Nutritional Qualities and Antioxidant Properties of *Hypsizygus marmoreus* as Affected by Light Source on Cultivation

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***Abstract*—*Hypsizygus marmoreus* (Peck.), also called bunashimeji and hon-shimej, is successfully cultivated and commercially available in Taiwan. Illumination with a Cold Cathode Fluorescent Lamp ($CCFL_S$) and Light Emitting-Diodes (LEDs) has lately become a more available technology and a more economic and energetically efficient way for light treatment on mushrooms. In this study, effects of illumination light source and wavelengths on the fruit body's production and content of nutritional qualities and antioxidant properties were investigated. The Biological Efficiency (BE) of the CCFLs lights was the highest at 94.71%. Based on the fresh weight (fw), CCFLs, LED_S 2W1B (180) and LED_S 2W1B (240) light cultivation contained higher amount of crude ash, crude protein, crude fat and crude fiber, whereas the LED_S 3W (180) contained higher amount of carbohydrate. For all samples, the content of polysaccharide ranged from 0.30 to 0.36 g/100 g fw. The total hydrolysis amino acid content in four light cultivation of *H. marmoreus* ranged from 1477.22 to 1709.33 mg/100 g fw and were in the descending order of LED_S 2W1B (180) > LED_S 2W1B (240) ~ CCFL > LED_S 3W (180). EC_{50} values were 4.54-6.67 mg/mL, 2.78-2.81 mg/mL, and 0.32-0.34 mg/mL for reducing power, scavenging ability on DPPH radicals and chelating ability on ferrous ions, respectively. The major antioxidant components found in ethanolic extracts were total phenols (9.39 to 11.50 mg/g). Based on the results obtained, light of different wavelengths and sources had little influence on nutritional qualities and antioxidant properties of *H. marmoreus* fruit bodies.**

***Index Terms*—*Hypsizygus marmoreus*, cold-cathode fluorescent lamps, light-emitting diodes, nutritional compounds, antioxidant**

I. Introduction

Hypsizygus marmoreus (Peck.) H. E. Bigelow, is a popular edible mushroom with a small, light-brown. Fruit bodies of *H. marmoreus* are usually produced in polypropylene bottles containing sterilized sawdust [1]. The mushroom is packaged by placing an entire cluster or several clusters into each overwrapped package. The cap of fruit bodies is dark tan and its color turns to gray brown to creamy brown when mature [2]. The mushroom has become increasingly popular in Taiwan due to its mildly sweet nutty flavor and crunchy texture, and probably the existence of some physiologically beneficial components [3]. Cultivated worldwide, *H. marmoreus* has exceptional medicinal applications, including as an antitumor agent, in addition to its popular/exotic culinary status [4].

Environmental factors such as light, pH, CO_2, temperature, and nutrients are important elements in the production of high-quality mushroom [5]. Light also induces the development of fruiting bodies, including stipe elongation and cap formation. Up till now, various light sources, such as incandescent, fluorescent, metal halide or high-pressure sodium lamps, were adopted as supplementary lights for cultivation of mushroom [5]. Cold cathode fluorescent lamps (CCFLs) are an effective light source due to their characteristics, including low heat emission, low energy consumption, long service life, light weight and small diameter [6]. In recent years, a newly developed light technology, the Light Emitting Diode (LED), has just begun to be adopted as a supplementary light source for the cultivation of mushroom. The use of blue light for oak mushroom has been shown to induce pigmentation of the primordial stage [7].

There have been few comparative studies about how combinations of different wavelengths of LED lights can affect the growth or quality of mushroom during cultivation in a growth chamber. The mushrooms were cultivated under CCFLs and LEDs under the same growth conditions. It is also an important determinant of a mushroom's composition in terms of proximate composition and amino acid components. The antioxidant properties of four light cultivation of mushroom, including their reducing power, scavenging ability of radicals, and chelating ability of ferrous ions, were determined. The total phenols of potential antioxidant components were also determined.

II. Materials and Methods

A. Mushroom Cultivation and Preparation

Four illumination lights *H. marmoreus* (including $CCFL_S$, LEDs 2W1B (180), LEDs 2W1B (240) and LEDs 3W (180)) were donated by Dai-Yang Farm Co.

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Ltd. (Taichung, Taiwan). An array of LED mixture used in the construction consists of white (W, 380 - 670 nm) and blue (B, 430 - 470 nm) lights. Four light treatments with different spectral composition were tested (Fig. 1). Three combinations of LEDs emitted different white/blue light ratio and bulb number: LED 2W1B 180, 120 bulb white light LEDs + 60 bulb blue light LEDs; LEDs 2W1B 240, 160 bulb white light LEDs + 80 bulb blue light LEDs; LED 3W 180, 180 bulb white light LEDs.

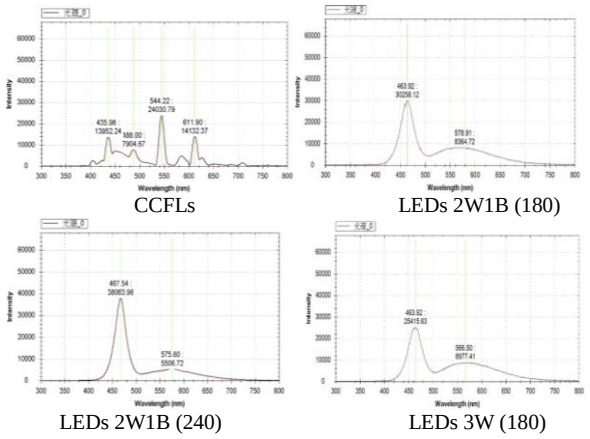

Figure 1. Spectral distribution of light in a greenhouse.

Figure 2. Lamp installation in the dimension of each layer is 60 cm×50 cm (a) and each layer is 30 cm in height; Picture of *H. marmoreus* fruit body in greenhouse cultivation (b).

Fig. 2 shows mushroom warehouses with grow lights for *H. marmoreus* cultivation in each one. The fruiting bodies of the *H. marmoreus* include the cap and stipe and are the edible part, which grow to approximately 10 cm in length under the CCFL and LEDs lights (Fig. 3). The Biological Efficiency (BE) was calculated from the substrate dry weight as follows: ([weight of fresh mushrooms harvested/substrate dry matter content] × 100). [8] Mushrooms grown under each light source were randomly selected and pooled into three samples of approximately 1 Kg each, freeze dried and ground with a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany) to obtain a coarse powder (60 mesh). The powdered samples were dispensed into matte plastic bags and stored in desiccator before use.

B. Proximate Composition, Polysaccharide and Amino Acid Analysis

The proximate composition of the mushrooms cultivated under the three lighting conditions, including the moisture, ash, crude fat, crude fiber, and crude protein, was determined according to AOAC methods. [9] The nitrogen factor used for the crude protein calculation was 4.38. [10] The carbohydrate content (%) was calculated by subtracting the crude ash, fat, fiber and protein contents from 100% dry matter. The energy was calculated according to the following equation: Energy (Kcal)= 4 × (g protein +g carbohydrate) + 9 × (g fat).

Polysaccharides were extracted and analyzed according to the method of Tseng *et al.* [11] Freeze-dried mushroom powder (1 g) was refluxed with 50 mL of deionized water for 30 min. The mixture was cooled to room temperature and filtered through Whatman No. 4 filter paper. The residue was then refluxed with two additional 10 mL portions of deionized water for 30 min, cooled and filtered. The combined filtrate was dialyzed in a Cellu Sep T2 tubular membrane (MWCO: 6,000-8,000, Membrane Filtration Products, Inc., Seguin TX, USA) for 24 h, resulting in a water-soluble polysaccharide sample. The polysaccharide content was determined by the phenol–sulfuric acid method according to Dubois *et al.* [12]

The total hydrolysis amino acid was extracted and analyzed according to the method of Huang *et al.* [13] with some modifications. Sample powder (500 mg) was shaken with 50 mL of 100 mmoL/L HCl (Union Chemical Co., Hsinchu, Taiwan) for 45 min at ambient temperature and filtered through Whatman No. 4 filter paper. The filtrate was then passed through a Millex-HV filter unit (13 mm), and filtered using a 0.45-μm PVDF filter. This filtrate was mixed with o-phthalaldehyde reagent (Sigma Chemical Co., St. Louis, MO) in an Eppendorf tube, shaken to facilitate derivatization and then immediately injected onto HPLC. The HPLC system was the same as for sugar and polyol analysis, but included a Hitachi L-7485 fluorescence detector with fluorescence excitation at 340 nm and emission at 450 nm, and a LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm, Merck, Darmstadt, Germany). The mobile phases were A, 50 mmoL/L sodium acetate (pH 5.7) containing 50 mL/L tetrahydofuran; B, deionized water; and C, methanol. The gradient was A: B: C 80:0:20 (v/v/v) to 33:0:67 for 0-38 min, 0:33:67 for 38-40 min, and 0:100:0 for 40-43 min. The flow rate was 1.2 mL/min. Each amino acid was identified using the authentic amino acid (Sigma) and quantified by the calibration curve of the authentic compound.

CCFLs

LEDs 2W1B (180)

LEDs 2W1B (240)

LEDs 3W (180)

Figure 3. Photographs of *H.* marmoreus fruiting bodies grown under different light cultivation.

C. Preparation of Extracts for Analysis of Antioxidant Properties and Components

Ethanolic extraction was performed using 10 g sample powder, and 100 mL 95% (v/v) ethanol was put into a glass beaker and shaken by rotary incubator shaker with extraction time at 25 ℃ and 150 rpm for 24 h and filtered. The filtrate was then evaporated on a rotary evaporator at 40 ℃ to dryness. The dried ethanolic extracts were stored at -20 ℃ until the analyses were carried out. Reducing power of extracts was determined, which followed the method describe by Oyaizu [14]. The ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) radicals was determined based on the work of Shimada *et al.* [15] The scavenging ability assayed is the ability of the extracts to react with DPPH radicals and to reduce most DPPH radical molecules. Chelating ability was determined according to the method described by Dinis *et al.* [16]. Chelating ability is the ability of the extracts to inhibit the complex formation of ferrozine with ferrous ions. The value (mg extract/mL) at an half-maximal effective concentration (EC_{50}) is the concentration at which the absorbance was 0.5 for reducing power; DPPH radicals were scavenged by 50%, ferrous ions were chelated by 50%. The EC_{50} values was obtained by interpolation from linear regression analysis.

Total phenols of extracts were determined according to the method of Taga *et al.* [17]. The absorbance was measured at 750 nm, with gallic acid (Sigma-Aldrich) used as standard. Results were expressed as milligram of gallic acid equivalents (GAE) per gram of extract.

D. Statistical Analysis

The EC_{50} values were obtained from linear regression analysis. A principal component analysis (PCA) was used to determine the correlations between the various compounds and their relationship with the lighting cultivation conditions. The PCA was accomplished with the XLStat 2018 (Addinsoft, Inc., Brooklyn, NY) software.

III. Results and Discussion

A. Proximate Composition

Total weight of four light cultivation *H. marmoreus* was in the range of 12.01 to 12.88 kg and peaked at CCFLs (Table I). The moisture content of mushrooms was in the range of 91.40 to 92.87%. Among their wet matters, the content of other components was in the order: carbohydrate (4.81-5.34%) > crude protein (2.18-2.46%) > crude ash (0.44-0.74%) > crude fiber (0.19-0.46%) > crude fat (0.17-0.41%). Obviously, mushrooms were high in carbohydrate and protein content but low in ash and fat content. Soluble polysaccharides were thought to the biologically active component in mushrooms, and their content was 0.30, 0.32, 0.32 and 0.36 g/100 g dw for CCFLS, LEDs 2W1B (180), LEDs 2W1B (240) and LEDs 3W (180), respectively. The discrepancy in the profiles of proximate compositions might be mainly due to the difference in the light cultivation used.

TABLE I. Proximate Composition of *H. marmoreus* from Different Light Source on the Cultivation

	CCFLs	LEDs 2W1B (180)	LEDs 2W1B (240)	LEDs 3W (180)
Total weight (kg) / box	12.88	12.01	12.37	12.08
BE (%)	94.71	88.31	90.96	88.82
	Content (g/100g fw)			
Moisture	91.69	91.40	91.76	91.87
Ash	0.71	0.74	0.67	0.44
Carbohydrates	4.92	4.99	4.81	5.34
Crude fat	0.33	0.41	0.39	0.17
Crude fiber	0.46	0.46	0.45	0.19
Crude protein	2.35	2.46	2.36	2.18
Energy	32.08	33.52	32.20	31.61
	Content (g/100g dw)			
Polysaccharide	0.30	0.32	0.32	0.36

B. Total Amino Acids Content

Total amino acid content ranged from 1477.22 to 1709.33 mg/100 g fw (Table II) and in descending order: Leds 2W1B (180) > Leds 2W1B (240) ~CCFL > Leds 3W (180). The amino acids with content more than 100 mg/ 100 g were in the descending order of methionine (113.93 mg/100 g) and tryptophan for CCFL, threonine (101.14 mg/ 100 g), leucine, methionine and tryptophan for Leds 2W1B (180), alanine (108.11 mg/ 100 g), methionine and tryptophan for Leds 2W1B (240), glutamic acid (110.65 mg/100 g) and tryptophan for Leds 3W (180). The results imply that the different light wavelength leads to an influence of amino acid composition in *H. marmoreus* cultivation.

C. Antioxidant Properties and Antioxidant Component

The antioxidant properties assayed herein are summarized in Table III, and the EC_{50} values (mg ethanolic extracts per mL) were calculated for comparison. The effectiveness of antioxidant properties correlates inversely with their EC_{50} values. Consistently in reducing power and chelating ability on ferrous ions, effectiveness was in descending order: LEDs 2W1B (240) > $CCFL_S$ > LEDs 2W1B (180) > LEDs 3W (180). Furthermore, the ethanolic extracts from CCFL light cultivation of *H. marmoreus* were more effective in scavenging ability on DPPH radicals. Among antioxidant

properties assayed, four ethanolic extracts were effective and that from CCFL and LEDs 2W1B (240) was more effective. Many total phenols are shown to have antioxidative activity, free radical scavenging capacity, and be hepatoprotective and anti-inflammatory. Phenols such as BHT (butylated hydroxytoluene) and gallate are known to be effective antioxidants. CCFL contained the highest total phenol content (Table III). A high amount of total phenol in ethanol extracts can explain its higher reducing ability and DPPH free radical scavenging ability. Due to their free radical scavenging abilities and ferrous ion chelating abilities, phenols may possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases.

TABLE II. AMINO ACID COMPOSITION OF *H. MARMOREUS* FROM DIFFERENT LIGHT SOURCE ON THE CULTIVATION

mg/100 g fw	CCFL	LED 2W1B (180)	LED 2W1B (240)	LED 3W (180)
Alanine	84.60	81.70	108.11	69.67
Arginine	75.79	79.81	69.22	93.01
Aspartic acid	41.88	46.35	37.00	45.45
Cysteine	36.73	39.47	61.96	33.25
Glutamic acid	64.07	97.18	74.16	110.65
Glycine	41.97	48.25	37.24	39.84
Histidine	29	31.82	29.09	31.06
Isoleucine	57.01	61.92	56.61	53.41
Leucine	94.90	103.03	94.10	90.89
Lysine	73.04	78.26	72.02	73.74
Methionine	113.93	112.83	109.92	94.23
Phenylalanine	43.96	47.90	43.51	47.07
Serine	82.52	97.09	113.30	85.83
Threonine	81.35	101.14	50.02	82.36
Tryptophan	541.81	579.64	519.12	432.43
Tyrosine	24.27	25.28	23.24	24.96
Valine	72.30	77.66	74.82	69.67
Total	1559.12	1709.33	1573.43	1477.22

TABLE III. EC_{50} VALUES OF ETHANOLIC EXTRACTS AND TOTAL PHENOL CONTENT FROM DIFFERENT LIGHT SOURCE ON THE CULTIVATION OF *H. MARMOREUS* IN ANTIOXIDANT PROPERTIES

	CCFLs	LEDs 2W1B (180)	LEDs 2W1B (240)	LEDs 3W (180)
	EC_{50} value [a] (mg extract/mL)			
Reducing power	4.58	5.26	4.54	6.67
Scavenging ability	2.78	2.81	2.83	2.80
Chelating ability	0.34	0.34	0.32	0.33
	Contents (mg/g)			
Total phenol	11.50	9.39	10.73	9.92

[a] EC_{50} value: the absorbance of 0.5 for reducing power; 50% of 2,2-diphenyl-1-picrylhydrazyl radicals scavenged; and 50% of ferrous ions chelated. The EC_{50} value was obtained by interpolation from linear regression analysis.

D. PCA Scrutinization

Despite the high number of statistically significant changes, it was not possible to identify overall trends, which might characterize the effects of light cultivation. Accordingly, the results were evaluated considering data for all samples and parameters simultaneously. PCA was conducted to gain an overview of the relationships among components and compounds of four light cultivation *H. marmoreus* (Fig. 4). The first two components (F1 and F2) explained 82.9% (60.23% + 22.36%) of the total variation. PCA score plot shows that light cultivation *H. marmoreus* was quantitatively distinguished as three sets, indicating that three light cultivation *H. marmoreus* had entirely different profiles of components and compounds. In addition, in plots for components and compounds, CCFLs, LEDs 2W1B (180) and LEDs 2W1B (180) had positive values in F1 where LEDs 3W (180) had negative value in F1. From the score and loading plots, CCFL and LEDs 2W1B (240) positively correlated with total phenol, scavenging ability, fiber and fat; LEDs 2W1B (180) positively correlated with ash, protein, energy, total amino acid content and chelating ability; LEDs 3W (180) positively correlated with polysaccharide, carbohydrates and reducing powder.

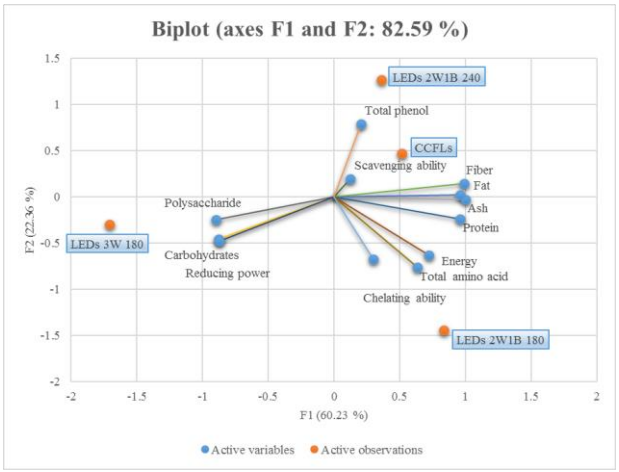

Figure 4. Principal component analysis of nutritional, amino acid and antioxidant of *H.* marmoreus from different light source on the cultivation.

IV. CONCLUSIONS

Based on the results obtained, cultivation of *H. marmoreus* under different light cultivation is useful for generating fruit body with good features, as well as high polysaccharide content, total phenol content and amino acid content. Ethanolic extracts from *H. marmoreus* at different light cultivation showed effective antioxidant properties. Therefore, considering nutritional qualities, antioxidant properties and the growth of mushrooms altogether, should be selected as the CCFL light for their cultivation.

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