Vol. 12, No. 4 (2023) : 818 - 830
DOI : http://dx.doi.org/10.23960/jtep-l.v12i4.818-830
JURNAL TEKNIK PERTANIAN LAMPUNG
ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Physicochemical Quality of Oyster Mushroom for Functional Food

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Article History :
Received : 24 May 2023
Received in revised form : 31 July 2023
Accepted : 15 August 2023

Keywords :
Ergothioneine,
Fibre-reinforced plastic house,
Internet of think,
Oyster mushrooms.

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ABSTRACT

This article presents studies on the physicochemical quality of oyster mushrooms grown within a housing and controlled by the Internet of Things (IoT). The goals of this study were to assess (1) the impact of indoor air quality on the growth and quality of mushrooms and (2) the antioxidant content of oyster mushrooms. In this study, the air temperature and humidity of oyster mushroom house per unit time was recorded and controlled automatically by an IoT system. Additionally, their physicochemical and microbiological quality were evaluated using physico-analytical instruments, and the potency of their ergothioneine (EGT) content was investigated using the HPLC method. The temperature of the air inside was between 29 and 35 °C, and the relative humidity was between 60% and 90%. The average texture of mushroom is soft. The average length, width, and height of the fresh mushroom were 41.5 mm, 60.0 mm, and 29.5 mm, respectively. The microbiological test confirmed that there was no salmonella infection in the collected mushrooms. The button-stage mushrooms have less total fungus than the bloom-stage mushrooms. The extraction standard method employs an EGT content of 0.674 mg/g as determined by chromatography data analysis. The oyster mushrooms can be consumed as a healthy meal, and the study of EGT showed also very prospective as one of immunotherapeutic food.

1. INTRODUCTION

The oyster mushroom is a type of mushroom that specifically grows in tropical countries such as Indonesia. Oyster mushrooms are grown on aggregate media one of them is the paddy waste from the paddy cultivation. Therefore, generally at the rice production centre area mostly becoming the centre for mushrooms production. This can be correlated with the availability of biomass as the main growing media for mushroom.

Cultivating high quality of the mushrooms required high-quality seeds, the high-quality media resources as well as the optimal environment due to the mushrooms are sensitive

to climate change. To fulfil the optimum condition for mushrooms cultivation, an adequate production house is required to be implemented by environment controller and high-quality safety materials for growing media and to avoid using industrial in order to produce free contaminant (Kopinski & Kwiatkowska-Marks, 2012).

A prototype of Fiber Reinforced Plastic (FRP) house (Rachmat & Horibe, 1999) is a proper construction for mushroom production housing, and it is implemented with an Internet of things (IoT) based connected to various devices microcontroller. The mushroom production house used IoT control system to make it easier to monitor the air condition inside the house online by mean of displaying the value generated from the installed sensors inside the house. According to Safitri & Lestari (2021) temperature and humidity in production house of mushrooms greatly affect the growth process of mushrooms. So that the mushrooms do not rot, brown and wilt, the humidity in the mushroom production house should not be too high or low, ranging from 75%-90% at the humidity level of the mushroom production house.

Research by Dahiya *et al*. (2019) who conducted an analysis of the efficiency of the edible mushroom stated that the growth of high consumption of mushrooms was due to the high protein content of edible mushrooms. The mushroom has good properties for the nutritional needs of the human body such as being rich in protein, vitamin C and various water-soluble vitamins (riboflavin, biotin and thiamine), fat (5.7%), carbohydrates (56,8%), amino acids, unsaturated fats, essential minerals (sodium, potassium and phosphorus) and has a low calorific value (Ahlawat *et al*., 2016).

Mushrooms contain various types of nutrients those are beneficial to the health of the human body, such as carbohydrates, proteins, and fats. Several types of essential nutrients found in edible mushrooms include amino acids (glutamic acid, proline, phenylalanine, lysine, histidine, etc.), fatty acids (lauric, palmitic, oleic, lenoleic, etc.), potassium, calcium, and phosphorus (Adiandri *et al*., 2018). One type of essential amino acid found in mushrooms is ergothioneine (EGT). EGT is a derivative of histidine which contains functional groups of sulfur atoms and hydrogen atoms (-SH) and it can only be synthesized by fungi and actinobacteria (Borodina *et al*., 2020; Halliwell *et al*., 2018).

Mushrooms as a food source have been reported to have the highest level of ergothioneine compared to any other food source (Dubost *et al*., 2006; Dubost *et al*., 2007). In an early report, it has been shown that if we consume mushrooms as our regular food, the bioavailability of ergothioneine remains in our body (Weigand-Heller *et al*., 2012). The ergothioneine chemical compound is accumulated in the fruiting bodies as well as in the mycelia of the mushroom (Borodina *et al*., 2020; Beelman *et al*., 2020). Several studies have been conducted with samples of mushrooms to describe improved ergothioneine production by the addition of several additives, such as aspartic acid, lysine, and methionine, which demonstrate increased ergothioneine biosynthesis (Lee *et al*., 2009). In addition, it has also been described that the production of ergothioneine increases with increased pH and is higher under alkaline conditions (Huang & Nie, 2015).

Research on the identification and isolation of EGT from several fungal varieties has been carried out. The content of EGT in mushrooms is quite high and has good antioxidant activity. In addition, differences in genus and species of fungi have different chemical compositions contained in them so that it affects the proportion of EGT in mushrooms (Kalaras *et al*., 2017). The results of the literature study indicate that the identification and isolation of EGT from edible mushroom has never been done. Therefore, research to explore the opportunity to use edible mushroom as a source of EGT needs to be done.

The provision of production house that meets food safety requirements is absolutely necessary to produce safe oyster mushrooms for consumption. Meanwhile the technology development of this mushroom production that meets food safety standards for the isolation of ergothioneine as an antioxidant is a potential matter in exploration of mushroom is still limited. This paper describes the research results of the prototype of FRP house with the air condition variation inside the house and the effect of air condition against mushroom quality. The effect of the IoT implementation in controlling the air condition system as well as minimizing the human contact during oyster mushroom growing during cultivation periods, and to explore the value added of mushroom through the ergothioneine (EGT) analysis as the of antioxidant content in mushroom. The scope of research includes (1) constructing a prototype of FRP house with IoT control system for oyster mushroom cultivation; (2) to evaluate the air condition variation inside the house and the effect of air condition against mushroom growth and quality; (3) Analysis of antioxidant content in mushroom.

2. MATERIALS AND METHODS

The research was conducted in 2022 at the field laboratory of Post Harvest Research Center of IAARD and analytical laboratory of University of Djuanda. The materials used in this research are analytical grade chemicals. The effectiveness of antioxidant properties on isolated EGT was measured by in vitro method. The main material and controlled apparatus respectively for (1) the construction material for building a prototype of FRP house with IoT control system for oyster mushroom cultivation; (2) The atmospheric air meter apparatus for investigating the air condition variation inside the house and the effect of air condition against mushroom growth and quality; (3) Analysis of antioxidant content in mushroom by HPLC method. The materials used in this research are analytical grade chemicals. The effectiveness of antioxidant properties on isolated EGT was measured by in vitro method.

2.1. FRP House

The Fibre Reinforced Plastic (FRP) house constructed (Figure 1) at an area of 650 cm x 350 cm with a light steel alloy frames and the roof is covered by a thin metal sheet roof and all each the wall blanketed by fibre reinforced plastic. (FRP), and white ceramic tile's floor. There are two shelves at inside the FRP house with of 400 cm length and 100 cm width respectively.

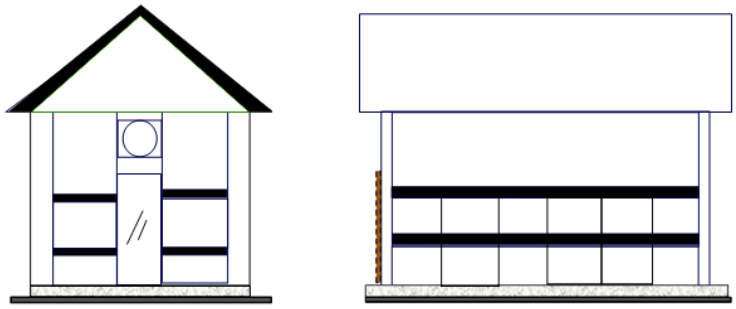


Figure 1. Prototype of FRP house model of straw mushroom (dimentions 3.0 m x 5.0 m x 3.0 m)

The FRP House implemented with an Internet of Think (IoT) system (Figure 2) to actuate a set of devices to measure the parameters of air temperature, air humidity,

moisture content of the media. The control devices are 30 nozzles of mist sprayer setup along the rack of shelves above the media which are connected with a special hose. Hot air temperatures above 35 ^{o}C, caused the exhaust fan is on, whenever a drastic drop of air temperature inside the FRP house below 29 ^{o}C, thus the.heater will operate automatically to increase the room temperature. The controller specification showed in Table 1 are set at day and night time with the elapsed time of intervals are enable to monitor the parameters conditions to avoid disturbance during mushrooms growth.

The Arduino Node MCU is an open-source platform based on IoT, which is able to implement various commands with programming languages. The application of the C programming language uses a sketch on the Arduino IDE (Integrated Development Environment), which is capable of programming various kinds of microcontrollers including Node MCU. The Node MCU has a small size compared to Arduino UNO, by using the ESP8266 module, there are GPIO (General-purpose input/output), PWM (Pulse Width Modulation), I2C, UART (Universal Asynchronous Receiver Transmitter), 1-Wire and ADC (Analog to Digital Converter).

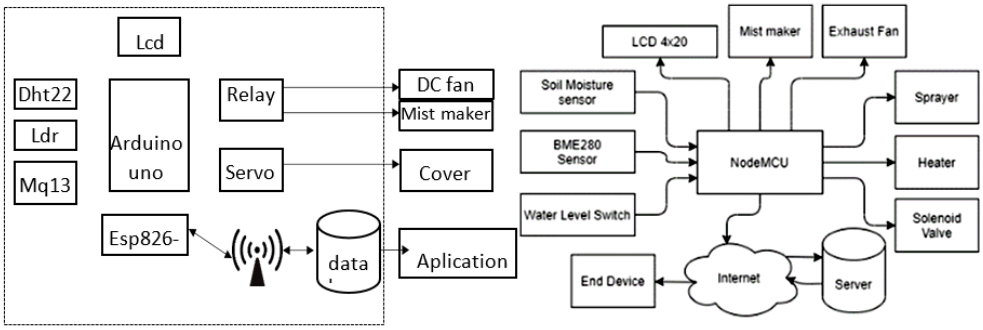


Figure 2. Architecture of IoT of production house in oyster mushroom

Tabel 1. Component and specification of control system

Component	Specifications
Sensor BME280	Air temperature and humidity sensor
Soil Moisture sensor	Soil moisture content controller
Water Level sensor	Water height controller
Mist-maker	Fog production and controller
Exhaust fan	Air humidity controller
Arduino IDE 1.8.9, fritzing	Temperature and humidity

2.2. Cultivation Process Procedure

Mushroom cultivation process procedure was carried out inside FRP House and utilized the organic components which are mixed and fermentation process for 4 days to be a compost, and then fill into bag log evenly in a plastic sacks assembled inside FRP House. The layers of compost were pasteurized inside FRP house with closed cover condition tightly and then fogging with hot air at temperature 60 to 65 ^{o}C for 8 hours. The mushroom seeds were inside over the bag logs of compost and then to be incubated for 3 days at around 29-30 ^{o}C of inside air temperature. The treatment during mushroom growth up to harvest periods comprised of the inside air temperature, humidity and soil water content. The treatment during mushroom growth up to harvest periods comprised of the inside air temperature, humidity by thermos-hygrograph and soil

water content sensor at every 20 min. The first harvesting conducted two weeks after seedling where the growth mushroom head is indicated by totally white head blooming.

2.3. Extraction of the Active Compound Ergothioneine

Ergothioneine is a complex compound of antioxidant. The isolation of ergothioneine EGT compounds from the harvested fresh mushrooms was carried out by the extraction method refers to the method developed by Ito *et al*. (2011) and Nguyen *et al*. (2012) with some modifications. Pre-treatment (blanching and non-blanching), mushroom before drying methods by electric oven drying.

The experimental factors of extraction employed the temperature 40°C and 60°C respectively with the variation of the extraction solvent (water and ethanol 30% (v/v), 50% (v/v), 70% ethanol (v/v), and ethanol 100 % (v/v)). The extraction procedure utilized 10 grams of dry mushroom powder and 200 mL of solvent which has been heated in a shaking water bath according to variations in temperature. The solvent along with the solute was continuously agitated at 100 rpm for 1 hour, then cooled at room temperature for 2 hours. Then it was centrifuged at 600 rpm for 15 minutes at 25°C. The achieved of the process is supernatant which was transferred to a conical tube for further evaporation using a vacuum evaporator at a temperature of 40°C to evaporate the remaining solvent up to be a paste of EGT extract. Furthermore, the paste of EGT was dried by the freeze-drying method. The dried EGT sample was then weighed to measure as the extraction yield.

2.4. EGT Concentration Analysis

Analysis of the concentration of EGT in the oyster mushroom extract used the HPLC method referring to the method used by Kalaras *et al*. (2017) with some modifications. The mushroom powder (0.5 g) and 7 ml of ethanol was put into a 15 ml centrifuge tube (consisting of 10 mM dithiothreitol, 100 M betaine, 100 M 2-mercapto-1-methyl imidazole) and 3 ml of distilled water was added and homogenized with a vortex for 10 seconds. A total of 2 mL of 1% SDS solution dissolved in ethanol was added and homogenized with a vortex for 10 seconds. Then the suspension was centrifuged for 20 minutes at 4000 rpm and 25°C. The supernatant formed was transferred to a 15 mL centrifugation tube. Then, 1 mL of the supernatant was put into a micro tube and allowed to stand for 24 hours. The solid formed from this process is then dissolved into 0.5 mL of aqua bides and homogenized using a vortex until it dissolves completely. Samples that have been stored in micro-tubes were centrifuged at 10,000 rpm for 1 minute. The supernatant formed was then filtered using a syringe filter (0.45 100 m) and put into a glass vial for HPLC analysis.

HPLC analysis was performed using column C18. The volume of the solution injected was 10 mL. The mobile phases used mobile phase A (3% acetonitrile, 0.1% TEA, 50 mM dibasic sodium phosphate dissolved in distilled water), mobile phase B (acetonitrile), and mobile phase C (water). The separation was performed (Kalaras *et al*., 2012) on the elution gradient as follows: 0 minutes 15:85 (B:C), 0-5 minutes set at a slope of 5:15:85 (A:B:C), 5-6 minutes set at a slope of 10:90 (B:C), 6-8 minutes at 3:97 (B:C), and 8-15 at 3:97 (B:C). EGT was detected at a wavelength of 254 nm with a peak retention time of 5.7 min which was quantified using an external standard curve.

The antioxidant activity of EGT from edible mushroom was carried out by measuring the radical scavenging activity of DPPH referring to the method of Pahila *et al*. (2019). The analytical procedure comprised of 50 L of straw mushroom extract is added to 50 L of DPPH solution (100 M in 85% ethanol) and stirred using a vortex. After 15 minutes, the solution mixture was put into a 50 L Pyrex NMR capillary tube, then the DPPH

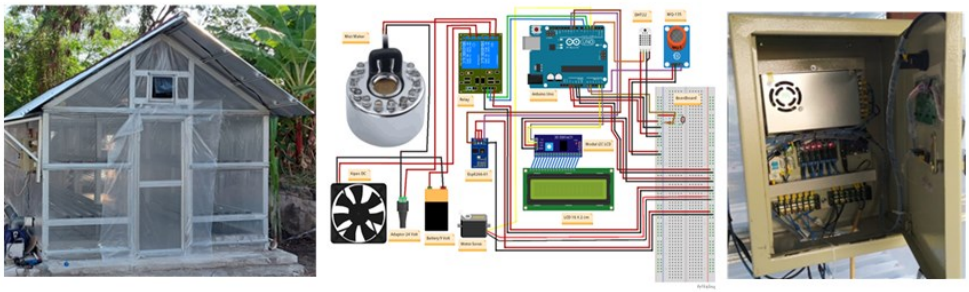
radical spin resonance was measured with a spectrometer at a wavelength of 517. The spectrometer settings as microwave power, 1.2 mW; microwave frequency 9149.3 MHz; field magnet, 325.5 25 mT; and the sweep time, 30 seconds. Various concentrations of EGT were measured for resonance to construct a standard curve. The radical scavenging activity of DPPH was expressed as mmol equivalent of EGT per mL of sample.

3. RESULTS AND DISCUSSION

3.1. FRP House

The prototype of the FRP house is shown in Figure 3. Condition of the air temperature, air humidity and water content of the media inside the FRP house during mushroom cultivation are monitored automatically. The variation of air temperature around 29-39^{o}C is relatively stable, while condition at day, night and rainy, resulted in high fluctuated of air humidity 40-98% with the water content of the media around 39-99%.

The devices inside the mushroom's FRP house consists of circuits, input blocks and output blocks which are joint by the Nodemcu microcomputer to control the air condition inside mushroom's FRP house. The input block is a soil moisture sensor and BME280 as a temperature, humidity and barometric detector. The sensor will generate an input value for the Nodemcu microcomputer, this value will be processed by Nodemcu which produces an output value to the connected devices. While the output block is a receiver a value generates by sensor through Nodemcu, such as a heater will operate if the temperature inside the mushroom's FRP house is low and the mist-maker will turn off if the humidity inside the mushroom's FRP house is sufficient. Nodemcu has a Wi-Fi module that is able to save the values and data in the database, this can make it easier to use a smartphone to access mushroom's FRP house condition even the values or data remotely. The IoT control system is setup for operating the hot air exhaust with the mist sprayer process taking place in parallel if the air temperature at above 39 ^{o}C with humidity below 60%. And when the opposite condition occurred (below 29 ^{o}C), thus the water heater will actively operate spraying hot air vapor into the FRP House. This methods of IoT control mechanism will reduce the human intervention so that the growth process of mycelium and pinhead of fungal embryos can achieve optimally.



(a) (b) (c)

Figure 3. (a) The prototype of the FRP house, (b) with IoT circuit, and (c) panel control box

The generated measuring instrument and sensors performance corresponded the proposed elapsed time through the interface to display's monitor. In table 2 showed the comparison of sensor values with measuring instruments. Digital measuring instrument used to compare sensor values such as hygrometer, lux meter and the average temperature difference of 0.92 ^{o}C and humidity of 2.1%.

Table 2. Temperature and humidity sensor testing

Temperature (°C)			Humidity (%)		
Sensor	**Device**	**Diff**	**Sensor**	**Device**	**Diff**
31.7	30.8	0.9	77	76	1
31.6	30.7	0.9	78.3	77	1.3
31.7	30.7	1	78.9	78	0.9
31.8	30.7	1.1	78.3	78	0.3
31.8	30.7	1.1	79.1	79	0.1
31.8	31.3	0.5	80.2	79	1.2
32.2	31.1	1.1	83.4	82	1.4
32.2	31.2	1	82	82	0
32.1	31.5	0.6	81.3	81	0.3
32.1	31.1	1	81.2	81	0.2

Table 3 is a comparison graph of sensor values with measuring instruments. Digital measuring tools used to compare sensor values such as mini-Hygrometer, lux meter and carbon detector. The test was carried out by taking samples 30 times by getting an average temperature difference of 0.91 °C, humidity 0.89%. Furthermore, the light sensor gets an average difference of 19.76% and gas content of 10.13 ppm. The second test evaluates the performance of the tool and obtains an accuracy rate of success of 93.3%.

Table 3. Light sensor and CO_2 content

Light			Gas Content		
Sensor	**Device**	**Diff**	**Sensor**	**Device**	**Diff**
192	175	17	21	10	11
187	144	43	33	12	21
182	152	30	16	9	7
103	100	3	24	16	8
170	145	25	22	20	2
-	-	-	-	-	-
-	-	-	-	-	-
144	124	20	18	7	11
169	123	46	10	3	7
118	115	3	15	8	7
124	121	3	24	16	8
128	122	6	30	20	10

3.2. Effect of FRP House on Mushroom Quality

Air conditions and lighting inside mushroom's FRP house day and night during the mushroom growth (Figure 4) contibute for the effect to the quality of harvested fresh mushroom. The results of quality analysis indicated that the range of phenotype length of 2.5-10 cm, diameter of 3-10 cm and weight 10-120 gram (Figure 5), in general the mushrooms growth from organic media are longer and heavier than non-organic growth media with more uniform dimensions except for the diameter of phenotype. Figure 4 is to show the effect of air condition mentioned by sensors to the growth of the mushroom.

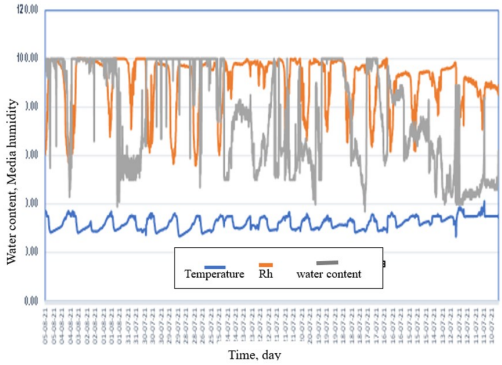


Figure 4. Fluctuation of air temperature, RH, and moisture content inside FRP House

Figure 5. Dimension of harvested oyster mushroom

The hardness test and the moisture content of the mushrooms were carried out by comparing the mushrooms at the farm level as shown in Table 4, indicating that the mushrooms in the FRP house were harder and less water content than that ones at the farmer mushroom's housing at the farm level.

Table 4. Characteristics of straw mushroom

No	Parameter	Average
1.	Texture	Soft
2.	Dimension	
	- Length (mm)	41.5±6.0
	- Width (mm)	60.0±4.0
	- Height (mm)	29.5±9.0

3.3. Micro Laboratory Analysis

The results of micro-laboratory analysis (Table 5) showed that the fresh harvested mushroom are negative from total fungus and salmonella.

3.3.1. Active Compound Ergothioneine

The results of the analysis on functional compounds of EGT in oyster mushrooms using the HPLC method, a graphic recording of the results of HPLC analysis of dried mushrooms with standard ethanol solvent. The average recording results from the EGT chromatography results were 0.674 mg/g as shown in Table 6.

Tabel 5. Analysis result of total fungus and salmonella

Code	Phase of fungus	Total fungus (CFU/g)	*Salmonella* (negative/25g)
A	Non organic mushroom, Bloom	$2.9x10^5$	negative
B	Non organic mushroom, fresh	$1.0x10^5$	negative
C	Organic mushroom, bloom	$3.5x10^4$	negative
D	Organic mushroom, fresh	$6.5x10^4$	negative
E	non organic mushroom, bloom	$1.7x10^6$	negative
F	non organic, mushroom, fresh	$5.4x10^4$	negative

Table 6. Results of chromatogram graphic data processing from mushroom extract

Code	Area	Intercept	Slope	V (ml)	fp	B.s (g)	C (ppm)	C (%)	mg/g DW
Organic, et 70%	2.005	0.1501	0.0643	20	250	0.2006	719032.64	71.90	0.799
	2.0309	0.1501	0.0643	20	250	0.2006	729072.50	72.91	0.810
Non organic, et 70%	2.2289	0.1501	0.0643	20	250	0.2688	601370.99	60.14	0.668
	2.2626	0.1501	0.0643	20	250	0.2688	611119.98	61.11	0.679
Ethanol 96%	2.2255	0.1501	0.0643	20	250	0.2034	793432.33	79.34	0.882
	2.0676	0.1501	0.0643	20	250	0.2034	733066.64	73.31	0.815
Powder, organic	1.3702	0.1501	0.0643	20	250	0.2022	469216.53	46.92	0.521
	1.3862	0.1501	0.0643	20	250	0.2022	475369.69	47.54	0.528
Powder, anorganic	1.3431	0.1501	0.0643	20	250	0.2009	461763.43	46.18	0.513
	1.2709	0.1501	0.0643	20	250	0.2009	433817.65	43.38	0.482

The extraction process breaks down the bonds among the molecules of the material so that EGT and phenol compounds are produced with varying concentrations depending on the type of solvent used (Table 7). The results of the standard solution analysis showed that the EGT compound had a retention time of 3.33 ± 0.06 minutes, while the mushroom sample showed a retention time of 3.28 to 3.30 minutes.

Table 7. Recapitulation of EGT characterization data from edible mushrooms

Solvent	Concentration EGT (mg/g DW)	Total Phenol (mg/g DW)
Ethanol 96%	0.805 ± 0.01	0.015 ± 0.00
Ethanol 70%	0.848 ± 0.05	0.025 ± 0.00

The extraction process using 70% ethanol produced higher concentrations of EGT and phenol than the extraction process using 96% ethanol. This shows that 70% of ethanol solvent is more effective used to bind EGT and phenol compounds in oyster mushrooms than that in pure solvents. This result is in line with the previous study conducted by Nguyen *et al*. (2012). The process of extracting the *Flammulina velutipes* mushroom using 70% ethanol was able to produce EGT and phenol compounds with higher concentrations than that the extraction using 50% and 100% of ethanol, with values of 2.05 ± 0.18 mg/g and 3.65 ± 0, 08 DW, consecutively.

The extraction process that has been carried out can break down the bonds between the molecules of the material so that EGT and phenol compounds are produced with varying concentrations depending on the type of solvent used (Table 8). The results of the standard solution analysis showed that the EGT compound had a retention time of 3.33 ± 0.06 minutes, while the straw mushroom sample showed a retention time of 3.28 to 3.30 minutes.

Table 8. Methods and varying concentrations type of solvent for EGT

Mushroom species	Methods	Ergothioneine concentration (mg/g dw)
Flammulina velutipes (Nguyen *et al*., 2012)	Water	1.50 ± 0.21
	Ethanol 70%	2.05 ± 0.18
	Methanol 70%	1.86 ± 0.04
	Acetone 70%	1.92 ± 0.07
Pleurotus eryngii	Hot water (Yen *et al*., 2018)	0.86
Pleurotus citrinopileatus	Hot water (Yen *et al*., 2018)	3.73
Oyster Mushroom	Ethanol 70%	0.67 ± 0.01

The extraction process for active compounds (Table 9), such as EGT and phenol, is influenced by several factors, such as solvent polarity and process conditions. A mixture of solutions has the ability to bind the active compound better than the pure solvent. This is due to the mixing of nonpolar solvents with water which can increase the polarity index of the solvent so that the extraction efficiency increases.

In addition, the increase in extraction temperature was positively correlated to the increase in antioxidant activity and polyphenols (flavonoids and total phenol), while the increase in extraction time was not positively correlated to the increase in antioxidant activity and polyphenols. In addition, the presence of agitation during the extraction process also contributes to increasing the extraction yield. This happens because the agitation process can increase the rate of diffusion and minimize the barriers to mass transfer from the solid surface into the solution (Muhamad *et al*., 2014).

Table 9. Results of chromatograph analysis of mushroom samples

Code	Area	V (ml)	fp	B.s (g)	C (ppm)	C (%)
Organic	2.0050	20	250	0.20	719032.6	71.90
Non organic	2.2289	20	250	0.27	601371.0	60.14
Ethanol 96%	2.2255	20	250	0.20	793432.3	79.34
Powder (organic)	1.3702	20	250	0.20	469216.5	46.92
Powder (unorganic)	1.3431	20	250	0.20	461763.4	46.18

Table 10. Concentration of ergothioneine in different mushroom species (Lee *et al*., 2009; Ito *et al*., 2011)

Mushroom Species	Ergothioneine Content*	Mushroom Species	Ergothioneine Content*
Fomitopsis pinicola	0.07 ± 0.01	*Polyozellus multiplex*	0.51 ± 0.01
Ganoderma applanatum	0.06 ± 0.02	*Ramaria botrytis*	0.29 ± 0.03
G. lucidum	0.08 ± 0.02	*S. granulates*	0.09 ± 0.03
G. neo-japonicum	0.07 ± 0.00	*Lampteromyces japonicus*	0.43 ± 0.16

*mg/g dw; Mean ± SD.

Table 10 showed that the role in synthesizing EGT is the methyltransferase enzyme which is encoded by the EGT gene and specifically remodels histidine into EGT compounds (Stampfli *et al*., 2020). The results of another study stated that the biosynthesis of EGT is a complex synthesis process involving various types of amino acids and carbon sources. However, in principle there are three types of amino acids that play an important role in EGT biosynthesis, namely methionine, histidine, and cysteine. Histidine acts as a starting material that plays a role in the whole series of EGT biosynthesis. Cysteine serves to provide a source of sulfur atoms, while methionine which contains the protein S-adenosyl-l-methionine (SAM) acts as a precursor in reactions involving methyl groups. In supporting its growth, cells can use dextrose, fructose, glycerol, maltose, and sucrose as carbon sources. A suitable carbon source for EGT biosynthesis is glycerol because it is able to maintain cells in a longer stationary growth phase. In the glycolysis cycle, glycerol is broken down into glycerol-3 phosphate by the enzyme glycerol kinase. Furthermore, glycerol-3 phosphate is broken down by the enzyme glyceraldehyde-3 phosphate dehydrogenase to glyceron-P which is then overhauled by triosephosphate isomerase to glyceraldehyde 3 phosphate (GAP). GAP acts as a precursor in the glycolysis process to produce pyruvic acid which will also be a substrate in the Krebs cycle. Consumption of large amounts of pyruvic acid can stimulate enzymatic reactions of amino acids to produce EGT (Yu *et al*., 2020).

4. CONCLUSSION

The research results showed that the cultivation of oyster mushroom in an FRP house with ceramic floors and plastic walls and an IoT management. The temperature of the air inside was between 29 and 35 degrees Celsius, and the relative humidity was between 60% and 90%. The average texture of fresh oyster mushroom was soft. The average length, width, and height of the fresh mushroom were 41.5 mm, 60.0 mm, and 29.5 mm, respectively. The microbiological test confirmed that there was no salmonella infection in the collected mushrooms. The stage of mushrooms have less total fungus than the bloom-stage mushrooms. The extraction standard method employs an EGT content of 0.674 mg/g as determined by chromatography data analysis. The oyster mushrooms can be consumed as a healthy meal, and the study of EGT showed also very prospective as one of immunotherapeutic food.

REFERENCES

Adiandri, R.S., Hidayah, N., dan Nugraha, S. (2018). Identification of amino acid, fatty acid, mineral content and respiration rate of straw mushroom in different stage of maturity. *Proceeding the International Seminar on Tropical Horticulture*, 239-248.

Ahlawat, O.P., Manikandan, K., & Singh, M. (2016). Proximate composition of different mushroom varieties and effect of UV light exposure on vitamin D content in *Agaricus bisporus* and *Volvariella volvacea*. *Mushroom Res*., ***25***(1), 1-8.

Beelman, R.B., Kalaras, M.D., Phillips, A.T., & Richie, J.P. (2020). Is ergothioneine a 'longevity vitamin'limited in the American diet? *Journal of nutritional science*, ***9***, e52. https://doi.org/10.1017%2Fjns.2020.44

Borodina, I., Kenny, L.C., McCarthy, C.M., Paramasivan, K., Pretorius, E., Roberts, T.J., van der Hoek, S.A., & Kell, D.B., (2020). The biology of ergothioneine, an antioxidant nutraceutical. Nutrition Research Reviews, 33(2), 190-217. https://doi.org/10.1017/s0954422419000301

Dahiya, S., Satyanarayana, T., & Singh, B. (2019). Thermophilic fungal diversity in sustainable development. *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Volume 1. Microbial Diversity in Normal & Extreme Environments*, 187-224.

Dubost, N.J., Beelman, R.B., Peterson, D., & Royse, D.J. (2006). Identification and quantification of ergothioneine in cultivated mushrooms by liquid chromatography-mass spectroscopy. *International Journal of Medicinal Mushrooms*, ***8***(3), 215-222. http://dx.doi.org/10.1615/IntJMedMushr.v8.i3.30

Dubost, N.J., Ou, B., & Beelman, R.B., (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity. *Food Chemistry*, ***105***(2), 727-735. https://doi.org/10.1016/j.foodchem.2007.01.030

Halliwell, B., Cheah, I.K., & Tang, R.M.Y. (2018). Ergothioneine – A diet-derived antioxidant with therapeutic potential. *FEBS Letters*, ***592***(20), 3357-3366. https://doi.org/10.1002/1873-3468.13123

Huang, X., & Nie, S., (2015). The structure of mushroom polysaccharides and their beneficial role in health. *Food & function*, ***6***(10), 3205-3217. https://doi.org/10.1039/c5fo00678c

Ito, T., Kato, M., Tsuchida, H., Harada, E., Niwa, T., & Osawa, T. (2011). Ergothioneine as an anti-oxidative/anti-inflammatory component in several edible mushrooms. *Food Science and Technology Research*, ***17***(2), 103-110.

Kalaras, M.D., Beelman, R.B., Holick, M.F., & Elias, R.J. (2012). Generation of potentially bioactive ergosterol-derived products following pulsed ultraviolet light exposure of mushrooms (*Agaricus bisporus*). *Food chemistry*, ***135***(2), 396-401. https://doi.org/10.1016/j.foodchem.2012.04.132

Kalaras, M.D., Richi, J.P., Calcagnotto, A., & Beelman, R.B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. *Food Chemistry*, *233*, 429-433. https://doi.org/10.1016/j.foodchem.2017.04.109

Kopinski, L., & Kwiatkowska-Marks, S. (2012). Utilization of waste newspaper using oyster mushroom mycelium. *Industrial & Engineering Chemistry Research*, ***51***(11), 4440-4444. https://doi.org/10.1021/ie202765b

Lee, S.H., Hwang, H.S., & Yun, J.W., (2009). Antitumor activity of water extract of a mushroom, Inonotus obliquus, against HT-29 human colon cancer cells. *Phytotherapy Research*, ***23***(12), 1784-1789. https://doi.org/10.1002/ptr.2836

Muhamad, N., Muhmed, S.A., Yusoff, M.M., & Gimbun, J. (2014). Influence of solvent polarity and conditions on extraction of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*. *Journal of Food Science and Engineering*, ***4***, 255 -260.

Nguyen, T.H., Nagasaka, R., & Ohshima, T. (2012). Effects of extraction solvents, cooking precedures and storage conditions on the contents of ergothioneine and phenolic compounds and antioxidative capacity of cultivated mushrooms *Flammulina velutipes*. *International Journal pf Food Science & Technology*, ***47***(6), 1193-1205. https://doi.org/10.1111/j.1365-2621.2012.02959.x

Pahila, J., Ishikawa, Y., & Ohshima, T. (2019). Effects of ergothioneine-rich mushroom extract on the oxidative stabiity of astaxanthin in liposomes. *Journal of Agricultural and Food Chemistry*, ***67***(12), 3491-3501. https://doi.org/10.1021/acs.jafc.9b00485

Rachmat, R., & Horibe, K. (1999). Solar heat collection characteristics of a fiber reinforced plastic drying house. *Transactions of the ASAE*, ***42***(1), 149-157.

Safitri, S.A., & Lestari, A. (2021). Uji produktivitas jamur merang (*Volvariella volvaceae*) bibit F4 asal Cilamaya dengan berbagai konsentrasi media tanam substitusi tongkol jagung. *Agrotekma: Jurnal Agroteknologi dan Ilmu Pertanian*, ***5***(2), 122-131. https://doi.org/10.31289/agr.v5i2.4670

Stampfli, A.R., Blankenfeldt, W., & Seebeck, F.P. (2020). Structural basis of ergothioneine biosynthesis. *Current Opinion in Structural Biology*, ***65***, 1-8. https://doi.org/10.1016/j.sbi.2020.04.002

Weigand-Heller, A.J., Kris-Etherton, P.M., & Beelman, R.B. (2012). The bioavailability of ergothioneine from mushrooms (*Agaricus bisporus*) and the acute effects on antioxidant capacity and biomarkers of inflammation. *Preventive Medicine*, ***54***, S75 -S78. https://doi.org/10.1016/j.ypmed.2011.12.028

Yen, M.T., Chang, Y.H., Huang, S.J., Cheng, M.C., & Mau, J.L. (2018). Extraction of ergothioneine from *Pleurotus eryngii* and *P. citrinopileatus* (*Agaricomycetes*) and preparation of its products. *International Journal of Medicinal Mushrooms*, *20*(4), 381-392. https://doi.org/10.1615/intjmedmushrooms.2018025953

Yu, F., Song, J., Liang, J., Wang, S., & Lu, J. (2020). Whole genome sequencing and genome annotation of the wild edible mushroom, *Russula griseocarnosa*. *Genomics*, ***112***(1), 603-614. https://doi.org/10.1016/j.ygeno.2019.04.012