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GROWTH REVIVAL OF GREY OYSTER (*PLEUROTUS PULMONARIUS*) POWDER CULTURE MUSHROOM FROM THE EFFECT OF SPRAY DRYING TEMPERATURE

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ABSTRACT: In mushroom cultivation, spawn is among the most important factors that ensure a successful production. Considering several drawbacks from both solid and liquid spawn in shelf life and preservation matters, it is suggested that the dry powder form of spawn is a good potential to explore. A study of powder culture formation using a spray dryer involved various inlet temperatures of 80 °C, 90 °C, 100 °C, 110 °C, 120 °C and 130 °C. The yield % of the dried powder culture was measured and it was found that 130 °C yielded the highest percentage of 50.33%. The lowest temperature yielded the lowest percentage. On the other hand, the lowest temperature of 80 °C revived the highest mycelium dry weight at 1.68 g which was obtained on the 10th day of the incubation periods. The revival ability was decreased with the increase of temperature. The study proved that the powder culture of *P. pulmonarius* was able to perform and revive whereby it holds a potential to be preserved over a longer period, which is beneficial for the mushroom cultivator.

ABSTRAK: Dalam penanaman cendawan, benih yang baik adalah antara faktor penting yang memastikan penghasilan tinggi. Terdapat beberapa kelemahan daripada benih pepejal dan cecair dalam memastikan jangka hayat berpanjangan serta memastikan benih dalam keadaan berkualiti sepanjang masa. Oleh itu, penghasilan benih cendawan dalam bentuk serbuk kering sangat berpotensi untuk diterokai. Kajian mengenai penghasilan kultur serbuk menggunakan semburan kering telah melibatkan pelbagai suhu salur masuk seperti 80 °C, 90 °C, 100 °C, 110 °C, 120 °C dan 130 °C. Peratus penghasilan kultur serbuk kering telah diukur dan didapati pada suhu 130 °C menghasilkan peratusan tertinggi iaitu sebanyak 50.33%, manakala suhu terendah menghasilkan peratusan terendah. Sebaliknya, suhu terendah pada 80 °C menumbuhkan semula berat kering miselium dengan bacaan tertinggi iaitu sebanyak 1.68 g diperoleh pada hari ke-10 tempoh inkubasi. Keupayaan menumbuh berkurangan dengan peningkatan suhu. Kajian membuktikan bahawa kultur serbuk *P. pulmonarius* mampu berfungsi dan tumbuh semula di mana ianya berpotensi disimpan dalam tempoh lama, ini berfaedah kepada penanaman cendawan.

KEYWORDS: *spray drying; mushroom powder culture; grey oyster mushroom; growth revival*

1. INTRODUCTION

Mushrooms are an important food and medicinal commodity that has been cultivated on a large scale worldwide. Generally, in any mushroom species cultivation, spawn is among the

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most important factors that ensure a successful production [1]. Commercially, spawn is prepared in grain substrate such as corn, wheat, millet and others for easy injection in the mushroom substrate bag and transportation. This type of spawn is popularly known as solid spawn. Once matured or ready for use, the spawn life span is only about a month at room temperature. When kept at 4 ºC, the life span may be longer, up to 3 months, with the risk of reduction in quality. On the other hand, the production of solid and liquid spawn may also carry a high risk of contamination, require a large production area, and involve a longer incubation period. As a result, the process requires a significant investment in terms of time and money, leading to challenging cultivation and management [2].

Another type of spawn is liquid form in which is prepared for immediate use with the aim of faster growth. The advantage of this type of spawn is that it can be dispersed equally [3]. Nevertheless, it is difficult to transport and store for long periods and is prone to contamination [4]. Considering several drawbacks from both solid and liquid spawn, it is suggested that other forms of spawn, such as in dry powder form, should be explored.

Preparation of dry form live cultures for eventual revival is quite challenging. The most reliable technique so far for live samples is a spray drying technique. Several studies show that this technique is possible and very useful in extending shelf life and protecting biological properties [5]. Besides, dry form condition contributes to the easy transportation process and has a long survival period. This technique is further improved with incorporation of the microencapsulation process using suitable biopolymer to cover the cultures and therefore can protect them from heat damage. Many studies in this area used maltodextrin polymer and obtained satisfactory results [6]. The most critical factor in using this technique is the temperature since high temperature is used to convert liquid form of cultures into powder.

The dry powder form of a biological control agent of *Trichoderma spp*. conidia has been reported in Cameroon [7]. The lowest temperature identified so far was 80 ºC with *Bifidobacterium lactis* culture [8] and the highest was 200 ºC in *Lactobacillus spp*. studies [9]. Studied on *Trichoderma asperellum* shows that at temperature of 170 ºC, a high survival rate of 62.8% was obtained [10]. To date, there is no such study ever conducted on macro fungi and this would be challenging since the cells are more complex than bacteria and micro fungi. Temperature within 80–170 ºC is considered suitable to be applied in most microorganisms.

Pleurotus spp. is the largest mushroom production in Malaysia and second in the world [11]. The most popular is the *P. pulmonarius*, known for its chewy texture, aromatic flavor and delicious taste besides other benefits in nutrition and medicinal properties [12]. Therefore, this study aims to investigate the effect of drying temperature on powder formation and growth revival of the *P. pulmonarius* powder spawn.

2. METHODOLOGY

2.1 Mycelium Culture Preparation

P. pulmonarius strain used in this study was obtained from Universiti Malaysia Perlis mushroom farm research center. For the preparation of mycelium culture, fresh mushrooms were used. The mushroom was collected from the first cycle of healthy, well developed fruiting body. Firstly, the mushroom was cleaned from any impurity and sterilized by spraying with 70% ethanol. The mushroom tissue was dissected aseptically and inoculated into sterile potato dextrose agar (PDA) in a petri dish and incubated in the incubator for about 14 days for the mycelium to fully cover the petri dish.

2.2 Stress Medium and Liquid Culture Preparation

A 1 L stress medium was prepared with a composition of 5 g D-glucose, 130 mg monopotassium phosphate, 3.5 g soluble starch and 950 mL distilled water in a conical flask. A liquid culture was prepared by isolating a hyphae fragment from the mycelium culture using a saline solution. The saline solution was prepared by dissolving 0.85 g NaCl and 1% Tween® 80 in 100 mL distilled water and pipetting into the petri dish. The mycelium surface was then rubbed briskly using the pipette tip. A 5 mL of the hyphae fragment mixture was pipetted into the prepared sterile stress medium and incubated for 2 weeks [13].

2.3 Dry Powder Culture Preparation at Various Temperature

Prior to drying, 10% (w/v) of maltodextrin solution was added to the liquid culture as a protectant and stickiness prevention during the spray drying process. Lab scale spray dryer (BUCHI B-290) was used for the formation of powder culture at different temperatures (80 ºC, 90 ºC, 100 ºC, 110 ºC, 120 ºC, and 130 ºC). Other set up were the aspiration (100%), feed speed (3 mL/min) and air flow (30 m^3/min) [14]. The yield percentage was calculated from the formula shown in Eq. (1).

$$Weight(\%) = \left(\frac{Actual\ yield}{Theoretical\ yield}\right) \times 100 \quad (1)$$

where actual yield is the amount of a product that was obtained from the drying process as mentioned in section 2.3. The theoretical yield of a reaction is the quantity of product that is obtained if 100% of the reactant converts to product. The theoretical yield is determined by the limiting reactant.

2.4 Revival and Growth of Powder Culture

The powder culture (5 g) was inoculated into a 25 mL brown sugar-rice bran-malt-yeast extract (BRMY) medium in a sterile test tube covered with cotton and aluminum foil. The culture was incubated at room temperature (25±2 ºC) and in the dark for mycelium formation and growth. The dry weight of mycelium was measured every 2 days for 2 weeks. Mycelium was dried in an oven at 60 ºC for 24 hours until the constant weight was obtained [13]. This procedure was repeated for another four test tubes as replicates and compared with control culture which was prepared from mycelium culture in stress media. Statistical analysis of variance (ANOVA) was conducted to demonstrate the significant effect of the manipulated variable to the response. The R software version 4.3.1 was used for this analysis.

3. RESULTS AND DISCUSSION

3.1 Effect of Inlet Temperature on Yield Percentage of Fruiting Body

Dry powder cultures of *P. pulmonarius* were successfully obtained at all studied inlet temperatures. Data in Table 1 shows at fixed volume of liquid culture, the % yield of produced powder from tested temperatures was significantly varied which shows the influence of temperatures on the yield. Higher yield was obtained by increasing the inlet temperature. The highest yield percentage was obtained at 130 ºC with 50.33 % compared to 80 ºC to just 38.37 %. The best explanation for this situation is that an increase in drying temperature results in faster drying or evaporation rates, triggers an early structural formation, and prevents the particles from shrinking during drying [15]. Thus, a larger particle size resulted from lower temperature due to the greater swelling [16]. The large particle coincided with low yield

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percentage due to the loss of particles to the drying chamber wall. The non-efficient evaporation at the lower temperature caused the higher moisture content of the particles and increased its stickiness or adhesion force between the particles and drying chamber wall. With the relevance of residual moisture in the particles, the capillary liquid bridges were developed by creating the adhesion force between particles and drying wall surface [17]. This condition contributed to weight increment and high yield since the temperature coincided with the lower moisture content [15] and reduces the yield losses as the temperature increased.

Therefore, increase in temperature was attributed to low moisture content powder which translated to reduced adhesion on the wall of the drying chamber and increased the yield of the product [18]. Lower temperature caused a high moisture content product and increased the deposited powder on the wall of the drying chamber and reduced the yield. However, the success of the drying process will later be determined by the revival and growth ability of the powder culture. Similar results were obtained by Mansor et al. as they found that at low temperature, the particle size was higher due to the low evaporation process [19]. Low evaporation will cause high-density membranes, high water content, poor fluidity, and agglomeration of the particles. The time needed for the conversion shows no systematic pattern that is within 144 to 174 minutes.

Table 1: Powder culture percent yield at different inlet temperatures

Inlet temperatures (ºC)	Initial volume (mL)	Duration (Minutes)	Weight powder (g)	Yield (%)
Control	-	-	-	-
80	500	174	57	38.37±0.18
90	500	164	58	38.89±0.15
100	500	144	60	40.03±0.20
110	500	144	68	45.73±0.21
120	500	174	70	46.83±0.17
130	500	174	75	50.33±0.31

3.2 Effect of Powder Formation on Growth Revival Ability

The produced powder was successfully cultured in BRMY media, and the mycelium weight chart is shown in Fig 1. The results show that all inoculated powders from different temperatures survived in the BRMY media that was studied earlier for *P. pulmonarius* by Abdullah et al. [20]. This media is suggested since it can support the mushroom growth without interest from other fungi which helps to reduce contamination. As early as day 2, growth was initiated for all powders. However, significant growth ($P < 0.01$, Table 2) was observed from the powder produced at temperatures ranging from 80 to 100 ºC only. Powder produced at temperatures higher than 100 ºC appeared very slow in growth indicated by low mycelium weight. The highest weight obtained from this study was 1.68 g at 80 ºC on day 10 before approaching a steady decline. This was followed by 90 ºC with 1.62 g and both values exceeded the value of the control experiment, which is only 0.69 g.

Even though powder at all temperatures was able to initiate growth, it was found that only powder obtained at 80, 90, and 100 ºC was able to grow further after day 4 in the sense of the ability of cells to multiply and therefore increase the mycelium weight. Powder cultures

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produced from 110, 120, and 130 ºC were only able to initiate and support maximum mycelium growth up to 0.70 g, 0.34 g, and 0.25 g respectively, and able to retain the growth toward 14 days of incubation periods.

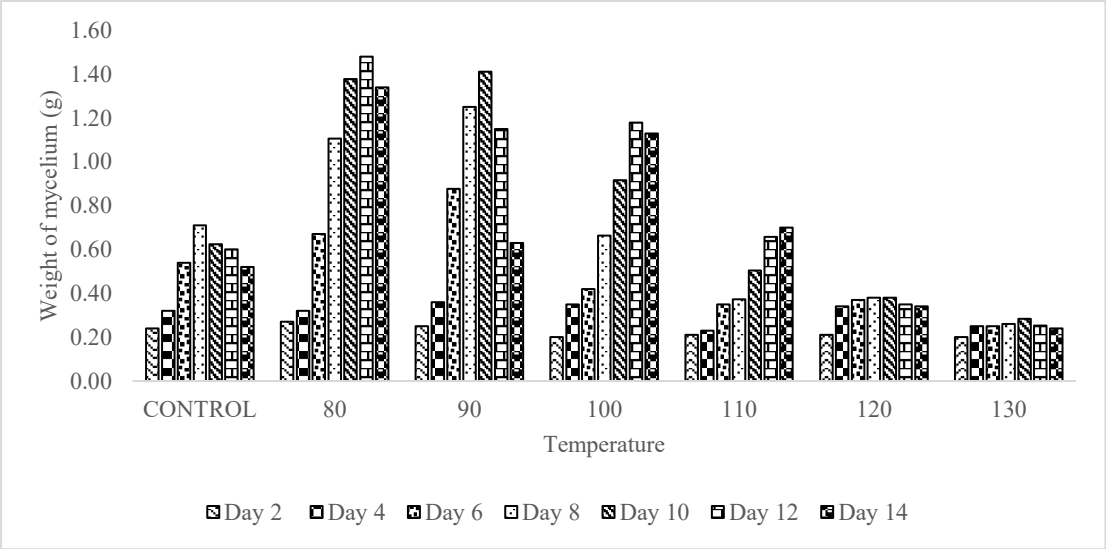

Fig 1: Differences of temperature in spray drying against mycelium weight from powder spawn within 14 days incubation.

Table 2: Analysis of Variance

Sources	Degree of freedom	Sum of squares	Mean square	F-value	Probability (>F)
X1	2	7.6279	3.814	59.811	< 2.2e-16
X2	2.000	9.2779	4.639	72.748	< 2.2e-16
Residual	142.000	9.0549	0.064		

3.3 Effect of Powder Formation on Mycelium Growth Curve

The whole picture on the effect of temperature on powder culture revival ability and mycelium growth is shown in Fig. 2. Powder culture at 90 ºC demonstrated the highest growth in early incubation days before declining after day 10, which may indicate that the heating level had affected the cell's metabolisms. This trend was also shown by *Trichoderma asperellum* conidia when a reduction in survival percentage was observed at high temperatures (80-90 ºC) and the survival was mostly supported by the application of a protectant [4]. Powder culture at 80 ºC was able to sustain growth up to 12 days before reducing.

It was presumed that cells had lost some of their proliferation ability due to the high heating effect. Interestingly, the powder culture at studied temperatures was still able to germinate, revive, and multiply over a certain incubation period. The retardation of growth was observed while maintaining its viability towards the end of observation period. The increased temperature caused damage to the mycelium cells, together with the limited availability of nutrients that led to a significant decrease in growth. These facts were also depicted in several studies on the *Lactobacillus salivarius* microbe that underwent various temperatures in the drying process [21]. Therefore, it can be stated that 80 ºC is the optimal temperature in this study as *P. pulmonarius* cells were able to revive and proliferate after the drying application. The temperature lower than 80 ºC had failed to form good powder from the initial screening.

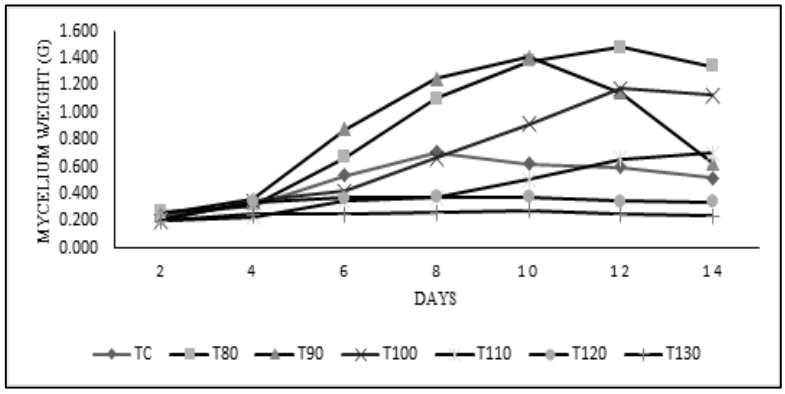

Fig. 2: Mycelium growth curve by different temperatures within 14 days incubation period.

4. CONCLUSION

The findings from this study achieved the main conclusion that the liquid culture of *P. pulmonarius* was successfully converted into a powder form by spray drying method. The most important finding was that the powder culture was able to revive and proliferate into mycelium culture and remain in the culture up to 14 days following the normal growth curve. This finding may bring a new development of spawn in mushroom industry.

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