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Analysis of Major Nutritional Components of *Pleurotus pulmonarius* During the Cultivation in Different Indoor Environmental Conditions on Sawdust

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ABSTRACT

Pleurotus pulmonarius was cultivated in three different environmental conditions, in ambient indoor environment (System 1), in humidifying without ventilation (System 2) and in humidifying with ventilation (System 3) to analyse the major nutritional contents. Sawdust was the main substrate for all the cultivation systems. The lowest temperature and the highest optimal humidity were found in System 3. The temperature and humidity had shown statistically significant among the three cultivation Systems. The highest numbers of flushes was found both in System 2 and System 3 but System 1 was produced mushrooms till 3rd flush. About 29.5%, 28.3%, 28.5% protein; 59.0%, 55.8%, 54.3% carbohydrate and 3.8%, 3.5%, 3.3% lipid were found in System 1, System 2 and System 3 respectively. The protein, carbohydrate, and lipid contents were shown statistically insignificant among the cultivation systems. The highest value of protein, carbohydrate and lipid were found for the sample of 1st flush in all the cultivation systems but the values were started to decrease with the increased numbers of flushes significantly. So, this study shown that, although the environmental conditions of the three cultivation systems were varied significantly but the protein, carbohydrate and lipid contents were existed their normal values in all cases but the values were decreased by the increased numbers of flushes.

Introduction

Mushrooms of *Pleurotus spp*. are commonly known as oyster mushrooms which occupy the second position among most cultivated edible mushrooms worldwide due to their nutritional values (Khan et al., 2008), grow in the wild in tropical and subtropical regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006; Chirinang and Intarapichet, 2009). Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries (Ma et al., 2014; Sufer et al., 2016). Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Khatun et al., 2015; Oei, 2003). *P. ostreatus*, *P. sajor caju*, *P. florida* and *Calocybe indica* were rich in proteins (20-25%) and fibers (13-24%), lipid (4-5%), carbohydrate (37 to 48%) and ash (8-13%). The moisture content of mushrooms were ranged from 86 to 87.5% (Alam et al., 2008).

P. sajor caju was cultivated in 25-30°C temperature and 70-80% relative humidity environmental conditions and found 90% moisture, 29.3% protein, 62.9% carbohydrate, 0.9% fat and other nutritional components (Gogavekar et al., 2014). Ashraf et al. (2013) studied about the effect of three different substrates (a) cotton waste (b) wheat straw and (c) paddy straw on the nutritive value of three different *Pleurotus spp* viz *P. sajor-caju*, *P. ostreatus* and *P. djmor* and nutritive contents of *P. sajor-caju* were found high in cotton waste substrate.

In another study, *P. sajor-cajui* was cultivated in four types of substrates (a) paddy straw (b) cotton waste (c) cotton waste with straw and (d) cotton waste with used tea-leaves and compared the nutritional value with *Agaricus bisporus*, *Lentinus edodes*, *P. ostreatus* and *Volvariella volvacea*. In that study researchers found that *P. sajor-caju* grown on cotton waste as substrate had higher protein content and comparable carbohydrate content to the four common types of mushrooms. The crude fats, ash, energy value, vitamin and mineral contents are lower and yet the differences are not great (Chang et al., 1981). *Ganoderma lucidum* grown on orange stump had higher nutritional value than mushrooms collected from nature (Turfan et al., 2016).

Presently sawdust is commonly used and is preferred medium at commercial scale. Different spawn of oyster mushroom was cultivated on sawdust as the fastest spawn running, pinhead formation, the highest mean yield and

maximum fresh yield percentage (Pathmashini et al., 2009). Very little study has performed on mushrooms nutritional contents by harvesting flushes and different environmental conditions. So, this study has initiated to investigate and analyse the major nutritional contents of *P. pulmonarius* (protein, carbohydrate and lipid) cultivated in different environmental conditions on sawdust as the main substrate.

Materials and Methods

Preparation of Mushroom Growing Bags

Substrates for growing mushroom bags were formulated by the mixture of saw-dust, rice bran and agricultural lime with the ratio of 100:10:1 (Abdul et al., 2011). About 800 g of substrate mixtures was filled in each autoclavable polypropylene bags (6×9 inch) and then the opening parts of the bags were closed by plastic cork, paper, rubber band and cap to protect loss of moisture content from inside the bags (Islam et al., 2016b). After that, the bags were sterilized at 20 psi pressure and 121°C temperatures for 6 hours to kill the spore of bacteria, fungi and other microorganisms (Christopher and Custodio, 2004). After sterilization, they were cooled to room temperature and injected with spawn prepared through tissue culture technique in Cell and Tissue Culture Laboratory, University Malaysia Perlis. Then the spawn injected bags were then placed vertically for 30-35 days in the dark for mycelium colonization.

Indoor Mushroom Cultivation

The experimental procedures were designed as ambient indoor environment without humidifying and ventilation (System 1), indoor humidifying environment without ventilation (System 2), indoor humidifying environment with ventilation (System 3) and indoor ventilating environment without humidifier (System 4). System 4 had no longer performed as in Malaysian environment where the indoor humidity is too low. Therefore, ventilation treatment absorbs the indoor humidity and tends to reduce it severely. In this condition, without any humidifying system mushrooms are not able to grow in Malaysia. So, this study had performed mushroom cultivation in System 1, System 2 and System 3.

The complete colonization 200 bags were transferred into the indoor growing room and arranged by rope as Islam et al., (2016a) for System 1, System 2 and System 3 consecutively. Four units of thermohygrometer were installed in different points to monitor and record the temperature and humidity.

No artificial method was applied during the cultivation of System 1 but System 2 and System 3 were followed in optimum humidity 80-90% by humidifier and ventilation (for System 3). For System 2, the humidifier was controlled by fixing with timer at 20 minutes running followed by 65 minutes interval to retain 80-90% humidity. And for System 3, the humidifier was controlled by fixing with timer at 25 minutes running followed by 35 minutes interval to retain 80-90% humidity. These humidifying durations were optimized by the optimization procedure (Islam et al., 2016b, 2016c). The temperature and humidity inside the indoor growing house were monitored and recorded by every hour. After maturation, the fruit bodies were harvested for nutritional analysis.

Mushroom Sample Preparation

About 500 g of the first flush fresh mushrooms from -"System 1"-, -"System 2"- and -"System 3"- were collected from the cultivation room. After that, they were transferred to biochemistry laboratory of University Malaysia Perlis (UniMAP) to follow the next procedures. The mushroom samples were placed in the laboratory oven for 3 hours at 50°C to remove all the water content without denaturation and degradation of protein. After 3 hours, the dried mushrooms were removed from the laboratory oven and weighed on digital weighing machine. Then, they were grinded in a grinder for 2 to 3 minutes at high speed in order to obtain mushrooms in powder form. Transparent plastic bags were used to keep the powdered mushrooms for biochemical analysis. This process was repeated for the intermediate and last flush of production as Table 1. This sample preparation procedure was followed by the extraction technique (Mizuno, 1995). In Table 1, sample of 1st and 3rd flush was collected from System 1 since no mushroom was found after 3rd flush of harvesting in this cultivation system.

Table 1 Sample collection from different cultivation systems for biochemical analysis

System	Sample 1	Sample 2	Sample 3
System 1	1st Flush	3rd Flush	
System 2	1st Flush	4th Flush	7th Flush
System 3	1st Flush	4th Flush	7th Flush

Determination of Total Protein

Preparation of protein standard curve: Total 200 mg of Bovine Serum Albumin (BSA) powder was dissolved in 100 ml distilled water and stirred to produce 2 mg/ml or 0.2% BSA stock solution. Then, 0.5 ml of BSA stock solution was pipetted into the test tube and distilled water was added to make up 5 ml total solution so that 0.2 mg/ml BSA concentration solution was made. This process was repeated for 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml, 1.2 mg/ml, 1.4 mg/ml, 1.6 mg/ml, 1.8 mg/ml and 2.0 mg/ml by increasing 0.5 ml BSA stock solution and reducing 0.5 ml distilled water one after another. Then, 5.0 ml analytical reagent (100 ml of sodium carbonate (Na_2CO_3) and NaOH mixture, 2 ml of copper sulphate ($CuSO_4$) solution and sodium potassium tartrate ($KNaC_4H_4O_6$) mixture) was added to each different BSA concentration solution. The mixtures were left at room temperature for 10 minutes to react. Next, 0.2 ml 50% (v/v) Folin-Ciocalteau phenol test solution was added into respective test tubes to react for another 30 minutes. After that, BSA concentration was measured using spectrophotometer at wavelength of 660 nm. Lastly, a graph was plotted using BSA concentration on the X-axis while the absorbance value on the Y-axis.

Total protein analysis: The protein concentration was analysed using Lowry's method (Lowry et al., 1951). About 10 mg of mushroom powder of each flush from -"System 1"- were weighed into different test tubes and 5 ml of 1 N (NaOH) was added. The test tubes were kept at room temperature for 24 to 48 hours for protein extraction. After 24 to 48 hours of extraction, the samples were centrifuged at 5,000 rpm for 10 minutes and 0.5 ml of each protein extract was pipetted into the test tubes containing 5 ml of analytical reagent and allowed to incubate at room temperature for 10 minutes. After 10 minutes of incubation, 0.5 ml of 1 N Folin-Ciocalteau phenol test solution was added into each test tube and incubated for another 30 minutes. 3 ml of sample from each test tube was pipetted into cuvettes for absorbance testing. The absorbance was determined at 660 nm using spectrophotometer and set to zero using blank sample. Concentration of protein for each sample was determined using the same procedure. This same procedure was repeated for the samples of -"System 2"- and -"System 3"- as in Table 1. All this process was triplicate for ANOVA.

Determination of Total Carbohydrate

Preparation of glucose standard curve: Total 100 mg of glucose was dissolved in 100 mL of water as stock solution. 10 mL of stock solution was diluted to 100 mL with distilled water to produce 0.1mg/ml working standard solution. Then 0.2,0.4,0.6,0.8 and1ml of working standard of glucose was taken in boiling tubes and the final volumes of each tube was made 1ml by adding distilled water. 1ml of 5% phenol and 5ml of 96% sulphuric acid was added one by one in each tube and shook well so that the phenol and sulphuric acid get mixed thoroughly with working standard. After 10 minutes all the tubes were placed in water bath at 25-30°C for 15 minutes. Blank was set with 1ml of distilled water and optical density of each tube was taken at 490 nm using spectrophotometer. Finally, a graph was plotted using glucose concentration on the X-axis while the absorbance value on the Y-axis.

Total carbohydrate analysis: The carbohydrate concentration was analysed using phenol sulphuric acid method (DuBois et al., 1956). About 100 mg of mushroom powder from each sample of -"System 1"- was added with 5 ml of 2.5N hydrochloric acid and put into boiling water for about 3 hours. After that the samples were being cooled at room temperature before being neutralized with solid sodium carbonate until effervescence ceases. The volume of each sample had been made as 100 ml and centrifuged. Then, 0.2 ml of sample solution from each centrifuged sample was pipette out and transferred to a test tube. After that, 0.8ml of distilled water was added to each test tube until it reaches 1 ml. 1 ml of phenol solution and 5 ml of 96% of sulphuric acid were added to each of the test tube and shaken thoroughly with the use of vortex. After 10 minutes of shaken, the test tubes was then placed into a water bath at 25-30 ˚C for 20 minutes before being read at uv-spectrometer at 490 nm wavelength to record optical density. This same procedure was repeated for the samples of -"System 2"- and -"System 3"- as in Table 1. All this process was triplicate for ANOVA.

Determination of total fat: Total lipid was determined by slight modified of Folch method (Folch et al., 1957). 5 g of ground mushroom powder from each sample of -"System 1"- was suspended in 50 ml of chloroform: methanol (2:1 v/v) mixture then mixed thoroughly and incubated for three days. The solution was filtrated and centrifuged at 1000 × g by a table centrifuge machine. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining was the crude lipid. This same procedure was repeated for the samples of -"System 2"- and -"System 3"- as in Table 1. All this process was triplicate for ANOVA.

Results and Discussion

Indoor Environment

The temperature and relative humidity persisting in all experimental methods inside the rooms are presented in Table 2a. Maximum, minimum and mean temperatures were varied greatly among the three different cultivation methods. The lowest mean temperature of 27.5±.6°C and the highest mean humidity of 89.1±2.9% were observed in System 3 whereas the highest temperature of 30.1±.9°C and the lowest mean humidity of 64.4±8.7% were observed in System 1. The mean temperature of System 2 was 28.9±.7°C which was lower than System-1 but higher compared to System 3. Whereas the mean humidity of System-2 was 84.3±3.6% which was higher than System 1 but also lower than System 3 (Table 2a). The overall mean temperature and humidity were shown significantly difference in one-way ANOVA where the significance level was $P<0.05$ (Table 2b).

Table 2a Descriptive statistics of temperature and humidity in different environmental conditions during cultivation

Parameter	Environmental Conditions	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Temperature	System 1	30.1	0.89	0.063	29.00	32.00
	System 2	28.9	0.69	0.048	28.00	30.00
	System 3	27.5	0.64	0.032	26.50	29.50
Humidity	System 1	64.39	8.74	0.618	41.00	77.00
	System 2	84.22	3.61	0.255	80.00	90.00
	System 3	89.06	2.91	0.145	80.00	90.00

Table 2b One way ANOVA of temperature and humidity in different environmental conditions during cultivation

Parameter	Compare	Sum of Squares	Df	Mean Square	F	Sig.
Temperature	Between Groups	861.4	2	430.7	822.8	0.000
	Within Groups	417.2	797	.52		
Humidity	Between Groups	82819.7	2	41409.9	1561.8	0.000
	Within Groups	21132.1	797	26.5		

Total Protein Analysis

Protein standard calibration curve analysis: The standard curve of protein was used as the calibration curve for total protein content in the sample. A graph of absorbance reading at 660 nm against protein concentration was plotted. The standard curve equation obtained was as equation (1) with the correlation coefficient, R^2 of 0.997.

$$y = 1.597x \quad (1)$$

Where *y* is the absorbance reading at 660 nm and x is protein concentration (mg/ml). Figure 1 shows the bovine serum albumin (BSA) standard curve.

Protein content in different cultivation systems: The protein content of 30.58±0.07%, 30.66±0.19% and 30.73±0.14% were found in the first harvesting flushes of System 1, System 2 and System 3 respectively. Sample 2 was collected from 3rd flush of System 1 and 4th flush of System 2 and System 3 since System 1 was not produced mushroom after 3rd harvesting flushes due to unfavourable environmental conditions. The protein content of sample 2 was found as 28.48±0.2%, 28.63±0.23% and 29.23±0.25% in System 1, System 2 and System 3 respectively. The sample 3 was collected from the 7th harvesting flushes of System 2 and System 3, the protein content was found 25.85±0.16% and 25.66±0.14% in the System 2 and System 3 respectively (Table 3). So, this result showed that the percentage of protein content of *P. pulmonarius* was decreased with the increased numbers of harvesting flush.

The overall 29.53±1.05%, 28.38±1.39% and 28.54±1.51% protein had found in System 1, System 2 and System 3 respectively. The mean value of System 1 was found as the highest protein content since the sample was collected only from 1st and 3rd harvesting flush (Table 3). The protein content of different flushes had showed statistically significant whereas insignificant result was found at 0.856 levels among the System 1, System 2 and System 3 in one-way ANOVA test where the significant level was considered at P≤0.05 (Table 4).

Total Carbohydrate Analysis

Standard curve calibration analysis: The purpose of standard curve was to obtain the equation which was required in order to calculate the concentration of glucose solution at different conditions. The standard was determined by measuring the absorbance of glucose solution at different concentrations. The wavelength of the absorbance was set at 490 nm. From the reading of the absorbance that had been recorded, the standard curve of glucose concentration versus absorbance was plotted and generated an equation (2).

$$y = 57.5 \times 0.0167 \quad (2)$$

By the equation (2), the absorbance value which was determined at wavelength of 490 nm was denoted as y and x refers to the concentration of glucose. The correlation coefficient, R^2 obtained was 0.9902, which is acceptable as the value closed to 1 (Figure 2). This standard curve equation was used to calculate the concentration of carbohydrate in the samples of this study.

Carbohydrate content in different cultivation systems: The carbohydrate content had found 62.5±0.432%, 63.0±0.647% and 62.5±0.912% in the first harvesting flushes of System 1, System 2 and System 3 respectively. Sample 2 was collected from 3rd flush of System 1 and 4th flush of System 2 and System 3 since System 1 had not produced mushroom after 3rd flush because of unfavourable environmental conditions. The carbohydrate content of sample 2 was found 55.5±0.910%, 56.0±0.647% and 54.0±0.648% in System 1, System 5 and System 3 respectively. The sample 3 was collected from the 7th flush of System 2 and System 3. The carbohydrate content 48.56±1.191% and 46.5±0.192% were in sample 3 of System 2 and System 3 respectively (Table 5).

The overall mean value of carbohydrate contents in System 1, System 2 and System 3 had found 29.53±1.05%, 28.38±1.39% and 28.54±1.51% respectively. The carbohydrate contents of System 1 was also found as the highest carbohydrate content since the sample was collected only from 1st and 3rd harvesting flushes. The carbohydrate content was found statistically significant among the different harvesting flushes where the values were started to decrease by increased number of harvesting flushes. But the overall mean values were found insignificant at 0.783 levels among the System 1, System 2 and System 3 in one-way ANOVA test where the significant level was considered at P≤0.05 (Table 6).

Lipid Analysis

The lipid content of 4.04±0.104%, 4.07 ± 0.174% and 4.14±0.152% were found in the first flush of System 1, System 2 and System 3 respectively. Sample 2 was collected from 3rd flush of System 1 and 4th flush of System 2 and System 3 since System 1 was not produced mushroom after 3rd flush because of unfavourable environmental conditions. The lipid content of sample 2 was found 3.61±0.046%, 3.58±0.064% and 3.3±0.092% in System 1, System 2 and System 3 respectively. The sample 3 was collected from the 7th flush of System 2 and System 3. The lipid content of sample 3 was found 2.70±0.52% and 2.56±0.086% in the case of System 2 and System 3 respectively (Table 7).

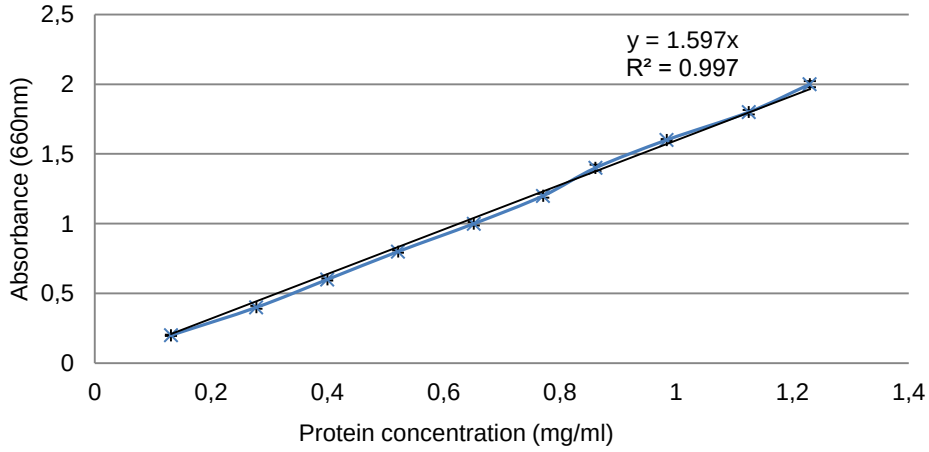

Figure 1 Bovine serum albumin (BSA) standard curve

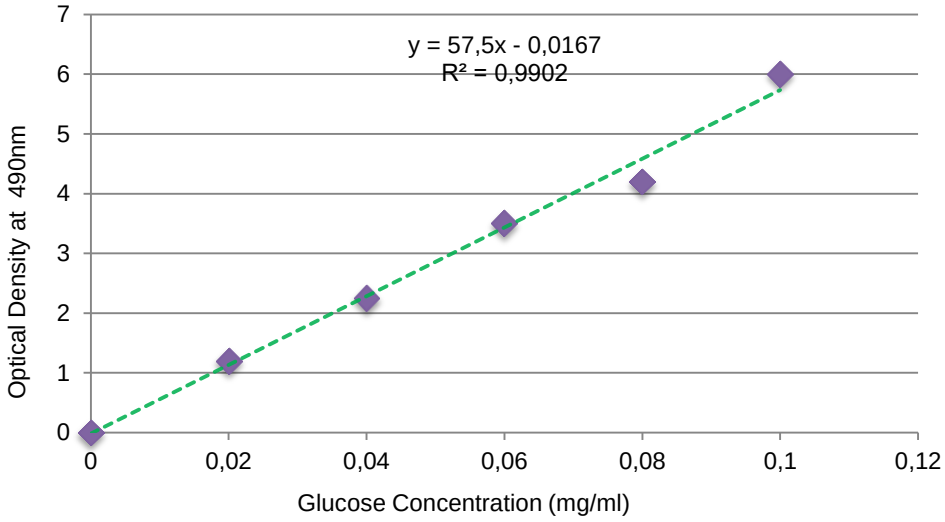

Table 3 Percentages of protein concentration in different cultivation systems and flushes (Dried Sample)

Condition	Sample (Flush)	Absorbance Mean ± SE	Protein concentration (mg/ml)	Percentages of Protein Contents (%)	
				Sample Mean ± SE (%)	System Mean ± SE(%)
System 1	Sample 1	0.814 ± 0.002	0.509±0.001	30.58 ± 0.07	29.531±1.05
	Sample 2	0.758 ± 0.006	0.475±0.004	28.48 ± 0.23	
System 2	Sample 1	0.816 ± 0.005	0.5110 ± 0.003	30.66 ± 0.19	28.38±1.39
	Sample 2	0.762 ± 0.006	0.477 ± 0.004	28.63 ± 0.23	
	Sample 3	0.681 ± 0.004	0.4264 ± 0.003	25.85 ± 0.16	
System 3	Sample 1	0.818± 0.004	0.5122±0.002	30.73 ± 0.14	28.54±1.51
	Sample 2	0.778± 0.007	0.4872±0.0041	29.23 ± 0.25	
	Sample 3	0.683± 0.004	0.4277±0.002	25.66 ± 0.14	

The results are the mean ± SEM of 3 replicates.

Table 4 One-way ANOVA analysis for protein contents of mushroom in different cultivation systems and flushes

Factors	Compared	Sum of Squares	Df	Mean Square	F	Sig.
In Flushes	Between Groups	28.845	2	14.422	209.435	0.000
	Within Groups	344	5	0.069		
	Total	29.189	7			
In Systems	Between Groups	1.759	2	0.879	0.160	0.856
	Within Groups	27.438	5	5.488		
	Total	29.197	7			

Values are significant at P≤0.05 level.

Table 5 Percentages of carbohydrate concentration in different cultivation systems and flushes (Dried sample)

Condition	Sample (Flush)	Absorbance Mean ± SE	Carbohydrate concentration (mg/ml)	Percentages of Carbohydrate concentration (%)	
				Sample Mean ± SE (%)	System Mean ± Std.
System 1	Sample 1	7.13 ± 0.033	0.125±0.0009	62.5±0.432	59.00±4.95
	Sample 2	6.37 ± 0.088	0.111±0.0018	55.5±0.910	
System 2	Sample 1	7.2 ± 0.058	0.126 ± 0.0012	63.0 ± 0.647	55.85±7.22
	Sample 2	6.4 ± 0.058	0.112 ± 0.0012	56.0 ± 0.647	
	Sample 3	5.57 ± 0.120	0.097± 0.0024	48.56± 1.191	
System 3	Sample 1	7.13± 0.088	0.125±0.0018	62.5±0.912	54.33±8.01
	Sample 2	6.20± 0.057	0.108±0.0013	54.0±0.648	
	Sample 3	5.33± 0.088	0.093±0.0018	46.5±0.912	

The results are the mean ± SEM of 3 replicates.

Table 6 One-way ANOVA analysis for carbohydrate contents of mushroom in different cultivation systems and flushes

Factors	Compared	Sum of Squares	Df	Mean Square	F	Sig.
In Flushes	Between Groups	278.859	2	139.430	156.48	0.000
	Within Groups	4.455	5	0.891		
	Total	283.314	7			
In Systems	Between Groups	26.359	2	13.179	0.256	0.783
	Within Groups	256.956	5	51.391		
	Total	283.314	7			

Values are significant at P≤0.05 level.

Table 7 Percentages of lipid concentration in different cultivation systems and flushes (g/100g Dried Sample)

Condition	Sample (Flush)	Percentages of Lipid Concentration (g/100g dried sample)	
		Sample Mean ± SE.	System Mean ± SE.
System 1	Sample 1	4.04±0.104	3.83±0.215
	Sample 2	3.61±0.046	
System 2	Sample 1	4.07 ± 0.174	3.45±0.401
	Sample 2	3.58 ± 0.064	
	Sample 3	2.70± 0.52	
System 3	Sample 1	4.14±0.152	3.33±0.791
	Sample 2	3.3±0.092	
	Sample 3	2.56±0.086	

The results are the mean ± SEM of 3 replicates.

Table 8 One-way ANOVA analysis for lipid contents of mushroom in different cultivation systems and flushes

Factors	Compared	Sum of Squares	Df	Mean Square	F	Sig.
In Flushes	Between Groups	2.535	2	1.267	86.17	0.000
	Within Groups	0.074	5	0.015		
	Total	2.608	7			
In Systems	Between Groups	0.302	2	0.151	0.327	0.735
	Within Groups	2.306	5	0.461		
	Total	2.608	7			

Values are significant at P≤0.05 level.

The overall mean value of lipid contents of System 1, System 2 and System 3 were found 3.83±0.215%, 3.45±0.401% and 3.33±0.791% respectively. The mean value of System 1 was found as the highest lipid content since the sample was collected only from 1st and 3rd harvesting flushes (Table 7). The protein content had showed statistically significant among the different harvesting flushes whereas insignificant result was found at 0.856 levels among the System 1, System 2 and System 3 in one-way ANOVA test where the significant level had considered at P≤0.05 (Table 8).

Within these three systems, the mean temperature varied approximately 1.1-2.5°C and the mean humidity varied approximately 19.9-24.7% which had shown statistically significant results with one-way ANOVA where the significance level was P<0.05 (Table 2b). In System 2, the temperature and humidity varied 1.05°C lower and 19.84% higher from System 1 respectively. Other hand in System 3, the temperature and humidity varied 2.46°C lower and 24.66% higher from System 1 respectively. So the temperature and humidity of System 3 was found 1.41°C lower and 4.88% higher (respectively) than System 2. The lowest indoor

temperature (26.5°C) was also found in System 3 whereas; the highest (32°C) was in System 1. Moreover in System 1 and 2, there was no proper air circulation although the humidity of System 2 was within optimal ranges. This result agrees with Thepa et al. (1999) that evaporative cooling and continuous ventilation system reduced the temperature and increased the relative humidity of air inside a mushroom house, which is also similar to the earlier report of Leiva et al. (2015). So, this result has shown that the environmental conditions of these three cultivation systems were significantly different from each other.

In this study it was noticed that the protein, carbohydrate and lipid contents of *P. pulmonarius* decreased with the increasing number of harvesting flushes. Although the cultivation systems had found significant different environmental condition but the nutritional contents were noticed decreased by increasing flushes in all systems of cultivation. Moreover, System 3 had provided the best environmental condition for mushroom growing but the same results also had found. Caglarırmak (2007) reported that, the nutritional contents of *Pleurotus species* had significantly decreased by increasing numbers of harvesting which is also supported to the report of Miles and Chang, (2004).

On the other hand, the overall mean value of protein, carbohydrate and lipid had found insignificant difference among the cultivation systems. The significant difference of environment among the systems had influenced the number of flushes and production but not influenced the nutritional contents of protein, carbohydrate and lipid. Kadlag et al. (1998) reported that the protein content of oyster mushroom varied between the ranges of 20% and 30% on a dry weight basis in different species. Some other studies also reported that the protein contents of *P. pulmonarius* varied from 25 to 30% (Ashraf et al., 2013; Gogavekar et al., 2014; Miles and Chang, 2004).Several studies also reported that the carbohydrate contents of *P. pulmonarius* varied from 30 to 50% (Ashraf et al., 2013; Maria et al., 2015). Very low value (2.5-4.07 g/100g) of lipid content in *P. pulmonarius* was found in this present study. The lipid content usually ranges between 2.6-5.5 g/100g among different *Pleurotus spp.* of oyster mushrooms (Alam et al., 2008; Khan et al., 2008). So, study proved that, the variation of environmental conditions influenced the number of flushes and production of *P. pulmonarius* but the protein, carbohydrate and lipid contents are independent of environment conditions. Therefore, the percentages of protein, carbohydrate and lipid were exit at their normal value cultivated on sawdust as substrate.

Conclusions

Based on the result of current study, it could be concluded that mushroom is able to be cultivated in controlled environment where the humidifying with ventilation system provided better growing environment than others. In view of different indoor environmental conditions, there was no significant difference had found in protein, carbohydrate and lipid contents which were exit at normal value cultivated on sawdust. But the protein, carbohydrate and lipid contents had found significantly decreasing with increasing numbers of flushes in all the cultivation systems. So, this study concluded that indoor environment can influence the mushrooms flush of harvesting and production but the protein, carbohydrate and lipid contents are independent of environmental conditions.

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