POTENTIAL OF SPENT COFFEE GROUND IN *Pleurotus sajor-caju* CULTIVATION

Truong Thi Dieu Hien

Ho Chi Minh City University of Food Industry

Email: *god2103truong@yahoo.com*

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ABSTRACT

Pleurotus sajor-caju is one of the popular edible mushrooms that contribute to the daily meals of East Southern Asia countries. Demand for this oyster mushroom is creasing and triggers challenges to the substrate supply. In this study, spent coffee ground (SCG) collected from various coffee shops located in Ho Chi Minh City, was employed as the main substrates in *P. sajor-caju* cultivation, mixed up with rice bran/sawdust (8% w/w) in different ratios (0, 25, 50, 75, and 100 %). Results showed that the ratio of SCG and rice bran/sawdust (8% w/w) at 50:50, exhibits the highest quantity of mushrooms (41 gr/embryos), shortening harvest time and maximizing the economic profit. Therefore, the deployment of spent coffee grounds in *P. sajor-caju* cultivation should be expedited, in order to utilize the available sources and to create the eco-vision in edible mushroom production.

Keywords: Economical profit, *Pleurotus sajor-caju*, recycle, sawdust, spent coffee grounds.

1. INTRODUCTION

Food production is faced with many challenges such as the land for crops narrowing, the change in weather, and global warming or topsoil erosion. These are creating more obstacles to the strategy of food provision in many countries. Edible mushrooms are safe choices for the environment, which help recycle food wastes and convert high lignocellulose substrate into protein-enriched food [1]. *Pleurotus sajor-caju* (grey oyster mushroom) is a mushroom that fruit bodies shape like oysters and encloses high nutrients such as carbohydrates, proteins, minerals, and vitamins while enabling serving as the choice for diet foods. The demand for the mushroom market is leveled up, serving foods and traditional medicine, especially in East Southern Asia. Many types of ingredients have been employed for *P. sajor-caju* cultivation such as rice hull, banana leaf, sugarcane bagasse, or corn cob [1], and resolving the demand for substrate resources. However, with increasing consumption, substrate supply is the key step to large-scale cultivation.

Viet Nam is the second producer of coffee, and coffee consumption takes important in the Vietnamese lifestyle [2]. This practice is releasing a huge amount of spent coffee grounds (SCG) into the environment. Many innovative ways to recycle the spent coffee ground are testified, including natural insect repellent [3, 4], cosmetic production [5], homemade fertilizer [6], and especially SCG serving as the substrate for mushroom cultivation [7]. The compositions of SCG are cellulose (59.2-62.94%), hemicellulose (5-10 %), lignin (19.8-26.5 %) [8], and some extra-nutrients such as nitrogen, fat, or carbohydrates. These ingredients prove that SCG could be an ideal substrate for *P. sajor-caju* cultivation [9].

Reports showed that coffee-derived substrates have been employed for mushroom cultivation such as coffee husk or parchment [10]. In addition, SCG is certified as a valuable source for edible mushroom cultivation [11-14]. Indeed, Alsanad *et al*. indicated that SCG as a nutritional supplement- increases the bio-efficiency of *Pleurotus ostreatus* in lignocellulose degradation if mixed up with wheat straw [11]. Furthermore, Carrasco-Cabrera *et al*. indicated that caffeine from SCG is metabolized and converted into xanthine by *P. ostreatus* mycelium, and the amount of either caffeine or its metabolites is extremely low [12]. Per the estimation of this study, the caffeine content in the consumption of 250 kg of fresh oyster mushroom is equivalent to one cup of espresso coffee. This finding guarantees the safety of oyster mushrooms grown on SCG-mixed substrate regarding health impact. Moreover, Fayssal *et al*. suggest that SCG mixed up with olive pruning resides at a low proportion (17% versus the whole substrate), would enhance nutrients with lower fat, increase proteins, and monounsaturated fatty acids as well as lower heavy metal accumulation [13]. Thanh *et al.* also pointed out that 30% cardboard: 70% SCG in the substrate, significantly increases mycelium density and the number of primordial formations of *P. eryngii* versus either cardboard or SCG alone [14]. Therefore, the potential of SCG recycling in oyster mushroom cultivation is countless. However, there is no evidence supporting SCG in feasibility for *P. sajor-caju* cultivation and different substrates or mushroom strains might generate different body fruit amounts. In addition, rice bran is a disposable source, believed to support mushroom growth, and verified by various studies due to its nutrient content [15]. Therefore, this study aimed to examine the feasibility of SCG recycling in different ratio combinations of sawdust plus rice bran and address which combination ratio exhibits the best profit. This finding would bring out the solution that minimizes the capital investment for *P. sajor-caju* cultivation while providing a new approach to recycling the SCG.

2. MATERIALS AND METHODS

2.1. Materials

P. sajor-caju, rice seeds, Magnesium Sulfate ($MgSO_4$, Millipore Sigma, MA, USA), and sawdust were provided by the Center of Scientific Research and Practice, Thu Dau Mot University, Binh Duong. The spent coffee ground was collected from various coffee shops located in Ho Chi Minh city. Rice bran C15 was purchased from PROCONCO (Bien Hoa, Dong Nai, Vietnam).

2.2. Methods

2.2.1. Experiment design

Experiment 1: Determination of rice bran percentage in *P. sajor-caju* cultivation

Firstly, an experiment was set up to uncover the amount of rice bran supplemented with the mushroom substrate (rice seeds). Rice seeds were soaked in water for 6h, followed by washing to get rid of dust. Rice seeds afterward, were cooked by autoclaving at 121^0C, 1atm/ 15min, and mixed with $MgSO_4$ as a ratio of 0.1% (w/w). This mixture would be added to rice bran with various percentages (0, 2, 4, 6, 8% w/w). Next, rice seeds and bran mixtures were autoclaved in a 100 mL glass bottle and proceeded to grow *P. sajor-caju*. Mycelium length, time point overcover substrate, and mycelium density to were noted.

Experiment 2: Determination of SCG quantity in *P. sajor-caju* cultivation

To examine the feasibility of SCG in mushroom cultivation, SCG collected from various shops, was sterilized with lime 3%, followed by a pH neutralization process. Sawdust was also treated with lime 3% within 1 day, mixed adequately with rice bran. The level of lime was 3%, instead of 0.5% as reported by Contreras *et al.* [16], to reach the pH level that facilitates *P.*

sajor-caju growth (pH 5.0-6.0). The mixture was added with SCG in different ratios as described in Table 1 below and dispensed into embryo bags for autoclave at 121°C, 1 atm. *P. sajor-caju* was randomly sown after 24 hours into embryo bags and indoor-grown at 25-30°C, ventilating with 8 air changes/hour and in the light with 1000-1200 lux. Air humidity was maintained at 70-95% and monitored by an indoor hygrometer.

Table 1. Combinations of the mixture of rice bran/sawdust and SCG in different groups

Groups	SCG 0	SCG 25	SCG 50	SCG 75	SCG 100
Spent coffee ground (%)	0	25	50	75	100
Mixture (Rice bran/ sawdust 8%)	100	75	50	25	0

2.2.2. Harvest

The expansion of mycelium was measured daily and the time point at body fruiting was noted. Body fruits in different experiments were collected and weighed. Bio-efficiencies of each experiment were calculated via the formulation: weight of harvest/weight of dry substrate (w/w) as proposed by Chang *et al.* [17]. The profit of experimental groups was evaluated via the gross and expenses.

2.2.3. Statistical analysis

Experimental differences were examined using ANOVA and Student's t-tests, as appropriate by Graphpad Prism 7.0. All values are expressed as mean ± SD (n = 3); *P*-value < 0.05 were considered to indicate statistical significance. Each of the experiments was repeated.

3. RESULTS AND DISCUSSION

3.1. Supplement of rice bran

Rice bran is believed to enhance the body's fruiting process [18]. To know which proportion of rice bran might accelerate mycelium growth, various rice bran amounts were deployed during material preparation and mycelium expansion was recorded every 3 days (Day 0-Day 9). Results showed that mycelium is able to grow in all groups from bran 0% (Br0) to bran 8% (Br8) (Figure 1A). However, the capacity of mycelium expansion is in contrast to rice on bran percentage. On Day 9, mycelium lengths in Br0, Br2, and Br4 are not significant to each other, while Br6 is lower than Br0 (*P*-value = 0.0307 < 0.05) (Figure 1B).

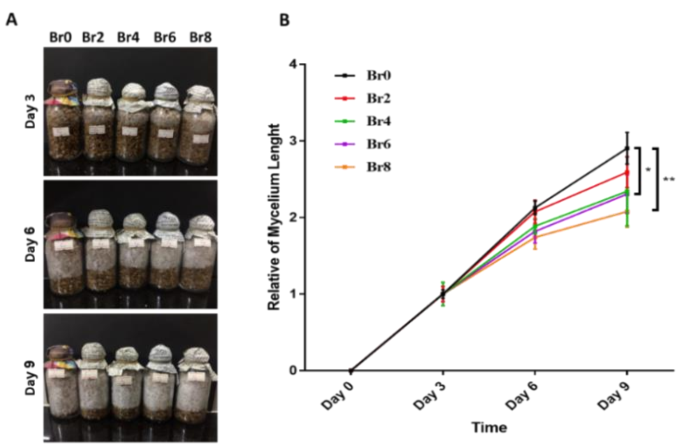

Figure 1. Optimization of rice bran in substrate preparation

The Br8 group exhibits the slowest mycelium expansion as compared to Br0 (P-value = 0.0018 < 0.01). This result suggests that the supplement of rice bran slows down mycelium expansion. Nevertheless, it is obvious that mycelia in the Br8 group are dense, and which in turns facilitates body fruiting processing [19].

3.2. Effects of SCG amendment to mycelium surmounting

To evaluate the potential of SCG recycling in *P. sajor-caju* cultivation, SCG was blended with rice bran/sawdust mixture at different ratios (w/w) as described in Table 1. Since, the mycelium in Br8 (rice bran 8%) presents the highest thickening, rice bran 8% was served as a supplement for all further experiments. Results showed that the addition of SCG does not alter the surmounting time at ratios SCG 25, SCG 50, and SCG 75 versus SCG 0 (Figure 2A-B). By contrast, SCG 100 (100% spent coffee ground) extends the surmounting time of mycelium significantly (Figure 2, P-value = 0.0026 < 0.01). This result suggests that the addition of SCG to rice bran/sawdust would not restrain the surmounting process of mycelium and SCG could be the alternative choice to save the sawdust consumption during *P. sajor-caju* cultivation.

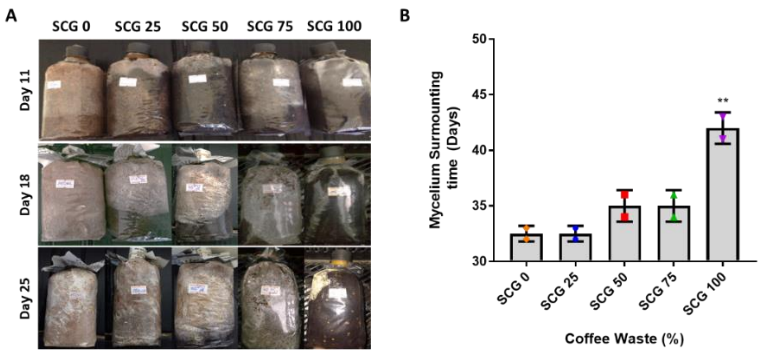

Figure 2. Mycelium surmounting time in different combinations

Fan *et al.* indicated that caffeine and tannins in coffee husks might harm mycelial growth and decrease biomass production [20]. The study has been conducted on *P. ostreatus* LPB 09 showed that caffeine dose-dependently reduces mushroom production and no growth observed at 2500 mg/L caffeine. In addition, tannin under 100 mg/L plays a stimulating role in mycelium expansion, however, the tannin limit should not be over 500 mg/L. Therefore, in large-scale *P. sajor-caju* cultivation, it is necessary to analyze the caffeine and tannin amount before mixing SCG into rice bran/sawdust, in order to guarantee maximal profit.

3.3. Harvest time and body fruits weight

Harvest time and body fruit weight are critical factors determining the efficiency of mushroom cultivation. Since *P. sajor-caju* cultivation nowadays is able to conduct in-house, controlled by the optimal conditions and therefore, shortening the harvest time would allow for more harvests per year. Furthermore, the quantity of mushroom products is also important to the cultivation and reflects the success of condition optimization. In this study, we look at how the combination between SCG and rice bran/sawdust in different ratios, affects harvest time and mushroom product. Results showed that supplements with the SCG to rice bran/sawdust at a ratio of 50:50 (SCG 50) exhibited the shortest harvest time, significantly compared to other combinations (P-value < 0.05, Figure 3B). Moreover, this combination also created the highest production versus 100% rice bran/sawdust (P-value = 0.0466 < 0.05, Figure 3A-B). Other combinations lower down mushroom harvested (below the baseline, Figure 3B). The increase in SCG percentage tends to diminish body fruiting, which is partly shown by the lower productivity in SCG 75 and SCG 100 (*P*-value = 0.0053 < 0.01 and < 0.001 respectively, Figure 3B). These data suggest that a combination of SCG and rice bran/sawdust at a ratio of 1:1 could be the ideal approach to recycling SCG in mushroom cultivation and yield production.

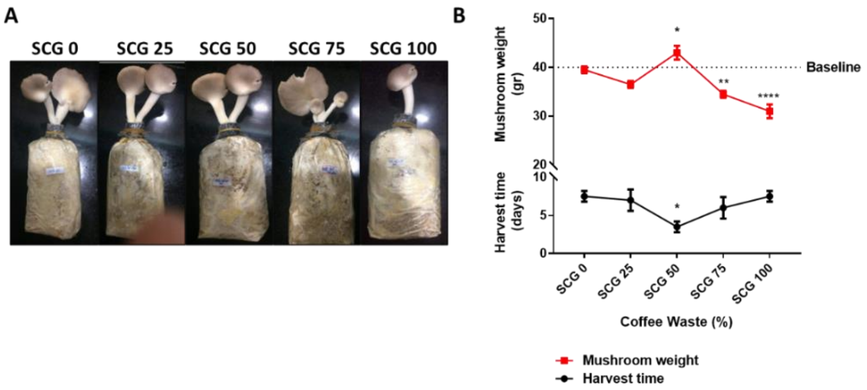

Figure 3. Harvest time and gained mushroom weight

Despite the SCG deployment to *P. sajor-caju* cultivation would provide a certain amount of nutrients, however, the effects of SCG on mushroom growth are not fully evaluated in this study. Since the SCG is highly acidic, that might have negative impacts on *P. sajor-caju* growth as reported by Chai *et al.* [7]. To fix this issue, there is necessary to examine the feasibility of the adjuvant agents serving as a neutralizer in an acidic substrate environment. Moreover, Chai *et al.* also indicated that the limit tolerance to SCG amendment is 30% (w/w), and over this tolerance would lead to the failure of body fruiting in different mushroom strains, including *P. pulmonarius,* and *P. floridanus*. In this study, *P. sajor-caju* was employed to transform SCG and rice bran/sawdust and the ratio at SCG50 exhibits the highest yield. Hence, it is probable that *P. sajor-caju* gains higher tolerance to the SCG as compared to the above mushroom strains and this strain could be a good model for mushroom cultivation regarding SCG recycling.

3.4. Bio-efficiency and profit

Bio-efficiency is a simple method to grade the effectiveness of one given mushroom strain in a way of using a single substrate or combination of substrates and transforming them into a mushroom body. Therefore, bio-efficiency is usually employed to generally compare among body fruiting of mushroom strain or indicate the capacity of substrate utilization [21]. Among experimental groups, SCG mixed with rice bran/sawdust (1:1) exhibited the highest profit as compared to SCG 0 baseline (serving as 100% rice bran/sawdust, *P*-value = 0.0059 < 0.01). However, all the rest of the combinations evidenced a significant deficit versus baseline (Figure 4). Furthermore, there was not much difference in bio-efficiency among combinations (Figure 4). These data suggest that SCG50 proved itself the most beneficial option in terms of recycling spent coffee grounds in mushroom cultivation. Recently, Martinez *et al*. pointed out that the fermentation of coffee by microbials could be an answer to the acidic environment induced by caffeine [22]. Indeed, coffee beans fermented with a yeast starter, tend to produce volatile compounds while bacterial starter generates organic acid compounds. This phenomenon allows for predicting and selecting the flavors of coffee beans. Therefore, the pre-treated SCG with a yeast starter could be the ideal option to deplete the acidic environment and naturally flavor the mushroom products. Moreover, the addition of the SCG-fermentation step prior to being mixed with rice bran/sawdust mixture would probably change the bio-efficiency and profit. Hence, screening of optimal microbial strains employed for SCG fermentation is a critical step to maximize bio-efficiency and profit on a large scale.

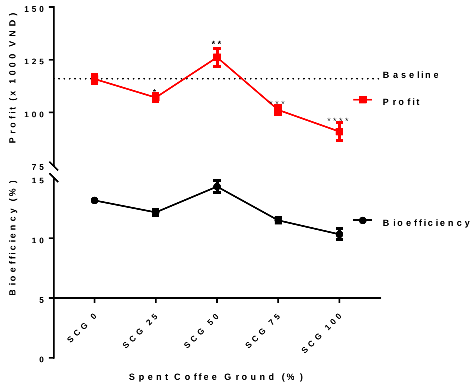

Figure 4. Profit and bio-efficiency of different combinations

4. CONCLUSION

P. sajor-caju is an edible mushroom that is favored in East Asia and portions significantly in daily meals. Optimization of the substrate would yield an economic profit. In this study, SCG served as a supplement to rice bran/sawdust, a conventional substrate of mushroom cultivation. Results showed that the addition of rice bran would benefit mycelium thickness and consequently supports the body fruiting process. The augment of SCG tends to delay mycelium surmounting time at 100% SCG. However, at the ratio SCG25 - SCG 75, there was not much difference versus SCG 0. In addition, results showed that the mushroom amount gained in SCG 50 group, was highest while reducing the harvesting time. SCG50 also exhibited the highest profit as compared to other combinations, despite indifference to bio-efficiency. Taken together, SCG50 or a combination between 50% SCG and 50% rice bran/sawdust is the best choice and re-emphasize the feasibility of SCG recycling in *P. sajor-caju* cultivation.

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TÓM TẮT

TIỀM NĂNG SỬ DỤNG BÃ CÀ PHÊ TRONG TRỒNG NẤM BÀO NGƯ XÁM NHẬT (*Pleurotus sajor-caju*)

Trương Thị Diệu Hiền
Trường Đại học Công nghiệp Thực phẩm TP.HCM
Email: *god2103truong@yahoo.com*

Nấm bào ngư xám Nhật (*Pleurotus sajor-caju*) là một trong những giống nấm được ưa chuộng trong những bữa ăn hằng ngày tại các nước Đông Nam Á. Khi nhu cầu sử dụng loại nấm này tăng lên đã đặt ra những thách thức lớn trong khâu cung cấp nguyên liệu nuôi trồng loại nấm này. Trong nghiên cứu này, tác giả sử dụng bã cà phê từ các nguồn sẵn có, thu thập được từ các quán cà phê trên địa bàn Thành phố Hồ Chí Minh và kết hợp với cám gạo/mùn cưa (8% w/w) - là chất nền truyền thống trong nuôi trồng nấm. Với các tỷ lệ 0, 25, 50, 75 và 100% bã cà phê/ mùn cưa được khảo sát, kết quả nghiên cứu cho thấy, khi phối trộn bã cà phê với hỗn hợp cám gạo/mùn cưa (8% w/w) với tỷ lệ 50:50 thu được kết quả đáng mong đợi ở lượng sản phẩm nấm (41 gram/ phôi nấm), đồng thời cũng rút ngắn thời gian thu hoạch, với chi phí đầu tư thấp hơn. Do vậy, việc sử dụng bã cà phê cần được nghiêm túc đầu tư và xúc tiến, nhằm tận dụng các nguồn cơ chất sẵn có, cũng như tạo nên "tầm nhìn xanh" trong nuôi trồng nấm bào ngư xám Nhật và các loại nấm khác.

Từ khóa: Bã cà phê, hiệu quả kinh tế, mùn cưa, *Pleurotus sajor*-caju, tái sử dụng.