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The Growth Morphology and Yield of Grey Oyster Mushrooms (*Pleurotus sajor-caju*) Subjected to Different Durations of Ultrasonic Sound Treatment

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Abstract. Grey oyster mushroom (*Pleurotus sajor-caju*) is a type of edible mushroom that suitable to be cultivated in Malaysia. There is an increasing demand in the market due to its high nutritional values. In this study, effects of different durations of ultrasonic treatment on the growth morphology, yield enhancement and quality of mushrooms were investigated. Four different durations of ultrasonic treatment had been applied on the mushroom bags which were 1.5, 3.0, 4.5 and 6.0 minutes and 0 minutes act as control. The results showed that all ultrasonic treated bags had shorter time for mycelium to fill-up the mushroom bags as compared to control. Ultrasonic treated bags was observed could promote and activate the pinhead emergence and fruiting bodies formation. Besides, all ultrasonic treated bags also showed higher yield and better quality in terms of the largest pileus diameter of mushrooms as compared to control. For the color of mushrooms, there was no significant different ($P > 0.05$) observed in L^* values. However, treatment of 1.5 minutes had the highest a^* (redness) and b^* (yellowness) values. Different durations of ultrasonic treatment did not showed any significant different in texture and moisture content of mushrooms. In summary, 1.5 minutes was the best treatment duration as it took the shortest time for different growth stages with the greatest yield of mushrooms.

Keywords: Grey oyster mushroom (*Pleurotus sajor-caju*), ultrasonic sound treatment, growth performance, yield, physico-chemical properties.

1. Introduction

Pleurotus sajor-caju is also known as grey oyster mushrooms due to their physical appearance which looks like an oyster shell. The Latin *Pleurotus* means the sideways growth of stem with respect to the cap [1]. It has unique flavor, aromatic properties and consist of high amount of fiber, carbohydrates, vitamins, protein and minerals [2]. Sound vibration was found could stimulate seed germination and plant growth [3]. Ultrasound is the sound waves that have the frequency that exceeds the limits of human hearing which is about 20 KHz [4]. Several research have reported on the effects of ultrasound on tomato plants, barley and other vegetables in improving crop yield and quality. The frequency, durations and intensity of sound would affect the growth response of plants [3]. In this study, the



effects of different durations of ultrasonic sound treatment on the growth morphology and yield of grey oyster mushrooms (*Pleurotus sajor-caju*) were investigated.

2. Methodology

In the preparation of substrate bags, the substrate is a mixture of calcium carbonate, rice bran and sawdust at a ratio of 1:10:100 (38g: 380g: 3800g) respectively. The substrate was mixed manually by using both hand and around 740 ml of water was added into the substrate. Each polyethylene bag was filled with 500 g of the substrate (dry weight basis) and enclosed with cotton stuffed cap. Then, each substrate bag was sterilized in autoclave at 121°C for 30 minutes. The process of inoculation was carried out in a laminar flow. About 10 g to 15 g of the mushroom spawns were transferred into the mushroom bag. The cap was closed immediately to prevent contamination. Then, the mushroom bag was agitated gently in order to allow the mushroom spawns transferred down fully to the substrate bag. All the mushroom bags were labelled according to the treatment treated and arranged on the racks in the mushroom cultivation chamber with suitable conditions for spawn-running process.

2.1 Different Durations of Ultrasonic Sound Treatment

After 5 days from inoculation, each bag was treated with ultrasonic sound treatment at four different durations which were 1.5 minutes, 3.0 minutes, 4.5 minutes and 6.0 minutes. The treatment was carried out at every 5 days interval. Mushroom bags which were not treated with ultrasonic sound treatment act as control. The ultrasonic sound treatment was conducted in laboratory using ultrasonic bath at 37 KHz.

2.2 Spawn-running Stages

In the spawn-running process, the substrate bags were placed vertically on the racks in the mushroom cultivation chamber to accelerate the process due to pull of gravity force. It took around 25 to 35 days for mycelium to fill-up the bags. During spawn-running process, the temperature in the mushroom cultivation chamber was maintained at 28-30°C with relative humidity of 75-85% and only little light was required (about 10%).

2.3 Mushroom Growth Process and Harvesting

Once the mycelium had filled-up the bags, the mushroom bags were transferred to the mushroom house. The caps were opened and the mushroom bags were placed horizontally on the racks for pinhead emergence and fruiting body formation. The temperature in the mushroom house was maintained at 25°C to 30°C, relative humidity of 80% to 85% and about 30% of light. In the harvesting process, the fruiting bodies of mushroom were pulled from the substrate bags. Then, the mushroom bags were enclosed with caps and left for 7 days for the next fruiting cycle to give time for the injured mycelium to accumulate the nutrients and for tissue repaired.

2.4 Mycelium Growth Rate Determination

Mycelium growth rate (cm/day) in each bag for different durations of ultrasonic sound treatment and control were determined. During the spawn-running process, the growth of mycelium in each bag was measured from the neck of the bag until the bag is fill-up mycelium by using a ruler in term of length at every 5 days interval.

2.5 Mycelium Growth Performance

The number of days took for the mycelium to fill-up the bags, pinhead emergence and fruiting body formation were recorded started from the date of inoculation. The mushroom bags were monitored frequently in order to record the days taken for the mycelium to fill-up the bags, pinhead emergence and fruiting body formation.

2.6 Yield of Fruiting Bodies

Once the fruiting bodies of mushroom reached maturity, it could be harvested from the substrate bags. The yield of mushrooms were analyzed in terms of total fresh weight of fruiting bodies, number of fruiting bodies and percentage biological efficiency from 5 harvestings.

2.7 Substrate Utilized (g) for Every 100g Mushrooms Produced

After each harvesting, the weight of substrate bag was weighed and recorded. The amount of substrate utilized (g) for every 100g of mushrooms produced was calculated after 5 harvesting cycles. It could be determined how much the substrate utilized to produce 100g of mushrooms.

2.8 Pileus Size Determination

The largest and smallest sizes of harvested mushroom's pileus for different durations of ultrasonic treatment and control were chosen. The diameter measurement would be from each end side of the mushroom's pileus. The diameter of the largest and smallest sizes of the pileus were measured by using a ruler (cm) and recorded.

2.9 Texture (firmness) Determination

Texture analyzer TA.XT plus (Stable Micro Systems) was used to determine the firmness of the harvested mushrooms by using P/2 stainless steel probe. From the same surface of the harvested mushroom's pileus, three different points were taken to analyze the firmness. In the penetration test, cylinder probe of 2 mm diameter was used to puncture the harvested mushroom cap from top to 5 mm depth. During the analysis process, Texture Expert Software would captured the force, time and distance which gave a texture profile of the penetration. The maximum positive force was recorded which indicates the firmness value.

2.10 Color Determination

In the color analysis, the pileus of the harvested mushroom was analyzed using Minolta Chromameter with CIELAB Color parameter of L*, a*, b*. Three different points were taken from the same surface of the harvested mushroom's pileus. The L*, a*, b* values of the mushroom's pileus were measured and recorded. L meaning that monochrome scale expressing the amount of reflected light. If L of 100 was the pure white and L of 0 was matt black. While for 'a' was greenness (-) to redness (+) and 'b' was blueness (-) to yellowness (+).

2.11 Moisture Content Determination

The fresh weight of harvested mushrooms were weighed and placed on a pan in a moisture analyzer MX-50 at 105°C for around 1 hours. The moisture content of mushroom was determined directly from the moisture analyzer.

2.12 Experimental Design and Statistical Analysis

The experiment was designed based on Completely Randomized Design (CRD) with 4 different durations of ultrasonic sound treatment and one control. Each treatment was represented by 5 replicates. All the data obtained were analyzed using statistical package SAS version 16.0 software. One-way Analysis of variances (ANOVA) was used to analyze the differences among the treatments. The significant means ($P < 0.05$) was tested through Tukey's Studentized Range Test.

3. Results and Discussion

This section would focus on the analyses and discussions of the results obtained in this study in order to determine the effects of ultrasonic treatment at different durations on growth performance, yield and quality of harvested grey oyster mushrooms.

3.1 Mycelium Growth Rate

Mycelium growth of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment at 37 KHz frequency are presented in Figure 1. It showed that all the ultrasonic sound treated bags had significantly ($P < 0.05$) higher rate of mycelium growth compared to control. From Figure 1, it can be seen that the fastest rate of mycelium growth to fill-up the bags reaching 23 cm of bag length was when applied with 1.5 minutes of ultrasonic treatment which took about 25 days.

The slowest rate of mycelium growth showed by the control which no ultrasonic treatment applied on the mushroom bag and it took about 35 days to reach 23 cm. This showed that ultrasonic treatment had significant effect on the mycelium growth of the mushrooms. [5] also described that the carrot cell growth with the ultrasonic treatment (28 KHz) for 2 to 40 seconds had greater growth rate of cells than the control (untreated bag).

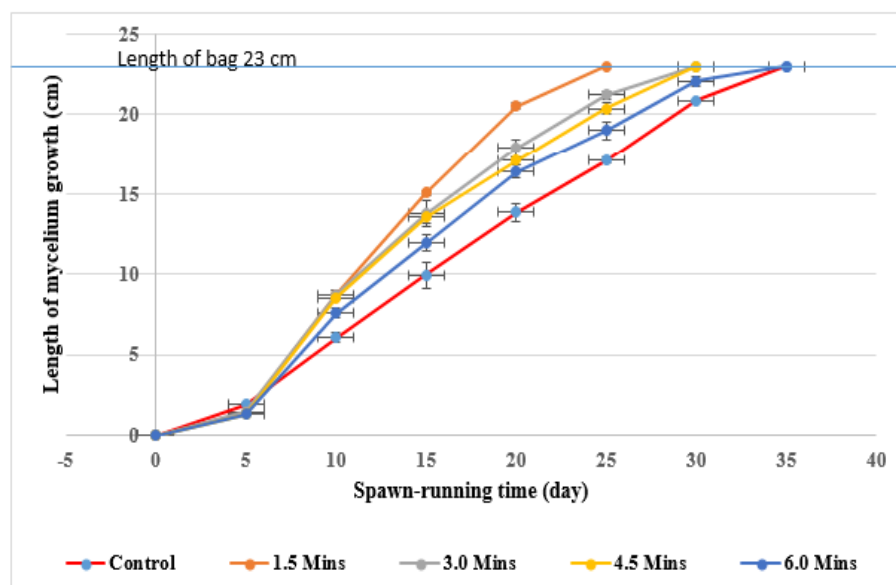


Figure 1: The mycelium growth of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment. Vertical bars represent standard errors.

Table 1: The means of mycelium growth rate (cm/day) of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment.

Ultrasonic treatments	Mycelium growth rate (cm/day)
Control	0.696 ± 0.041^c
1.5 mins	1.040 ± 0.055^a
3.0 mins	0.878 ± 0.097^b
4.5 mins	0.812 ± 0.045^{bc}
6.0 mins	0.766 ± 0.075^{bc}

Note: Values are means of 5 replicates. Means ($n=5$) \pm standard deviation.

a-c : Values bearing the different superscript within the same column are significantly different at 5% level ($P < 0.05$).

The mycelium growth rate (cm/day) for ultrasonic treatment with 1.5 minutes was significantly the fastest ($P < 0.05$) which was 1.040 cm/day followed by 3.0 minutes (0.878 cm/day), 4.5 minutes (0.812 cm/day) and 6.0 minutes (0.766 cm/day). While, the control took the slowest mycelium growth rate (cm/day) which was only 0.696 cm/day (Table 1).

[6] also reported that the longer ultrasound treatment could inhibited the growth and proliferation of the cells. With comparing to this study, ultrasonic treatment of 4.5 and 6.0 minutes took longer time for mycelium growth as compared to 1.5 and 3.0 minutes.

3.2 The Number of Days for Mycelium to Fill-up the Bag, Pinhead Emergence and Fruiting Body Formation

There were significant differences ($P < 0.05$) in the number of days taken for mycelium to fill-up the mushroom bags among all of the different durations of ultrasonic treatment (Figure 2). It can be seen that the ultrasonic treated bags had shorter time for mycelium to fill-up the bag compared to control. Ultrasonic treatment with durations of 1.5 minutes was significantly ($P < 0.05$) the fastest for mycelium to fill-up the substrate bags which took 22.2 days followed by 3.0 minutes (26.4 days), 4.5 minutes (28.4 days) and 6.0 minutes (30.2 days).

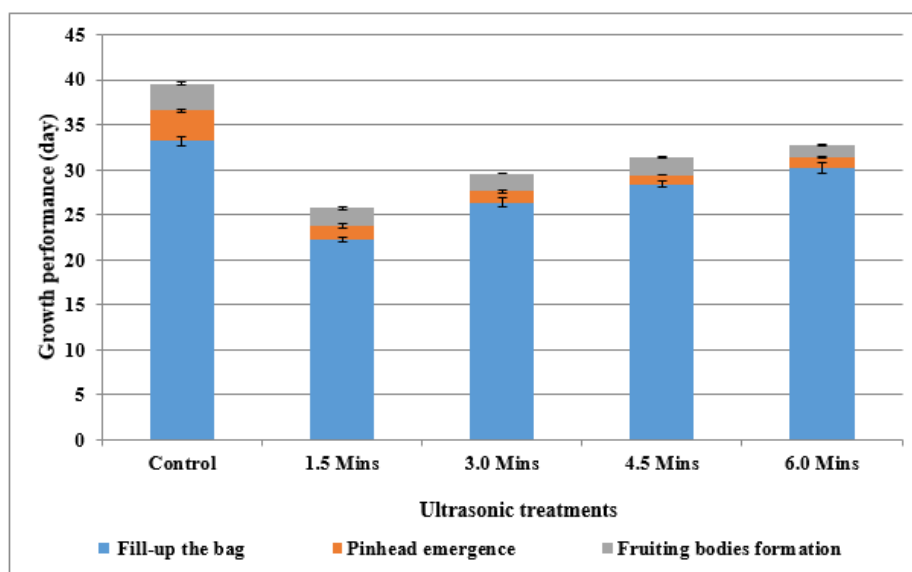


Figure 2: The number of days for mycelium to fill-up the bag, pinhead emergence and fruiting body formation of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment. Vertical bars represent standard errors.

[7] also reported that ultrasonic sound stimulation could increase the enzyme activities and the growth of cell by stimulating their physiological activities under appropriate intensity, frequency and durations of ultrasound. Similar results were also obtained in this study where control took 33.2 days which was the longest time for mycelium to fill-up the bags.

There were significantly ($P < 0.05$) longer time needed for pinhead emergence for control as compared to ultrasonic treated bags (Figure 2). The time taken for pinhead emergence were 1.0 to 1.6 days for ultrasonic treated bags. Whereas, control took 3.4 days which was the longest time for pinhead emergence. However, there was no significant different ($P > 0.05$) obtained among the different durations of ultrasonic treatment for pinhead emergence.

The number of days for fruiting bodies formation was counted from the pinhead emergence until the matured mushrooms had been harvested. There were significant differences ($P < 0.05$) in fruiting bodies formation among all the different durations of ultrasonic treatment (Figure 2). The ultrasonic treated bags had faster time for fruiting bodies formation as compared to control. The time recorded for fruiting bodies formation were 1.4 to 2.0 days for ultrasonic treated bags. While, the control took 3.0 days which was significantly ($P < 0.05$) the slowest time for fruiting bodies formation.

In overall, ultrasonic sound treatment of 1.5 minutes was the fastest time for mycelium growth rate and the shortest time taken to complete the different growth stages. In this study, the enzymes activity of mushrooms actively stimulated by ultrasonic treatment under suitable durations and thus actively promoted the growth of mushrooms.

3.3 Yield

For the number of fruiting bodies, there were significant differences ($P < 0.05$) among all the durations of ultrasonic treatment (Table 2). All the ultrasonic treated bags had higher number of fruiting bodies as compared to control.

Table 2: The yield of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment.

Ultrasonic treatments	No. of fruiting bodies	Total weight of fruiting bodies (g)	Biological efficiency (%)
Control	18.200±6.261 ^b	173.000±25.642 ^b	34.600±5.128 ^b
1.5 mins	34.600±10.644 ^a	377.000±73.981 ^a	75.400±14.796 ^a
3.0 mins	27.600±5.128 ^{ab}	332.500±10.897 ^a	66.500±2.179 ^a
4.5 mins	29.200±9.385 ^{ab}	326.500±39.394 ^a	65.300±7.879 ^a
6.0 mins	32.600±8.554 ^{ab}	327.500±57.228 ^a	65.500±11.446 ^a

Note: Values are means of 5 replicates. Means ($n=5$) \pm standard deviation.

a-b : Values bearing the different superscript within the same column are significantly different at 5% level ($P < 0.05$).

The ultrasonic treatment of 1.5 minutes had significantly ($P < 0.05$) the highest number of fruiting bodies which was 34.6 followed by 6.0 minutes (32.6), 4.5 minutes (29.2) and 3.0 minutes (27.6) for 5 harvesting cycles. Whereas, the control had the lowest number of fruiting bodies which was 18.2 only.

Ultrasonic treatment (20 KHz) of 1, 3, 5, 7, 9, and 11 minutes which had been applied on tomatoes resulted in an increased in germination rate and the yield of plants were also increased by 6 to 14% [8]. The similar results were also observed in this study where the number of fruiting bodies for all ultrasonic treated bags were greater than control.

There were significantly ($P < 0.05$) the lowest total weight of fruiting bodies obtained for control as compared to ultrasonic treated bags (Table 2). All the ultrasonic treated bags had greater total weight of fruiting bodies (ranged from 377.0 g to 326.5 g) as compared to control which only 173.0 g. However, there was no significant different ($P > 0.05$) observed among the different durations of ultrasonic treatment.

It was also reported that application of ultrasonic treatment (22 KHz) with durations of 1, 5 and 10 minutes on germination of carrot seeds (*Daucus carota L.*) would increase the germination rate by 17% and fresh weight by 22% [8]. With comparing to this study, all ultrasonic treated bags have greater total weight of fruiting bodies as compared to control.

Biological efficiency was determined from the ratio of total fresh weight of mushrooms per dry weight of substrate and expression in percentage. There were significantly ($P < 0.05$) the lowest biological efficiency obtained for control as compared to ultrasonic treated bags (Table 2). From the results, it can be seen that control (34.6%) tend to show lower biological efficiency than all the ultrasonic treated bags (ranged from 75.4 % to 65.3 %). This showed that all ultrasonic treated bags had a greater yield potentials of mushrooms converted from the substrate. Whereas, there was no significant different ($P > 0.05$) observed among the different durations of ultrasonic treatment (Table 2).

3.4 Substrate Utilized (g) for Every 100g of Mushrooms Produced

In terms of substrate utilized for every 100g mushrooms produced, there were significant differences ($P < 0.05$) among all the different durations of ultrasonic treatment (Table 3). It can be seen that all ultrasonic treated bags had lower amount of substrate utilized as compared to control for every 100g of mushrooms produced.

Table 3: The substrate utilized (g) for every 100g mushrooms produced when subjected to different durations of ultrasonic treatment for 5 harvesting cycles.

Ultrasonic treatments	Substrate Utilized (g) for Every 100g Mushrooms Produced
Control	246.818±57.089 ^a
1.5 mins	159.742±35.829 ^b
3.0 mins	196.824±24.649 ^{ab}
4.5 mins	159.018±20.220 ^b
6.0 mins	167.222±28.083 ^b

Note: Values are means of 5 replicates. Means ($n=5$) ± standard deviation.

a-b : Values bearing the different superscript within the same column are significantly different at 5% level ($P < 0.05$).

The best treatment duration of ultrasonic treatment would have lower substrate utilized for every 100g mushrooms produced. This means that all ultrasonic treated bags were able to utilize efficiently the lignocellulosic materials in the substrates for fruiting bodies formation and thus have a rapid growth.

For all ultrasonic treated bags, the amount of substrate utilized for every 100g of mushrooms produced ranged from 159.02g to 196.82g. Whereas, control had substrate utilized of 246.82g for every 100g of mushrooms produced.

[7] also reported that application of ultrasonic treatment on plants could results efficient nutrient transportation and absorption of useful elements from soil promoted by modifications of cellulose membrane. With comparing to this study, application of ultrasonic treatment at suitable duration on *Pleurotus sajor-caju* could promote the mushrooms to utilize lignin and cellulose as its carbon source and turning it into fruiting bodies.

3.5 Physico-chemical Properties

The pileus size of *Pleurotus sajor-caju* was determined by pileus diameter. There were significant differences ($P < 0.05$) in the largest pileus diameter of harvested mushrooms among all the different durations of ultrasonic treatment (Table 4).

Table 4: The pileus diameter (cm) of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment.

Ultrasonic treatments	Largest Pileus diameter (cm)	Smallest Pileus diameter (cm)
Control	7.100±1.903 ^b	2.480±1.458 ^a
1.5 mins	9.740±1.764 ^a	3.520±0.581 ^a
3.0 mins	9.420±0.455 ^{ab}	3.080±1.119 ^a
4.5 mins	7.740±0.594 ^{ab}	2.700±0.660 ^a
6.0 mins	9.720±1.103 ^a	2.500±0.308 ^a

Note: Values are means of 5 replicates. Means ($n=5$) ± standard deviation.

a-b : Values bearing the different superscript within the same column are significantly different at 5% level ($P < 0.05$).

All ultrasonic treated bags produced larger pileus diameter than control (Table 4). The largest pileus diameter for all ultrasonic treated bags were ranged from 7.74 cm to 9.74 cm. However, control had the largest pileus diameter of 7.10 cm only. Ultrasonic treatment of 1.5 minutes had the largest pileus diameter of 9.74 cm followed by 6.0 minutes (9.72 cm), 3.0 minutes (9.42 cm) and 4.5 minutes (7.74 cm). This showed that ultrasonic treatment had significant effect on the largest pileus diameter of the mushrooms.

For the smallest pileus diameter of the mushrooms, there was no significant different ($P > 0.05$) observed among all different durations of ultrasonic treatment (Table 4). This showed that ultrasonic treatment had no significant effect on the smallest pileus diameter of the harvested mushrooms.

The physico-chemical characteristics in terms of color of the harvested mushrooms were analyzed. L^* values represent the lightness of the color, if L of 100 was the pure white and L of 0 was matt black. There was no significant different ($P > 0.05$) observed in L^* values among all different durations of ultrasonic treatment (Table 5). In this study, control and all different durations of ultrasonic treatments for L^* values presented almost the same value which was ranged from 60.607 to 68.343. Ultrasonic treatment had no significant effect on the L^* values of the harvested mushrooms.

Table 5: The physico-chemical properties of *Pleurotus sajor-caju* subjected to different durations of ultrasonic treatment.

Ultrasonic treatments	Color			Firmness (gf)	Moisture Content (%)
	L^* value	a^* value	b^* value		
Control	68.343± 3.895 ^a	3.621±1 .085 ^b	15.417± 1.775 ^{ab}	69.469± 17.308 ^a	88.967±0.843 ^a
1.5 mins	62.607± 5.123 ^a	5.200±0 .939 ^a	17.372± 1.834 ^a	79.727± 22.472 ^a	88.573±0.643 ^a
3.0 mins	63.256± 4.988 ^a	3.978±0 .670 ^b	13.721± 1.536 ^b	71.280± 15.905 ^a	88.620±0.522 ^a
4.5 mins	65.389± 8.480 ^a	4.486±0 .678 ^{ab}	14.167± 1.389 ^b	85.378± 18.026 ^a	88.380±0.563 ^a
6.0 mins	60.607± 8.580 ^a	4.328±0 .743 ^{ab}	13.702± 1.885 ^b	80.392± 18.390 ^a	88.777±0.698 ^a

Note: Values are means of 5 replicates. Means ($n=5$)±standard deviation.

a-b : Values bearing different superscript within the same column are significantly different at 5% level ($P < 0.05$).

There were significant differences ($P < 0.05$) in a^* values of harvested mushrooms among all different durations of ultrasonic treatment (Table 5). For color a^* values, it was ranged from -60 (greenness) to +60 (redness). Ultrasonic treatment of 1.5 minutes had significantly ($P < 0.05$) the highest a^* value (redness) of 5.200 and followed by 4.5 minutes (4.486), 6.0 minutes (4.328), 3.0 minutes (3.978) and control (3.621).

In terms of b^* values, there were significant differences ($P < 0.05$) among all different durations of ultrasonic treatment (Table 5). For b^* values, it was ranged from -60 (blueness) to +60 (yellowness). The results showed that ultrasonic treatment of 1.5 minutes had significantly ($P < 0.05$) the highest b^* value of 17.372 and followed by control (15.417), 4.5 minutes (14.167), 3.0 minutes (13.721) and 6.0 minutes (13.702).

From the results, it showed that ultrasonic treatment of 1.5 minutes had the highest b^* value (more yellowness) as compared to other durations of ultrasonic treatment. In this study, ultrasonic treatment had significant effect on the a^* and b^* values of the harvested mushrooms.

There was no significant different ($P>0.05$) observed in among all different durations of ultrasonic treatment (Table 5) for firmness of the mushrooms. This showed that ultrasonic treatment had no significant effect on the firmness of the harvested mushrooms.

The firmness values obtained from control and all the different durations of ultrasonic treatment were in the range of 69.469 gf to 85.378 gf. The almost same values of firmness obtained in this study might due to same species of grey oyster mushroom.

In terms of moisture content, there was no significant different ($P>0.05$) observed among all different durations of ultrasonic treatment and control (Table 5). This showed that ultrasonic treatment had no significant effect on the moisture content of the harvested mushrooms. In this study, the percentage of moisture content obtained from control and all different durations of ultrasonic treatment were in the range of 88.967% to 88.380%.

4. Summary

In this study, the effects of different durations of ultrasonic treatment on the growth performance, yield enhancement and quality of *Pleurotus sajor-caju* were investigated. Ultrasonic treated bags had faster time in mycelium growth rate than control. It could be concluded that the mushrooms treated with ultrasonic sound treatment took shorter time to complete the growth stages (spawn-running process, pinhead emergence and fruiting bodies formation). Besides, it was also found that ultrasonic treated bags showed improvement in yield of mushrooms and better quality in terms of larger pileus diameter than control. There was no significant effect ($P>0.05$) observed in L^* values for all durations of ultrasonic treatment. Ultrasonic treatment with 1.5 minutes was obtained to be the highest a^* (redness) and b^* (yellowness) values. The quality of texture and moisture did not showed any difference in all grey oyster mushrooms when subjected to different durations of ultrasonic treatment. In conclusion, ultrasonic treatment with 1.5 minutes was the best treatment duration on enhancement of growth performance and yield of mushrooms as it would produce the greatest production of mushrooms at the shortest time taken.

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