The effects of UV light on vitamin D_2 production among various mushroom species have been recently reported by a number of authors. These studies have shown that exposure to UV light can increase vitamin D_2 content in mushrooms from non-detectable level to over 100% [9, 12].

Moreover, oyster mushroom has high amounts of antioxidants components such as ascorbic acid, β-carotene, tocopherol and phenolic compound[13]. Antioxidants play an important role in maintaining human health due to their ability to scavenge free radicals in the bodies. Although human body is designed to have its own defense and repair systems that have evolved to protect against oxidative damage, these systems are insufficient to entirely prevent damages[14, 15].The consumption of supplementary antioxidants in foods such as in fruits, vegetables and mushrooms are useful to reduce oxidative damage in human body[16].Several researchers have found that high amounts of antioxidants in mushrooms may prevent the oxidative stress caused by the presence of free radicals which lead to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis[17, 18].

Although, there are many studies on the conversion of ergosterol to vitamin D_2 by UV-B light, they have not been studied the proximate chemical composition and antioxidant properties in oyster mushrooms after UV-B irradiation. There is also a lack of information available about the degradation of vitamin D_2 in irradiated oyster mushrooms during storage. Therefore, the objective of this research were to investigate, firstly, the effect of UV-B exposure duration on the conversion of vitamin D_2 and antioxidant activities of oyster mushrooms, secondly, to determine the proximate, chemical compositions (amino acid and fatty acid) and antioxidant activities of irradiated oyster mushrooms. The vitamin D_2 degradation in irradiated mushrooms during storage conditions was also examined. This research mainly aimed to investigate the optimal time of UV-B irradiation for production of the recommended daily allowance of vitamin D_2 with minor effect on chemical compositions, antioxidant activities and vitamin D_2 retention in oyster mushroom during storage.

MATERIALS AND METHODS

Preparation of Samples and Irradiation Procedure

Oyster mushrooms (*Pleurotus ostreatus* Fries.) were obtained from local farms in KhonKaen province, Thailand. They were packed in plastic bags and refrigerated at 1-2°C before treatment. Prior to the treatment, the stored mushrooms were left at room temperature for 4 h and placed on aluminum tray. The samples were placed 15 cm away from the irradiation source and exposed to the UV-B (ultraviolet-B) in an irradiation chamber.The UV-B unit was used with 8 UV-B lamps (313±12 nm, Philips TL-D18W) 604 cm in length and the total treatment area was 100x120 cm^2. Mushrooms were treated with UV-B irradiation for 15, 30, 60, 90, 120, 150 and 180 min. The rates of irradiation dose received by the mushrooms under UV-B light were 45.86, 91.72, 183.44, 275.16, 366.89, 458.60 and 550.32 J/cm^2 which calculated by multiplying intensity (J/cm^2) by times. All experiments were carried out at 25 – 28°C in triplicate. After UV-B irradiation mushrooms were separately freeze – dried, grounded to powder with a blender and then stored at -20°C until analysis.

Post-harvest of mushrooms after UV-B treatment

After UV-B irradiation for 15, 60 and 180 min, oyster mushrooms were placed in plastic containers (3-5 mushrooms per pack) and wrapped with plastic sheets. Mushrooms were placed in refrigeration at 1-2°C for 1, 3, 6, 9 and 12 days. After harvest of each period, mushrooms were lyophilized, homogenized with a blender and stored at -20°C before vitamin D_2 analysis.

Analysis of Ergosterol and Vitamin D_2

Quantification of ergosterol and vitamin D_2 was extracted and analyzed according to the method described previously [9]. Freeze-dried mushroom of each sample powder (1 g) was weighed into 250 ml round bottom flask and mixed with 1 g of L-ascorbic acid, 50 ml of 99% ethanol and 25 ml of 50% potassium hydroxide. The mixture was shaken and saponified under reflux at 85°C for 30 min. It was immediately cooled to the room temperature and poured into a separating funnel. The mixture was first extracted with 10 ml of deionized water and 30 ml of *n*-hexane. The organic layers were washed three times with deionized water until neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 50°C and re-dissolved in 2 ml of a mixed solution of eluent (methanol/acetonitrile = 75:25 v/v) and isopropyl alcohol (2:1 v/v). The sample was passed through a 0.45 µm non pyrogenic filter. A volume of 20 µl of filtered sample was injected into the HPLC system (LC 20A, Shimadzu, Japan) and eluted through a reversed phase C18 column. The mobile phase was methanol/acetonitrile (75:25 v/v), at flow rate of 1 ml/min. The UV detection of elute was performed at 264 nm. Ergosterol and vitamin D_2 qualitatively analyzed by comparing the retention times of standards obtained (ergosterol and ergocalciferol, Sigma Chemicals, Steinheim, Germany) and quantification was done by using a calibration curve.

Antioxidant Activities

Ten grams of each freeze-dried powder of oyster mushroom, treated with UV-B for 0-180 min, were continuously extracted with 100 ml of 95% ethanol for 24 h. The ethanolic extracts were filtered through Whatman No. 1 filter paper and the extract was evaporated at 40°C to dryness. The dried extract was redissolved in ethanol and stored at 4 °C for further uses. Radical scavenging activity by antioxidant in oyster mushroom was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical and adopted according to the method described previously. Various concentrations (0.15-5 mg/mL) of mushrooms extracts were added to 1 mL of 0.5 DPPH radical solutions in methanol. The mixed was shaken vigorously and allowed to stand for 15 min at room temperature. A 95% methanol was used as control and the solution without extract. The absorbance was measured against the blank reagent at 517 nm using a UV/VIS spectrophotometer (UV-1700 Pharamspec). The assay were carried out in triplicate and half maximaleffective concentration (EC_{50}) value (concentration of sample required to scavenge 50%of DPPH free radical) was calculated. The scavenging effect on the DPPH inhibition of the DPPH free radical in percent (%) was calculated according to the equation: Percentage (%) of DPPH radical scavenging = (1-(Ac-As)) × 100 where,Ac is the absorbance of control which is the absorbance of the DPPH solution. As is absorbance of solution when the sample extract has been added at a particular level.

Proximate Analysis

Crude protein, crude lipid, moisture, fiber, and ash of non-irradiated mushroom and irradiated mushroom sample (UV-B irradiation for 180 min) were determined according to the Association of Official Analytical Chemists (AOAC) methods (AOAC, 2006). Total protein was determined by the Kjeldahl method with a conversion factor 6.25. The weight of fat extracted was determined by the ether extraction method using a Soxhlet extractor and dietary fiber by the enzymatic gravimetric method. The moisture content was determined by oven drying at 105°C until constant weight. The ash content was determined by muffle furnace at 550°C for 5 h or until light gray or white ash was obtained.Carbohydrate was estimated by subtraction of the percentage of protein, lipid, ash and fiber.

Amino Acid Analysis

Non-irradiated mushroom and irradiated mushroom sample (UV-B irradiation for 180 min) were analyzed of amino acid. Performic acid oxidation was undertaken prior to hydrolysis to oxidize cysteine and methionine. Sodium metabisulfite was added to decompose performic acid before hydrolysis. Amino acids were liberated from protein by hydrolysis with 6 M hydrochloric acid containing 1% phenol at 110°C for 24 h (AOAC, 2000 method 994.12). L-Norleucine was added as an internal standard. Hydrolysated solution was filtered through a sintered glass filter and then the filtrate was evaporated to 5 mL under a vacuum at 40°C using a rotary evaporator. Sodium citrate buffer was added to the evaporated test solution to adjust the pH to 2.2 with 2M NaOH. Amino acids were determined using GC-MS (GC:

Agilent technologies model 6890N made in Germany and MS: Agilent technologies model 5973 made in USA) equipped with a PhenomenexZebron ZB-AAA (10 m×0.25 mm) column. Helium was used as a carrier gas at a flow of 1.1 mL min^{-1}. Since tryptophan is destroyed by acid hydrolysis, alkaline hydrolysis of the protein sample was conducted for analysis of tryptophan (according to AOAC, 2000 official method 988.15). Protein from samples was hydrolysed for 22 h using 4.4M NaOH at 100°C for the determination of the tryptophan content.

Fatty Acid Analysis

Total lipids were extracted from non-irradiated mushroom and irradiated mushroom sample (UV-B irradiation for 180 min) by hydrolytic methods following the 996.06 official methods AOAC (2000). Fatty acid methyl esters (FAMEs) were prepared from the isolated lipids by heating with methanolic NaOH first and then with BF3 methanol for esterification. FAMEs were quantitatively measured by gas chromatography (Agilent technologies model 6890N made in Germany) equipped with a capillary column (100 m×0.25 mm ID, 0.20 µm film, fused silica capillary, SP-2560) using helium as a carrier gas. Injection was performed by the split method at 260°C. FAMEs were separated with the following oven program: 1) 140°C for 5 min; and 2) increase at a rate of 4°C min^{-1} up to 240°C. The fatty acids were identified with reference to the retention time and gas-chromatography (GC) library.

RESULTS AND DISCUSSION

The conversion of ergosterol to vitamin D_2 in oyster mushrooms after UV-B irradiation

After UV-B irradiation for 15-180 min, vitamin D_2 contents in oyster mushrooms constantly increased from 1.78 µg/g dry weight for the control (0 min) to 9.09-28.07 µg/g dry weight with the ratio of 510.67-1,576.97% (Table 1). The vitamin D_2 concentration was correlated with accumulative time of irradiation. The yields of vitamin D_2, after UV-B irradiation for 120-180 min were shown to be non-significant(p>0.05). It was obvious that this conversionwas completed within 120 min and prolonged irradiation does not contribute highly to vitamin D_2 level. In addition, an excessive UV dosage might lead to decreased quality of mushrooms such as surface discoloration and decreased moisture content[9].As shown in Table 1,the concentration of ergosterol in mushroom decreased to1.06-6.38% due to the extensive irradiation time.The ergosterol content in mushrooms decreased because some of ergosterol might be partially converted to vitamin D_2[10]. The conversion of ergosterol and vitamin D_2 in oyster mushrooms under this condition could be predicted from the following equations (Fig. 1):

Amount of ergosterol = $-0.063\ln(t) + 3.7414$
Amount of vitamin $D_2 = 11.111\ln(t) - 29.428$

Where amount of ergosterol is in mg/g dry weight and vitamin D_2 is in µg/g dry weight. t is the time of irradiation in min.

The conversion of ergosterol to vitamin D_2 was very low of quantity. Even through ergosterol in mushroom was found in milligrams, the yield of vitamin D_2 from this conversion was only in micrograms. The reason of this vitamin D_2 yielded lower than expected after treated with UV, could possibly be from the limitation of UV penetration into the mushroom tissues. UV-B is able to penetrate through the epidermal layer not deeper than 50 µm approximately from the surface of the mushroom[10]. However, the methods of exposing mushrooms to UV light have an effect on increasing vitamin D_2 concentration. Previous research reported that exposing sliced mushrooms to UV light could give higher vitamin D_2 level than that of whole mushrooms[7]. Because the UV irradiation acts only on the surface of the mushroom, it is important to fully expose sliced mushrooms or all sides of the mushrooms to the UV-B[7, 19].Moreover, exposing mushrooms to Pulsed UV uses a broad spectrum lamp along with high intensity pulses. It can enhance vitamin D_2 content more than continuous UV light systemswith intense pulses in short time of irradiation and with no negative effects on quality[12].

DPPH radical scavenging activity

Oyster mushrooms extract showed positive antioxidant activity by fading the violet color of DPPH solution to yellow and pale violet. The scavenging activities of radical were in direct proportion with the concentrations of the extracts. The DPPH radical scavenging activity of the ethanolic of oyster mushrooms(UV-B irradiation

for 15-180 min) extracts increased with increase in concentration. At 5 mg/ml extract concentration of all irradiated oyster mushrooms displayed the highest scavenging activity on DPPH radicles (78.93-82.44%).The results of antioxidant activity were presented as half maximal effective concentration (EC_{50}) value which is the concentration of extracts capable required scavenging 50% of DPPH free radical. The EC_{50} values of all samples tested were 2.71±0.41 to 2.94±0.40 mg/ml (Table 2). Compared to control (non-irradiation), EC_{50} values of all irradiated mushrooms were not significant(p<0.05). This suggested that UV-B irradiation for 15-180 min in this condition was not effective to antioxidant properties of oyster mushrooms. The result in this treatment is different from Pulsed UV treatment. Pulsed UV light can enhance vitamin D_2 in mushroom more than UV-B irradiation, however, Pulsed UV give lower antioxidant properties after irradiation. This loss could be attributed to the thermal damage caused by the use of high intensity[12].

The proximate and chemical compositions of oyster mushrooms after UV-B treatment

Comparisons of the proximate compositions of non-irradiated and irradiated oyster mushrooms (180 min) are shown in Table 3. Mushrooms contained high level of protein, carbohydrate and fiber. However, they had low level of lipid. Moisture, protein, lipid, carbohydrate, fiber and ash contentsin non-irradiated and irradiated oyster mushrooms were not significantly different (p>0.05). The amino acid profiles are presented in Table 4. Oyster mushrooms contained 10 essential amino acids (EAA). The most abundant amino acid in non-irradiated and irradiated oyster mushrooms was lysine (35.81 and 35.79 mg/g dry weight). It was no significant difference(p>0.05)in the amounts of amino acid profiles of non-irradiated

Table 1. The concentration of ergosterol and vitamin D_2in oyster mushrooms after UV-B irradiation

Time	Ergosterol		Vitamin D_2	
(min.)	Content (mg/g DM)	RelativePercentage(%)	Content(µg/g DM)	RelativePercentage (%)
0	3.57±0.54^{a}*	100	1.78±0.54^{f}	100
15	3.52±0.39^{b}	98.94	9.09±0.70^{e}	510.67
30	3.50±0.22^{c}	97.87	12.09±0.65^{d}	679.21
60	3.45±0.19^{d}	97.34	19.04±0.54^{c}	1069.66
90	3.43±0.12^{e}	94.95	26.44±0.63^{b}	1485.39
120	3.42±0.13^{e}	93.62	27.62±0.54^{a}	1566.85
150	3.42±0.15^{e}	93.62	27.87±0.72^{a}	1565.73
180	3.42±0.17^{e}	93.88	28.07±0.67^{a}	1576.97

*Means±SD with different letters within the same row are significantly different (p<0.05)

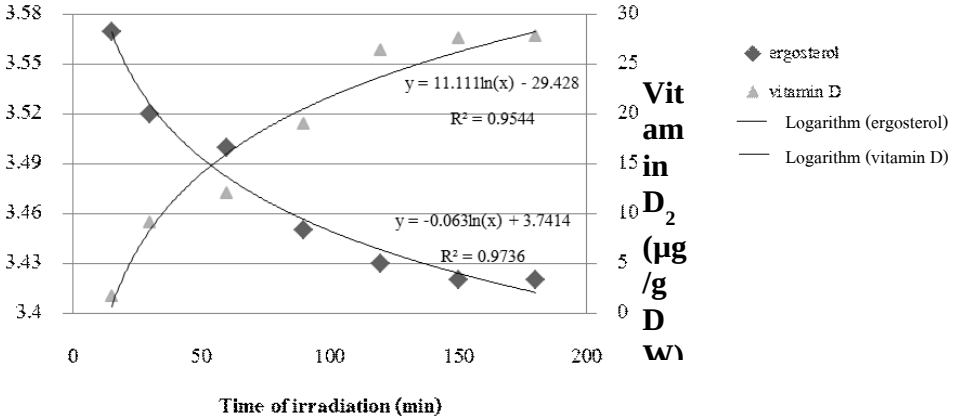

Fig. 1. Effects of UV-B irradiation on concentration of ergosterol and vitamin D_2 content in oyster mushrooms

and irradiated oyster mushrooms as shown table 4. In Table 5, Oyster mushrooms contained saturated and unsaturated fatty acid. The major fatty acids found in oyster mushrooms were palmitic acid, oleic acid and linoleic acid. In this study, fatty acid compositions of non-irradiated and irradiated oyster mushrooms were not significantly different(p>0.05). The data of proximate, amino acid and fatty acid compositions of oyster mushrooms after UV-irradiation for 180 min remained stable. This study demonstrated that UV-B irradiation did not affect the proximate and chemical composition of oyster mushrooms.

Table 2. Effective concentration at 50% (EC50) values in the antioxidant assay of oyster mushrooms irradiated by UV-B light for 15-180 min

Time of irradiation (min.)	EC_{50} value (mg extract/ml)
0	2.82±0.27 a*
15	2.93±0.52a
30	2.71±0.41a
60	2.81±0.30a
90	2.67±0.48a
120	2.76±0.49a
150	2.94±0.40a
180	2.71±0.46a

*Means±SD with different letters within the same row are significantly different (p<0.05)

Table 3. Proximate composition of non-irradiated and irradiated oyster mushrooms, Moisture, protein, lipid, carbohydrate, fiber and ash content are expressed as a percentage

Proximate composition	Non-irradiated mushrooms	Irradiated mushrooms
Moisture	3.12±0.09a	3.07±0.04a
Protein	22.75±0.14a	22.05±0.41a
Lipid	0.35±0.09a	0.32±0.17a
Carbohydrate	68.59±0.20a	68.93±0.30a
Fiber	47.72±0.21a	47.44±0.42a
Ash	5.19±0.03a	5.22±0.02a

Values in the same row with different letters are significantly different (p < 0.05, n = 3).

Table 4. Amino acid content of non-irradiated and irradiated oyster mushrooms (mg/g fresh weight)

Amino acids	Non-irradiated mushrooms	Irradiated mushrooms
Alanine	4.59±0.30a	4.53±0.70a
Arginine*	2.12±0.01a	2.20±0.01a
Aspartic acid	6.41±0.11a	6.39±0.16a
Cysteine	1.19±0.12a	1.22±0.14a
Glutamic acid	17.36±0.44a	17.58±0.38a
Glycine	3.35±0.12a	3.26±0.19a
Histidine*	15.56±0.34a	15.38±0.27a
Isoleucine*	8.40±0.47a	8.44±0.31a
Leucine*	15.46±0.48a	15.75±0.27a
Lysine*	35.81±0.88a	35.79±0.48a
Methionine*	1.81±0.31a	2.02±0.24a
Phenylalanine*	17.06±0.63a	17.23±0.30a
Proline	2.47±0.37a	2.92±0.44a
Serine	2.97±0.29a	3.13±0.13a
Threonine*	2.52±0.44a	2.29±0.43a
Tryptophan*	1.6±0.46a	1.49±0.37a
Tyrosine	13.69±0.49a	13.48±0.43a
Valine*	6.75±0.39a	6.58±0.78a
Total amino acid	159.12	159.68

Values in the same row with different letters are significantly different at p < 0.05, n = 3
*Essential amino acids

Table 5. Fatty acid composition of non-irradiated and irradiated oyster mushrooms (mg/g fresh weight)

Fatty acid	Non-irradiated mushrooms	Irradiated mushrooms
Saturated Fatty acid		
Butyric acid	2.31±0.51a	2.75±0.27a
Pentadecanoic acid	0.51±0.07a	0.55±0.04a
Palmitic acid	6.71±0.19a	6.54±0.28a
Heptadecanoic acid	0.07±0.02a	0.05±0.02a
Stearic acid	1.52±0.40a	1.38±0.27a
Behenic acid	0.24±0.06a	0.24±0.04a
Linoceric acid	0.60±0.07a	0.57±0.05a
Unsaturated Fatty acid		
Oleic acid	5.49±0.28a	5.59±0.27a
Eicosenoic acid	0.07±0.01a	0.07±0.01a
Nervonic acid	0.76±0.06a	0.81±0.06a
Linoleic acid	16.78±0.46a	16.49±0.44a
Eicosenoic acid	0.08±0.01a	0.08±0.01a
Eicosadienoic acid	0.08±0.01a	0.08±0.01a
Arachidonic acid	0.16±0.03a	0.18±0.03a
Trans Fatty Acid	0.17±0.01a	0.17±0.01a
Trans-9-Elaidic acid	0.17±0.01a	0.17±0.01a
Total Fatty acid	35.72	35.72

Values in the same row with different letters are significantly different at p < 0.05, n = 3

Table 6. Vitamin D_2 concentrations in irradiated fresh oyster mushrooms after storage for 1-12 days

Time of UV-B treatment (min)	Vitamin D_2 after storage (µg/g DW.)						Mean of vitamin D_2 in each UV-B treatment
	0 days	1 days	3 days	6 days	9 days	12 days	
0	1.93±0.50^{g}**	1.92±0.61^{g}	1.82±0.77^{g}	1.52±0.51^{g}	1.44±0.56^{g}	1.36±0.57^{g}	1.67±0.25^{D}
15	9.13±0.53^{f}	9.11±0.67^{f}	8.98±0.43^{f}	8.76±0.46^{f}	8.62±0.36^{f}	8.47±0.45^{f}	8.82±0.30^{C}
60	19.63±0.51^{d}	19.62±0.34^{d}	18.84±0.75^{de}	18.48±0.58^{e}	18.14±0.62^{e}	18.09±0.41^{e}	18.80±0.69^{B}
180	28.68±0.67^{a}	28.56±0.57^{a}	28.01±0.39^{ab}	27.68±0.46^{bc}	27.31±0.51^{bc}	27.11±0.40^{c}	27.89±0.64^{A}
Mean of vitamin D_2 in each storage time	14.84±11.74$^{(A)}$	14.80±11.70$^{(AB)}$	14.41±11.44$^{(BC)}$	14.10±11.42$^{(CD)}$	13.88±11.27$^{(D)}$	13.74±11.25$^{(D)}$	14.29±6.63

*All data showed Means±SD.

Vitamin D_2 in irradiated mushroom during storage

Overall, the amount of vitamin D_2 increased dramatically (p<0.01) after exposed to UV-B at different exposure times but decreased significantly(p<0.01) after storage started from 3-12 days. The amount of vitamin D_2 in mushrooms exposed to UV-B for 15 min and control treatment (0 min) did not decrease significantly (p>0.05) during the storage study (Table 6). However, irradiated mushrooms for 60 and 180 min performed a significant (p<0.01) decrease in vitaminD_2 concentration after storage for 6-12 days. This result suggested that irradiated oyster mushroom, exposed to UV-B light for 60-180 min, could cause the degradation of vitamin D_2 after 3-4 days of refrigerated storage. Similar to the previous studies showed 1-4 days of postharvest time did not have any impact on vitamin D_2 formation in *Portabella* mushrooms (exposed to UV-B light)[20].In addition, the other research showed exposing button mushroom to Pulsed UV light, vitamin D_2 decreased significantly after postharvest storage for 3 days[21]. Fresh mushrooms only have a short shelf life and lose their biological activitiesand commercial value within 3-4 days after storage, due to browning, water loss, senescence and reduction in the biochemical reaction rates[22, 23]. High irradiation intensity leads to the decrease in quality of mushrooms such as cell death, heat stress (oxidation) and moisture content which could be lead to degradation of vitamin D_2 content in mushroom during storage[9].UV-B irradiation for 15 min to fresh mushrooms was suitable for consumption and could be distributed to markets in fresh or refrigerated conditions. Because UV-B irradiation for 15 min was the optimum treatment for the production of vitamin D_2 recommended per day (15µg/day)[24]and also vitamin D_2 concentration remained relatively stable in fresh mushrooms during storage.The conversion to vitamin $D_2$was highest under UV-B irradiation for 180 min but vitamin D_2concentration in fresh mushrooms of this treatment was decreased after storage. This treatment could be develop a method for treating commercially viable quantities of fresh mushrooms to produce dried mushroom powders with high of vitamin D_2. A mushroom powder having high levels of Vitamin D_2 may be used as a condiment to be sprinkled on or into foods, or may be incorporated

into a consumable food product.The comparisons study of vitamin D_2 conversion and vitamin D_2 stability during storage between fresh and freeze-dried powder of oyster mushrooms (data did not show), the increase in vitamin D_2 proportion in freeze-dried mushrooms powder was higher than in fresh mushrooms after UV-B irradiation at the same times and vitamin D_2content also remained stable after storage over 90 days. Freeze-drying is the process of removal of moisture from fresh mushroom to such a low level that microbial and biochemical activities are checked due to reduced water activity[25].

CONCLUSION

In conclusion, UV-B light could be influential to the escalation of the vitamin D_2 concentration in oyster mushroom. The vitamin D_2 content was increased linearly correlated with increase in time of irradiation or the irradiation dose and the conversion was almost completed within 120 min. UV-B treatments in this condition were suggested since they increased the concentration of vitamin D_2 significantly without any deleterious effects on proximate, chemical composition concentration and antioxidant properties of oyster mushrooms. However, prolonged UV-B irradiation dose could lead to degradation of vitamin D_2 in oyster mushroom after 6 days of refrigerated storage. In this experiment, UV-B irradiation for 15 min to 10g oyster mushrooms was exceedingly sufficient to obtain the recommended allowance of vitamin D_2 each day for 1 to 70 years of age (15µg/day)[24]and vitamin D_2 concentration remained relatively stable during storage.

ACKNOLEDGMENTS

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster and Applied Taxonomic Research Center,KhonKaen University.

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