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Original Article

Evaluation of the nutritional value, mycochemicals, and antioxidant activities of *Hericium erinaceus* cultivated using jasmine rice

Sari Darmasiwi[1,3], Yaovapa Aramsirirujiwet[1], Ingorn Kimkong[1,2*]

[1]Department of Microbiology, Faculty of Science, Kasetsart University, 10900 Bangkok, Thailand

[2]Center for Advanced Studies in Tropical Natural Resources, National Research University – Kasetsart University, 10900 Bangkok, Thailand

[3]Faculty of Biology, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia

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**Corresponding author email:*
fsciiok@ku.ac.th

Abstract

Hericium erinaceus is a medicinal mushroom that has various health benefits. The cultivation of mushrooms with solid substrates was previously reported to improve their chemical compositions and bioactivities. The effect of the solid-state cultivation of *H. erinaceus* using jasmine rice media was evaluated with regard to the nutritional value, mycochemicals, and antioxidant activities of the cultivated products. White jasmine rice (R1) and a mixture of white and red jasmine rice (R2) were used as growth substrates for *H. erinaceus*. The products of their cultivation, such as rice-fermented mycelia (MR1 and MR2) and basidiomata (BR1 and BR2), were assessed to determine their nutritive contents, mycochemicals, and antioxidant activities compared to unfermented rice (R1 and R2) and commercial basidiome (BS). The nutritional values were measured quantitatively, while the mycochemicals were evaluated qualitatively. The antioxidant activity was measured using the level of DPPH scavenging activity. The results showed that *H. erinaceus* was successfully cultivated on jasmine rice media. The pH of the media was positively correlated with mycelia growth. The R2-fermented mycelium (MR2) product had higher protein levels (11.40 g/100g) compared to unfermented R2 (6.74 g/100g). Basidiome cultivated on R2 media (BR2) exhibited higher protein levels (15.06 g/100g) compared to commercial basidiome (BS) (10.45 g/100 g). The rice-fermented mycelia contained alkaloids, terpenoids, and saponins. The MR2 sample showed the highest level of antioxidant activity (IC_{50}=1.26 mg/ml). These findings suggested that cultivation on jasmine rice enhanced the nutritional value and mycochemical compositions of *H. erinaceus*, with beneficial antioxidant activity.

Keywords: *Hericium erinaceus*, Solid-state cultivation, Nutrition, Mycochemicals, Antioxidants

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Introduction

Food plays a very important role in contributing to human health by providing energy and preventing diseases. Recently, functional food has received a lot of attention due to its dual advantages as food and medicine. Mushrooms have long been consumed as sources of functional food that contain high amounts of proteins, carbohydrates, and other beneficial mycochemicals with nutraceutical and antioxidant properties (Cateni et al., 2021). One popular edible medicinal mushroom is Lion's mane (*Hericium erinaceus*), which contains recognized antioxidant, anti-inflammatory, anticancer, and antimicrobial activities (Friedman, 2015). Species belonging to the genus Hericium are considered to be economically significant and valuable, as they can be consumed as edible mushrooms and mass-processed into extracts for medicinal applications (Park et al., 2004, Lu et al., 2002).

Lion's mane mushroom is a native species in subtropical countries and commonly thrives as a saprotroph living on deadwood in deciduous forests (Sokół et al., 2015). The cultivation of mushrooms is required to satisfy the high demand for their consumption and to compensate for their limited availability in nature. So far, *H. erinaceus* has been successfully cultivated by using lignocellulosic substrates such as wood sawdust or several agricultural by-products as growing media (Atila, 2019).

Solid-state cultivation of mushrooms using starch and amylose as alternative substrates has recently attracted increased interest. The use of solid cultures in the cultivation of mushrooms has been reported to improve the nutritive value, the bioactive compounds, and certain biological activities of fungal products, such as substrate-fermented mycelia products and basidiomata. Solid-state cultivation has been previously investigated in many species of mushrooms. Zhai et al. (2021) reported that the cultivation of *Agaricus brasiliensis* and *A. bisporus* on wheat bran could enhance the antioxidant activities and phenolic compounds of the wheat-fermented mycelia products, while the cultivation of *Pleurotus eryngii*, *A. blazei*, and *Cordyceps sinensis* on rice grain could add the nutritional value and chemical contents of rice-fermented mycelia products (Bao et al., 2013; Zhai et al., 2015; Zhang et al., 2008). Additionally, the solid-state cultivation of *Inonotus obliquus* using corn grain and mulberry powder could enrich the level of bioactive components and antioxidant activity of basidiome, while the cultivation of *H. erinaceus* using cornmeal showed nutritional value improvements on the cultivation products (Chen et al., 2020; Han, 2003).

Rice (*Oryza sativa* L.) is a starchy material that could be used as an alternative substrate for mushroom cultivation to enhance their chemical and biological activities (Zhai et al., 2015). It is consumed as a staple food worldwide, particularly in Asian countries, numerous varieties have been developed (Sen et al., 2020). Jasmine rice is one rice variety with a strong aromatic fragrance, softness, and adhesiveness characteristics that determine its economic value for growers and consumers, making it a high-quality product in international trade (Attaviroj and Noomhorm, 2014).

Jasmine rice cultivars such as non-pigmented white jasmine rice (*Oryza sativa* L.ssp.*indica* cv.Khao Dawk Mali 105, KDML 105) and pigmented red jasmine rice (*Oryza sativa* L.ssp.*indica* cv.Hom Mali Daeng, HMD) are some of the most widely consumed varieties in the market (Charoenthaikij et al., 2021). The general nutrition content and chemicals found in jasmine rice include carbohydrates and protein (Phanurak, 2021), while the phenolic content and the levels of antioxidant activity in pigmented jasmine rice were reported to be higher than in non-pigmented jasmine rice (Vichapong et al., 2010). Phenolic compounds act as antioxidants that can reduce the risks of degenerative diseases (Sen et al., 2020).

In this study, *H. erinaceus* was subjected to solid-state cultivation using jasmine rice as the substrate to determine its nutritional content and antioxidant potential. The use of jasmine rice as an alternative substrate in *H. erinaceus* cultivation has not been previously studied. The effect of the solid-state cultivation of *H. erinaceus* using different formulas of jasmine rice media; non-pigmented (white) jasmine rice, and a mixture of non-pigmented (white) and pigmented (red) jasmine rice, was evaluated for growth and yield performance.

Nutritional compositions, mycochemical properties, and antioxidant activities of the fungal products, i.e., rice-fermented mycelia (MR1 and MR2) and basidiomata (BR1 and BR2), were also compared to unfermented rice (R1 and R2) and commercially available basidiome (BS). This preliminary work explored the use of jasmine rice as an alternative substrate for mushroom cultivation and its potential applications. Researchers hope that this study could offer an avenue for improvements in health and the

economic importance of *H. erinaceus* by using starch substrate, which is rich in nutrients and bioactive compounds, as an alternative cultivation media, as well as producing both basidiome and fermented mycelia simultaneously.

Material and Methods

Chemicals and material

Potato dextrose agar (PDA) medium, commonly used for mushroom maintenance, was purchased from HiMedia (India). A DNA extraction kit was bought from Tiangen (China) and a DNA purification kit was purchased from Thermo Fisher Scientific (USA). All the other chemicals were sourced from Merck (Germany) and Pierce (USA). Solid-state cultivation experiments were prepared using different compositions of jasmine rice: white jasmine rice (R1) and a mixture of white and pigmented red rice (R2) media. White jasmine rice (*Oryza sativa* L.ssp.*indica* cv.Khao Dawk Mali 105, KDML 105) and red jasmine rice (*Oryza sativa* L.ssp.*indica* cv.Hom Mali Daeng, HMD) were obtained from local products from Buriram, Thailand, and the commercial basidiome (BS) was purchased from Marayat Farm, Pathum Thani, Thailand. The investigated cultivation products included rice-fermented mycelia products (MR1 and MR2), basidiomata cultivated on rice media (BR1 and BR2), and commercial basidiome (BS).

Mushroom strain and maintenance

Mushroom spawn (HE-01) was obtained from the Thailand Mushroom Collection Center and transferred to the Department of Microbiology, Kasetsart University, Thailand, for further experimentation. Spawn was developed in the laboratory for 14 days at ambient temperature in the dark, before being transferred onto potato dextrose agar (PDA) slants and incubated for 14 days at room temperature (30 °C) under dark conditions. Mushrooms on the PDA slants were stored at 4 °C until required for further experiments.

Mushroom identification

The mushroom strain was identified in the laboratory by the molecular method. Mycelial DNA was extracted using a plant DNA extraction kit (Tiangen, Beijing, China) following the manufacturer's protocol. PCR was prepared with 25 µl volume mixtures of 10 × PCR buffer with 20 mM $MgCl_2$ (5 µl), 2 mM dNTP mix (2 µl), 5U Taq polymerase (0.25 µl), 10 µM of ITS-1 and ITS-4 primers (1 µl) each and 60 ng of the template (2 µl) in dH_2O. PCR reactions were conducted with the following conditions: 1 cycle of denaturation (94°C, 5 min), 30 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 45 s), extension (72°C, 2 min) and final extension (72°C, 1 min). PCR products were run on electrophoresis in 1% agarose in 1 × TAE buffer (100 V, 30 min), visualized under UV after staining with ethidium bromide (EtBr), purified by Gene Jet DNA Purification (Thermo Fisher Scientific, California, USA), and sequenced (GibThai, Bangkok, Thailand). Nucleotide sequences were aligned using Bioedit 7.0.5.3 (North Carolina State University, North Carolina, USA) and then compared with available sequences in GenBank using the BLASTn tool from the National Center for Biotechnology Information (NCBI). Phylogenetic analysis was performed using MEGA 6.0 (Pennsylvania State University, Pennsylvania, USA).

Pre-culture preparation

Pre-culture mycelium in broth was prepared by transferring 5 discs of 14-day-old mushroom mycelium grown on PDA (5 cm^2 diameter size) to 50 ml sterile yeast peptone dextrose (YPD) broth in 250 ml flasks. Pre-culture flasks were incubated in a rotary shaker (160 rpm) at 25-28 °C for 14 days and then transferred to the rice medium.

Mushroom solid-state cultivation on jasmine rice

Medium properties, such as pH, were analyzed following the method of Torres-León et al. (2019). Water activity (A_w) was measured using the method of Esteban et al. (1989) and the total C, N, and C/N ratio in the substrates were analyzed using an elemental analyzer (Leco 836 Series Elemental Analyzer, Michigan, USA). R1 medium was prepared from 20 g of white jasmine rice (School Rice, Buriram, Thailand) with the addition of 40 ml of nutrient solutions (5 g/l yeast extract, 5 g/l peptone, 20 g/l glucose, 200 mg/l thiamine, 200 g/l potato, 2 eggs/l and 1 liter of distilled water). The R2 medium contained a 20 g mixture of white jasmine rice (School Rice, Buriram, Thailand) and red jasmine rice (Hom Mali, Buriram, Thailand) (1:1) with the addition of 40 ml of nutrient solutions (15 g/l glucose, 15 g/l sucrose, 51 g/l potato, 2 eggs, and 1 liter of distilled water). The rice media and nutrient solutions were mixed and placed in 16 oz. mason jars with air-filtered plastic caps and were autoclaved at 121°C for 20 min. The

sterilized media were then inoculated with 5 ml of pre-culture liquid mycelium and incubated in an air-conditioned laboratory at 22 ± 2°C for 5-6 weeks.

Evaluation of growth and yield performance

Mushroom growth was determined by growth parameters, such as the number of days taken for mycelium appearance, mycelium colonization, primordia appearance and the length of basidiome. Biological yield efficiency was measured as total basidiome weight/weight of substrate x 100% (Debnath et al., 2019).

Nutritional analysis

Samples of basidiomata and rice-fermented mycelia were oven-dried at 45-50°C, and then ground into powder for nutritional analysis. The moisture content was determined using the gravimetric method by weighing the samples before and after drying until a constant weight was achieved (AOAC, 2005). Carbohydrate analysis was performed using phenol-sulfuric acid with glucose as the standard, fat was analyzed by the chloroform-methanol (Folch) method (Passari et al., 2016), and protein content was estimated by the Bicinchoninic acid assay (Pierce, USA) using BSA as the standard. Total C, H, and N were measured using an elemental analyzer (Leco 836 Series Elemental Analyzer, Michigan, USA).

Qualitative mycochemical analysis

Qualitative alkaloids were identified by Wagner's test. Saponins were confirmed by a foam test, following the method of Kaur and Arora (2009). Terpenoids were qualitatively identified using the method of Hossain et al. (2013).

Total phenolic contents

Samples for total phenolic contents and antioxidant activities assays were prepared from mushroom aqueous extracts, as described previously by Liu et al. (2012) with slight modifications. In brief, powdered samples were added with distilled water and stirred for 2 h (1:10, w/v). The solutions were then filtered, evaporated, and freeze-dried. The total phenolic contents were analyzed using the Folin-Ciocalteau method. About 20 µl of mushroom extracts (10 mg/ml) were mixed with 20 µl of Folin-Ciocalteau reagent diluted in water (1:4, v/v) and 160 µl of sodium bicarbonate (75g/l) in 96-well microplates.

The solutions were then incubated for 20 min at room temperature. The absorbance was measured at 680 nm (Multiskan, Thermo Fisher Scientific, USA), using gallic acid as the standard. The total phenolic contents were expressed as the milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) (Nowacka et al., 2014).

Antioxidant activities

Antioxidant activities were measured by a DPPH radical scavenging activity (RSA) assay. Briefly, 30 µl of aqueous extract from various concentrations was prepared, placed in 96-well microplates containing 170 µl DPPH reagents in methanol ($6x10^{-5}$ mol/l), and then incubated for 30 min in the dark. Absorbance was measured at 515 nm (Multiskan, Thermo Fisher Scientific, USA) using DPPH in methanol as the negative control and ascorbic acid as the positive control. The antioxidant DPPH radical scavenging activity was calculated using the formula: % RSA= $[(A_{negative\ control}-A_{sample})/A_{control}] \times 100$. IC_{50} was defined as the concentration required to scavenge 50% of DPPH radicals and was determined by plotting extract concentrations against % RSA (Heleno et al., 2015a).

Statistical analysis

All experiments were performed in triplicate with the results expressed as mean ± standard deviation. Statistical analyses by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) were conducted using SPSS (version 22, IBM, USA). A value of $p<0.05$ was considered significant. Pearson's correlation coefficient at a significance level of 5% was used to examine the correlation between physicochemical parameters and mushroom growth performance.

Results

Mushroom identification

The mushroom strain was grown on wood sawdust for spawn development. After being transferred to potato dextrose agar (PDA), the strain morphology appeared as a white-pinkish colony with an irregular pattern growing from the center of the colony to the edge. The mushroom colony reached 40-50 mm after 10 days of incubation at ambient laboratory temperature in the dark (25±2°C). Mushroom growths on sawdust and PDA media are presented in Figure 1.

(A) (B)

Figure-1: **Morphology of *H. erinaceus* grown on (A) sawdust spawn and (B) PDA**

To confirm the species identity, molecular identification was performed based on ITS regions. The amplification of mushroom ITS regions obtained 500-650 bp of DNA bands. BLAST analysis of the HE-01 strain confirmed a 99.83% identity match at 100% query sequence cover with *H. erinaceus* (Bull.) Pers. The sequence was then deposited under NCBI accession number MW 672510.1. A phylogenetic tree was constructed based on the neighbor-joining method using MEGA 6 software, as shown in Figure 2.

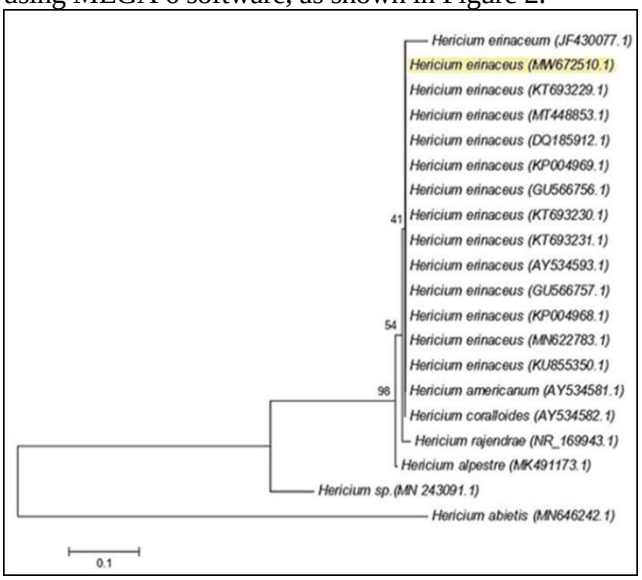

Figure-2: **Phylogenetic tree of *H. erinaceus* MW 672510.1**

Mushroom cultivation and growth performance

The physicochemical properties of the two jasmine rice media used in this study are shown in Table 1. Physicochemical characteristics such as the total C and N compositions, C/N ratio, water activity (A_w), and pH of both jasmine media were recorded as not significantly different. However, the R2 medium had a slightly higher total C content, C/N ratio, and pH value, but it had lower total N content and A_w value than the R1 medium.

Table-1: **Physicochemical properties of the two jasmine rice media used in this study**

Substrate	C (%)	N (%)	C/N (%)	A_w	pH
R1	42.43±0.28	1.33±0.02	31.86±0.20	0.63±0.00	6.51±0.01
R2	42.92±0.10	1.20±0.06	35.71±1.87	0.62±0.02	6.68±0.01

The growth performance of *H. erinaceus* during solid-state cultivation using jasmine rice media is presented in Table 2 and Figure 3. The R1 medium exhibited faster mycelium growth compared to the R2 medium, as indicated by the number of days taken for mycelium appearance and colonization. However, the R2 medium supported faster primordia appearance, better basidiome growth, and higher biological yield efficiency.

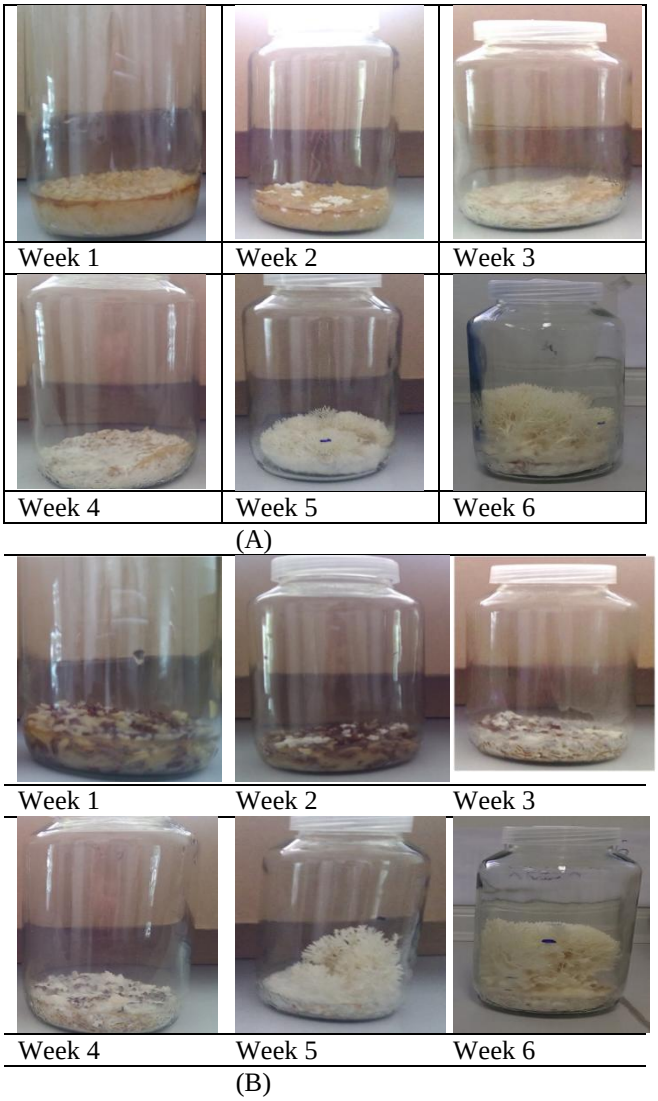

Figure-3: **Solid-state cultivation of *H. erinaceus* using jasmine rice (A) R1 medium and (B) R2 medium**

Table-2: Growth performance of *H. erinaceus* on the two jasmine rice media

No	Parameters	R1	R2
1.	Mycelium appearance (days)	4±0	4.67±0.58
2.	Mycelium colonization (days)	9.67±0.58	10.33±0.58
3.	Primordia appearance (days)	12.67±1.15	12±0
4.	Length of basidiome (mm)	33.33±0.58	38.33±0.58
5.	Biological yield efficiency (%)	38.9±1.92	41.9±2.4

Based on Pearson's correlation analysis, total N values were shown to be negatively correlated to the C/N ratio in the media (r^2= -0.997). The chemical compositions as total C, N, and C/N ratio of the substrates did not contribute to mushroom growth performance. Noticeably, the pH values of the media were positively correlated to the mycelia appearance (r^2=0.999), which demonstrated this factor's contribution to the mushroom growth performance.

Nutritional analysis

The nutritional contents of the cultivated products, i.e., rice-fermented mycelia products (MR1 and MR2) and basidiomata (BR1 and BR2), were compared with unfermented rice media (R1 and R2) and commercial basidiome (BS). The cultivated products are shown in Figure 4, while the nutritional contents are presented in Table 3. The solid-state cultivation of *H. erinaceus* revealed that protein contents increased significantly in rice-fermented mycelia and basidiomata cultivated on two jasmine rice media.

The rice-fermented mycelia, MR1 and MR2, showed protein compositions at 11.25 g/100 g and 11.40 g/100 g, respectively, while unfermented rice, R1 and R2, showed 6.67 g/100g and 6.74 g/100g protein compositions, respectively. Furthermore, the protein levels in basidiomata BR1 and BR2 were significantly higher ($p<0.05$) than those in BS, at 14.46 g/100g, 15.07 g/100g, and 10.45 g/100g, respectively.

(a) (b)

Figure-4: Cultivated products of *H. erinaceus* using jasmine rice as the substrate (a) rice-fermented mycelium and (b) basidiome

In addition, the levels of organic elements, C and N, in the rice-fermented mycelia and cultivated basidiomata were also improved when compared with those of unfermented mycelia and commercial basidiome. However, there were no differences in the H content between fermented and unfermented mycelia. Noticeably, the carbohydrate and fat contents of the rice-fermented mycelia were found to be lower than unfermented rice, while cultivated basidiomata presented higher fat but lower moisture content than commercial basidiome.

Qualitative mycochemical properties

Mycological assays of cultivated products of mushroom are presented in Table 4. The unfermented media contained alkaloids and terpenoids, while both rice-fermented mycelia contained alkaloids, terpenoids, and saponins. Cultivated basidiomata contained terpenoids and saponins, while commercial basidiome comprised only terpenoids.

Table-3: Nutritional contents of *H. erinaceus* on dry weight basis

Samples	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Moisture (%)	H (%)	C (%)	N (%)
R1	6.67 ± 0.17^{a}	1.56 ± 0.00^{ab}	11.40 ± 0.02^{c}	39.66 ± 1.91^{a}	6.75 ± 0.11^{b}	42.43 ± 0.28^{b}	1.33 ± 0.01^{a}
MR1	11.25 ± 0.17^{b}	1.07 ± 0.00^{a}	7.50 ± 0.016^{b}	53.70 ± 4.77^{c}	6.85 ± 0.07^{bc}	44.23 ± 0.20^{e}	2.95 ± 0.02^{c}
R2	6.74 ± 0.06^{a}	1.70 ± 0.00^{b}	15.16 ± 0.25^{d}	43.88 ± 1.33^{b}	6.84 ± 0.03^{bc}	42.92 ± 0.10^{c}	1.20 ± 0.06^{a}
MR2	11.40 ± 0.06^{b}	1.38 ± 0.00^{ab}	7.52 ± 0.10^{b}	$47.43+5.92^{c}$	6.86 ± 0.02^{bc}	43.73 ± 0.10^{d}	2.48 ± 0.03^{b}
BR1	14.46 ± 0.40^{c}	2.32 ± 0.00^{c}	4.23 ± 0.06^{a}	52.87 ± 4.07^{c}	7.06 ± 0.02^{d}	43.24 ± 0.01^{c}	7.68 ± 0.07^{f}
BR2	15.06 ± 0.32^{c}	2.42 ± 0.00^{c}	4.45 ± 0.10^{a}	45.55 ± 4.52^{c}	6.92 ± 0.00^{c}	41.25 ± 0.05^{a}	7.43 ± 0.11^{e}
BS	10.45 ± 0.06^{b}	1.58 ± 0.00^{ab}	5.68 ± 0.01^{ab}	87.30 ± 0.69^{d}	6.25 ± 0.03^{a}	42.14 ± 0.04^{b}	3.97 ± 0.04^{d}

Values are means with standard deviations (n = 3). Means with different letters in the same column were significantly different by Duncan's Multiple Range Test at the level of $p<0.05$.

R1 = unfermented R1; R2: unfermented R2; MR1=R1-fermented mycelia; MR2=R2-fermented mycelia; BR1=basidiome cultivated on R1; BR2=basidiome cultivated on R2; BS= commercial basidiome

Table-4: Qualitative mycochemical properties of *H.erinaceus*

Samples	Alkaloids	Terpenoids	Saponins
R1	+	+	-
MR1	+	+	+
R2	+	+	-
MR2	+	+	+
BR1	-	+	+
BR2	-	+	+
BS	-	+	-

Total phenolic contents and antioxidant activity

Table 5 shows the total phenolic contents and antioxidant activities of mushroom products. MR2 extract had the highest total phenolic content at 12.39 mgGAE/g, followed by BR2 with 12.12 mgGAE/g. The solid-state cultivation of mushrooms on jasmine rice improved the total phenolic contents of the cultivation products, particularly in the R2 medium.

Table-5: Total phenolic contents and IC_{50} values for antioxidant DPPH radical scavenging activity of *H. erinaceus*

Samples	Total Phenolic (mgGAE/g)	DPPH Radical Scavenging Activity IC_{50} (mg/ml)
R1	1.46 ± 0.59^{a}	7.77^{g}
MR1	11.08 ± 0.47^{bc}	1.45^{b}
R2	2.47 ± 0.26^{a}	7.24^{f}
MR2	12.39 ± 0.89^{d}	1.26^{b}
BR1	11.18 ± 1.22^{c}	3.15^{d}
BR2	12.12 ± 0.14^{cd}	2.04^{c}
BS	9.96 ± 0.02^{b}	5.03^{e}
Control (+)		0.07^{a}

Rice-fermented mycelia products had higher radical scavenging activity than basidiomata, with IC_{50} values of 1.45 mg/ml and 1.26 mg/ml for MR1 and MR2, respectively. Moreover, basidiomata cultivated in rice media had higher antioxidant activity than commercial basidiome, with IC_{50} values of 3.15 mg/ml, 2.04 mg/ml, and 5.03 mg/ml for BR1, BR2, and BS, respectively. In this study, rice-fermented mycelia exhibited promising antioxidant activities, with their IC_{50} values close to ascorbic acid as the positive control, while their basidiomata products were also good antioxidant sources, as indicated by their IC_{50} values.

Discussion

Mushrooms from the genus Hericium are considered rare. They have high medicinal value and are generally found in subtropical regions. Species belonging to this genus include *H. abietis, H. alpestre, H. americanum* and *H. erinaceus*. These species are difficult to distinguish under morphological and microscopic examination. Therefore, molecular identification is usually employed to support species identity.

The mushroom strain used in this study was considered slow-growing, with a 40-50 mm diameter in PDA after 10 days of incubation. This result concurred with the findings of Julian et al. (2018), who recorded an average mycelia growth of *H. erinaceus* of 56.95 mm, *H. americanum* of 27.62 mm, and *H. corraloides* of 47.65 mm on PDA after 10 days of incubation. PDA is the most common media used for growing and fungal maintenance. Moreover, it is also reported as the best option to support *H. erinaceus* mycelia growth (Julian et al., 2018). In this study, researchers recorded the length of the mushroom spine to be 30 mm (Figure 3), agreeing with the findings of Hallenberg et al. (2013) who reported a 10-40 mm spine length in *H. erinaceus,* a 5-15 mm spine length in *H. americanum*, and <20 mm in *H. alpestre*.

Based on molecular identification, the mushroom strain utilized in this study was closely related to other strains of *H. erinaceus*, with 99.83% similarity and 100% query cover. Park et al. (2004) and Lu et al. (2002) evaluated the application of ITS sequences to determine Asian isolates of *H. erinaceus* and found that the ITS sequences could differentiate Hericium species from another fungus by PCR. Additionally, Raja et al. (2017) and Dulay et al. (2020) revealed that >80% query cover with 97-100% similarity and a 3-5% cut off could be performed to determine the species name against mushroom isolates in the GenBank database.

H. erinaceus species are commonly found in subtropical countries, with hardwood being their preferred substrate. Here, in the tropical conditions of Thailand, an alternative starchy substrate, such as jasmine rice, was used to successfully grow the mushroom. Chemical parameters such as the total C, N, and C/N ratio in the media did not correlate with mushroom growth performance in this research. Interestingly, Jin et al. (2018) reported that a high C/N ratio could be responsible for the slow growth in *P. ostreatus*.

Here, we found that the pH of jasmine rice media positively correlated with *H. erinaceus* growth performance, and particularly with mycelia appearance. The pH values of the rice media were 6.51 and 6.68 for R1 and R2, respectively. Imtiaj et al. (2008) reported that *H. erinaceus* grew well in a pH range from 5.0-9.0, with the optimal pH being 6.0. Increasing the pH of the media generally slows down mycelial growth. Therefore, our results concurred with previous research findings.

Nutritional composition determines potential development as a nutraceutical or functional food. In this investigation, rice-fermented mycelia had almost double the protein, total nitrogen, and carbon content compared to both unfermented rice medium controls. Zhai et al. (2015) reported an increase in protein and amino acid nitrogen levels in rice-fermented mycelia of *A. blazei*, while Zhang et al. (2008) reported the improvement of protein, fat, and carbohydrate concentrations in rice-fermented mycelia of *C. sinensis*. The depletion of carbohydrate and moisture contents in the cultivation products found in this research was probably due to carbohydrate and water utilization during the process of cultivation. The loss of carbohydrates and moisture was also previously reported in the solid-state cultivation of *Lentinus polychrous*, where tangerine was used as a substrate (Nitayapat et al., 2015).

In this study, the protein content in the cultivated basidiome of *H. erinaceus* was found to be comparable with a previous study by Heleno et al. (2015b) and Rodrigues et al. (2015) which recorded about 15.40 g/100g and 18.8 g/100g proteins of *H. erinaceus* basidiome, respectively. These two studies also reported fat levels of *H. erinaceus* within the range of 1.29%-2.9%, corresponding with our study. This evidence showed that *H. erinaceus* is a good source of protein with low fat content. However, these nutrient contents also likely depend on the origin of the mushroom strains, medium composition, and cultivation conditions.

The mycochemicals in *H. erinaceus* are recognized for their potential to act as antioxidant, antimicrobial, anticancer, antidiabetic, and immunomodulatory agents (Sokół et al., 2015). Mycochemicals previously reported in *H. erinaceus* included alkaloids (Wang et al., 2015), phenolics, flavonoids (Gąsecka et al., 2016), saponins, triterpenoids (Vi et al., 2018), sterols, and aromatic compounds (Li et al., 2017; Ma et al., 2010). In this research, alkaloids, terpenoids, and saponins were qualitatively detected, especially in rice-fermented mycelia samples.

The rice-fermented mycelia and cultivated basidiomata were observed to contain a greater variety of bioactive compounds than unfermented rice and commercial basidiome. Adebayo et al. (2019) determined an increase in mycochemical contents such as alkaloids, saponins, and phenols after the cultivation of *P. ostreatus* on castor seed. Therefore, solid-state cultivation could enrich the bioactive compounds found in mushroom products.

A total phenolic assay and DPPH radical scavenging activity assay were used in this work to study the effects of cultivation on bioactive compounds and their antioxidant properties. The solid-state cultivation of *H. erinaceus* on jasmine rice media yielded better antioxidant activity than previous studies that used submerged cultivation. Wong et al. (2009) reported that the antioxidant activity, as indicated by the IC_{50} of *H. erinaceus* mycelia cultivated by the submerged method, was 13.67 mg/ml, whereas the IC_{50} of the cultivation products evaluated in this study ranged from 1.26-3.15 mg/ml.

Interestingly, we found that rice-fermented mycelia products had higher total phenolic contents and antioxidants than basidiomata; MR2 had the highest total phenolic content (12.39 mgGAE/g) and antioxidant activity level (IC_{50}=1.26 mg/ml) compared to the other samples. In accordance with a previous study by Liang et al. (2013), the adlay and buckwheat-fermented *Pleurotus eryngii* possessed higher total phenolic content and antioxidant activity compared to the *P. eryngii* basidiome and regular mycelia. These results revealed that fermented mycelia products could be used as promising antioxidant sources. Furthermore, cultivated basidiomata also showed higher antioxidant activity than commercial basidiome. These findings suggested that the utilization of grains with high antioxidant activity as mushroom growth substrates, particularly with the addition of pigmented red rice, probably contributed to the antioxidant properties as also mentioned previously by Liang et al. (2013). Overall, jasmine rice showed promise as an alternative substrate for the solid-state cultivation of *H. erinaceus*, showing potential in the development of cultivated products that could be promising sources of proteins and mycochemicals with high antioxidant activities.

Conclusion

Jasmine rice showed potential as an alternative

substrate for *H. erinaceus* solid-state cultivation. The pH of the media was correlated with mushroom growth. Solid-state cultivation using jasmine rice improved the nutritional value and increased the levels of bioactive compounds and antioxidant activities in the mushroom products. Cultivation products, especially rice-fermented mycelia in the R2 medium (MR2), were good sources of protein that were rich in mycochemicals and antioxidant compounds. Further research is required to evaluate other nutraceutical applications and the bioactive compounds that contribute to their bioactivities.

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Contribution of Authors

Darmasiwi S: Literature review, data collection and analysis and manuscript writing.
Aramsirirujiwet Y & Kimkong I: Designed and supervised the research, provided facilities, manuscript proof reading and approval.