

Optimum Formulation Substrate for Oyster Mushroom Cultivation Using Linear Programming Model

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Abstract The oyster mushroom (*P. ostreatus*), also known as the grey oyster mushroom, is currently one of the popular edible wood mushrooms consumed by people in Malaysia. Under Malaysia's National Agro-Food Policy (2011-2020), mushrooms have been identified as high-value commodities. Mushroom cultivation has become an immense potential agriculture activity in Malaysia, where oyster mushrooms are looked at as highly valued crops with low-cost technology that can bring high returns within a short time. Commonly, mushroom cultivation uses sawdust as oyster mushroom media. However, due to the increasing price of the commercial substrate of sawdust, growers are looking for a low-cost alternative substrate. Currently, researchers are looking at the potential of agriculture waste as an alternative medium for mushroom cultivation. For example, agriculture waste of empty oil palm fruit bunch and rice straw as a substrate for oyster mushrooms. Using Microsoft Excel Solver, the linear programming method was used to represent the substrate formulation. The results from this model produced an optimum 1 kg substrate formulation at a minimum cost of RM0.56 per kg. This proposed formulation satisfied the minimum and maximum nutrient requirements for oyster mushroom growth.

Keywords Linear Programming, Substrate

Formulation, Oyster Mushroom

1. Introduction

There are more than 1000 species of oyster mushrooms (*Pleurotus sp.*) worldwide. The oyster mushroom (*P. ostreatus*), also known as the grey oyster mushroom, is currently one of the popular edible wood mushrooms consumed by people in Malaysia. Under Malaysia's National Agro-Food Policy (2011-2020), mushrooms have been identified as high-value commodities. This is reflected by the local markets, where it is reported that the daily demand for fresh mushrooms is around 50,000kg while the supply is only 24,000kg [1]. The oyster mushroom commodity will be grown intensively with government support.

Like any other plants, oyster mushrooms also need nutrients to grow, such as carbohydrates (cellulose, hemicellulose and lignin), protein, fat, minerals, and vitamins. Commonly, in Malaysia, the commercial cultivation of oyster mushrooms utilises rubber tree sawdust as the medium. According to NST Business [2], there is a shortage of rubber tree sawdust due to the limited availability of rubber trees. For example, in Austria there

have also been spiking trends in the price of sawdust for the past twenty years as reported in Kranzl et al. [3] and it is still increasing. This price increasing trend is similar in Malaysia, one of the global exporters of rubber trees and other timbers. The price of sawdust increased due to many factors: the limited source and the logistic cost to deliver the materials.

Oyster mushroom growers, especially small-medium growers, are having a hard time looking for another sawdust alternative where it should be low in price and sustainable. A study of substitution materials that can replace sawdust as the main ingredient of the mushroom oyster plant media needs to be done. The selected substitute material should have characteristics like sawdust and sufficient nutrient content to support the growth of oyster mushrooms. In present studies, researchers actively look at agriculture waste as a medium to grow oyster mushrooms.

2. Substrate Ingredients for Oyster Mushroom Cultivation

Mushrooms can be grown on a wide range of substrates, and the selection of substrates is based on their availability and cost. A substrate is an essential part of mushroom production. According to Onyeka et al. [4], the period of mycelium running, pinhead development, the quantity of fruiting, the cropping time, the primordial diameter, and the biological efficiency of oyster mushrooms are all influenced by the substrate medium. Hence, the ingredient selection in substrate formulation for mushroom cultivation is vital to ensure the mixed planting media can provide different kinds of nutrients and minerals required for mushroom growth.

2.1. Main Substrate

Sawdust is a by-product of lumber and wood industries and has been commonly used as a medium substrate for oyster mushroom cultivation. In a study by Shah et al. [5], sawdust reached maximum yield, biological efficiency, and quantity of fruiting bodies, making it the ideal substrate for growing oyster mushrooms. Oyster mushroom is being grown commercially in Malaysia using sawdust from rubber trees as the foundation media. However, the shortage of rubber trees has created a significant challenge for mushroom growers [6]. A new alternative substrate should be investigated to alleviate the lack of sawdust from rubber trees. Therefore, much research has been conducted to find the effectiveness of other agricultural by-products on mushroom growing. As described by Muswati et al. [7], mixing substrate can maximise mushroom yield by optimising compositional features such as water holding capacity and enhanced medium structure to achieve an optimal carbon-to-nitrogen (C/N) ratio that increases substrate efficiency. Hence,

farmers can achieve their goal yields by combining substrates that are in scarce supply.

Other lignocellulosic wastes from oil palm wastes can be utilised as an alternate substrate to cultivate the oyster mushroom. Some studies have done work on the potential of growing oyster mushrooms on palm oil mesocarp fibre, and the result proved it as an excellent substrate for *Pleurotus* species cultivation [6,8,10]. Empty fruit bunches (EFB), palm press fibre (PPF), an oil palm frond (OPF), sugarcane bagasse, and corn cob have a significant amount of hemicellulose, cellulose, and lignin. They can be used as a low-cost alternative to growing oyster mushrooms [8]. EFB is made up of 26.49% lignin, 32.57% cellulose, and 27.7% hemicellulose [9]. Besides containing cellulose, hemicellulose, and lignin, EFB also lacks sap, a wood component that may inhibit mushroom development [10]. The combination of rubber tree sawdust with both shredded PPF and EFB showed tremendous potential as substrates for developing oyster mushrooms [6]. However, EFB and PPF are ineffective as individual substrates for oyster mushroom cultivation. In a study conducted by Tabi et al. [11], Substrate A (100% EFB) has an insufficient amount of nitrogen (0.2%) for mycelium development, while Substrate C (100% PPF) has the lowest mushroom yield due to the low carbon content (47.2%). Therefore, fewer nutrients are available for the fruiting bodies to grow.

Rice straw is a common *Pleurotus* substrate in Asia due to its composition of slow-digesting carbohydrates [12]. The nutrients in rice straws are similar to those in sawdust. Rice straw is made up of 27% hemicelluloses, 39% cellulose, 13% lignin, and 9% dust [13]. Somashekhar [14] have presented research on several agricultural wastes, and according to the findings, ragi straw yielded 1.41 kg of mushroom, followed by 1.23 kg of rice straw. Finger-millet husk and rice straw performed better in terms of the number of days required for an entire spawn run in an experiment conducted by [12]. According to Utami [13] the concentration of rice straw addition that can be used to replace sawdust in oyster mushroom planted media was 15%:60%.

2.2. Supplementation

Lignocellulosic wastes are often deficient in protein, making them unsuitable for mushroom cultivation that requires the addition of nitrogen, phosphate, and potassium [15]. Adding supplements to the mushroom substrate is essential to improve mushroom development and yield, especially for substrates with low protein content [16]. Supplementation positively affects mycelia growth and mushroom production [17].

Nitrogen supplementation is critical for mushroom growth and yield because the C/N ratio affects spawn running and fruiting body growth [15]. Most nitrogen level in substrates is between 0.5-0.8 %, as in Chanakya et al.

[18]; therefore, adding organic nitrogen helps to increase mushroom yields. Organic materials such as rice bran, wheat bran, and molasses are part of additives. Additives are protein and nitrogen-rich ingredients added to substrates to help mushrooms grow and produce more [19]. The effects of seven different additives, wheat bran, rice bran, soybean flour, de-oiled soybean meal, mustard cake, cotton seed cake, and cotton seed meal on mushroom yield, were investigated by [20]. The result concluded that adding 1% de-oiled soybean meal and 2.5% cotton seed cake to wheat straw is the best way to increase *Pleurotus ostreatus* var. *Florida* yields. Salama et al. [21] evaluated the effects of wheat bran, rice bran, urea and zinc sulphate on oyster mushroom cultivation. The result shows that oyster mushroom yield and quality were improved by adding either wheat or rice bran to rice straw substrates.

Rice bran has been used widely as a nitrogen source for oyster mushroom substrate [22,10,23,9]. However, the growth of mycelium at the first stage of mushroom growth may be hampered by a high amount of rice bran [10]. Guerrero et al. [24] assessed the effect of different levels of rice bran supplementation on oyster mushroom growth and production. The results show that supplementing fermented sawdust with 15% of rice bran together with 1% brown sugar and 1% lime is the best for growing oyster mushrooms like *Pleurotus Florida* and *Pleurotus Sajor-caju*. Khan et al. [26] suggested utilising wheat bran and rice bran at a rate of 10%, while for cottonseed meal, soybean cake and groundnut cake at a rate of 3-6% on a dry weight basis of the substrate.

Lime (CaCO_3) is a crucial component in mushroom cultivation and has been utilised frequently in various studies to improve the pH of the substrate. For a successful oyster mushroom harvest, pH is critical. Most mushrooms thrive at pH levels close to neutral or light basic [26]. Khan et al. [26] concluded that oyster mushrooms produce a sufficient yield on cotton waste containing 2% of lime.

This study aims to evaluate the suitability of several types of lignocellulosic wastes that are ample in Malaysia for replacing rubber tree sawdust in oyster mushroom cultivation. Because of the differences in the ability of such substrates to improve nutritional and environmental requirements, as well as variations in cellulose, hemicellulose, and lignin content, productivity and biological efficiency were increased in some mixtures when compared to wheat straw alone [23]. Following that, this study aimed to find the optimal formulation substrate mixture to assess oyster mushroom growth, yield, and economic feasibility of small-scale production by utilizing only locally accessible agro-industrial by-products.

2.3. Nutritional Requirement for Oyster Mushroom Growth

Pleurotus spp. get their nutrients from a host substrate or agricultural wastes high in lignin, cellulose and

hemicellulose [20]. Carbon, nitrogen as well as other minerals in the substrate such as S, Mg, Ca, K, P, and some lower-level minerals such as Mn, Fe, Zn, Mo, and Cu with an ash percentage of 2.5 to 15.7, is essential as a source of nourishment for *Pleurotus spp* [27]. Mushrooms need carbon and nitrogen for structural and energy requirements [28]. Cellulose and hemicellulose are carbon sources, while protein and amino acids are nitrogen sources [29]. Therefore, any agricultural waste containing cellulose, hemicellulose, or lignin can be a suitable substrate for growing oyster mushrooms [30].

Total nitrogen (N), total carbon (C), and the carbon/nitrogen ratio (C/N) are essential elements that influence mycelium colonisation and fruiting body development in oyster mushrooms [12]. Depending on the mushroom species, the required amount of each nutritional component varies.

2.3.1. Nitrogen Content

Nitrogen is an essential nutrient for mycelium growth [11]. Nitrogen is required for cellular development and various metabolic processes, including the synthesis of proteins and enzymes [20]. Supplementing nitrogen with organic sources helps to improve mushroom biological efficiency [31]. However, an excessive amount of nitrogen in the substrate can encourage the growth of mould Mishra et al. [25] and thus prevent mushroom growth [7]. Supplement addition can raise the temperature of the substrate by 2-3 degrees Celsius or even more [25]. Therefore, it is crucial to find the optimal nitrogen level in the media culture for mushrooms depending on their species.

2.3.2. Carbon Content

Carbon sources are essential components of the nutritional media to promote the best growth of fungi [32]. The carbon compounds that fungi use for sustenance offer the energy the fungus needs to carry out its life functions [33]. In straw nitrogen, carbon can easily be obtained from cellulose, hemicellulose, and lignin; however, it is mainly bound and cannot be accessed until it is released by enzymes [20]. Palm pressed fibre, sugarcane bagasse, and corncob have high levels of lignin, hemicellulose, and cellulose, which contribute to the carbohydrate or carbon source needed for the growth of oyster mushrooms [8].

2.3.3. C/N Ratio

C/N ratio is crucial in determining the best substrate composition for oyster mushrooms. C/N ratio is essential as it influences the fermentation process Abella et al. [34], mycelium growth, fruiting body formation, and development [30]. Though oyster mushroom demands more carbon and less nitrogen, most of the primary substrate materials, such as cereal straw, cotton waste, and sawdust, require the addition of nitrogen sources such as wheat and rice bran to achieve the appropriate C/N ratio for

oyster mushroom [34]. Each mushroom species needs an optimal C/N ratio in the culture substrate for growers to attain the best output in the shortest time possible [35]. According to Miles and Chang [33], a C/N ratio of 32- 150 is best to produce *Pleurotus* spp. To attain an optimal C/N ratio, the kinds, and formulations of substrates for mushroom culture should have a balance of carbon and nitrogen [36]. The ideal nutrient ratio of culture media components stated in a study by Lee and Cho [37] is a medium with total nitrogen and carbon levels of 0.65-1.11 % and 47.0-49.1 %, respectively.

3. Method to Formulate Substrate

Multiple studies have utilised mixture design to find the mushroom substrate's optimal proportion. Mixture design is a response surface experiment used to see whether there is a combination of ingredients that create an optimal response to either maximise or minimise a property [38]. Simplex-centroid designs are one of the common methods in mixture design for identifying a unique collection of components at various centroids that maximise or minimise the response variable based on the goal function. Kasina et al. [39] applied the simplex-centroid designs method to find the optimal local substrate mixture that maximises oyster mushroom yield. Simplex-centroid designs were proven highly efficient and successfully identified and established the ideal substrate combination for oyster mushroom production.

The simplex-lattice design is the most often utilised approach for substrate screening among all mixture-design methods as it provides a thorough analysis of the relationships in different aspects and the goal values, as well as the quantitative relationships between the various substrates and evaluation indicators, which are produced by using regression analysis [40]. D-optimum approach of the simplex-lattice design was used to optimise *Grifola frondosa* cultivation on crop straw (corn cob, corn straw, rice straw, and soybean straw) as a substrate, and the optimised model determined the use of crop straw as a substitute for sawdust in the substrate composition [41]. Wu et al. [42] applied a simplex-lattice design to their study to find the optimal proportion of agro-residues consisting of wheat straw, corn straw and soybean straw as the primary substrate to replace sawdust and cottonseed hulls in the production of *Pleurotus pulmonarius*.

A mathematical model is a popular approach to finding the ideal proportional ingredients mixture. The standard optimisation methods used widely in finding an optimal composition for fertiliser are the goal programming model [43-45] and the linear programming model [45,46]. However, the use of this method in formulating mixture substrate has been less applied in mushroom cultivation. Only one study can be found utilising goal programming in finding the optimal medium composition for oyster

mushroom growth which was reported in [48]. Therefore, in this study, the optimum formulation substrate for oyster mushrooms by using a linear programming model is discovered.

3.1. Methodology

The model for this study is taken from a study done by Aldeseit [46] where the author utilised the LP technique in formulating the optimal composition of the three synthetic fertilisers. The developed model's primary goal is to reduce the cost of formulated oyster mushroom substrate. The mathematical model in this study is built using the sets and parameters listed below. Let,

n = Total number of ingredients used in the model

u = Index for the type of ingredients in the model, where $u = 1, 2, 3, \dots, n$

v = Index for the type of nutrient in the model, where $v = 1, 2$

y_u = The cost (per kg) of ingredients u

x_u = The amount of ingredient u in the formulated substrate

a_{uv} = The amount of nutrient v contain in ingredient u

c_v = Maximum requirements of nutrient v in the formulated substrate

b_v = Minimum requirements of nutrient v in the formulated substrate

d_u = The maximum inclusion rate of ingredient u in the formulated substrate

e_u = Minimum requirements of nutrient u in the formulated substrate

The model is provided in the following format:

Minimize

$$Z = y_u x_u \tag{1}$$

Following are the limitations that apply to the objective function:

$$\sum_{u=1}^n x_u = 1 \text{ kg} \tag{2}$$

Constraint (2) represents the total amount of formulated substrate for oyster mushroom must satisfy 1 kg.

$$c_v \geq \sum_{u=1}^n a_{uv} x_u \geq b_v \tag{3}$$

Constraint (3) represents the nutrient requirement limit of nutrient v contain in the formulated substrate for oyster mushroom.

$$d_u \sum_{u=1}^n x_u \geq x_u \geq e_u \sum_{u=1}^n x_u \tag{4}$$

Constraint (4) represents the maximum and minimum inclusion rate of ingredients in the substrate.

$$x_u \geq 0 \tag{5}$$

Constraint (5) is a non-negativity constraint that ensures that the optimal value of ingredients in the substrate is not negative.

The model's expansion is written as follows:

x_1 = The amount of rice straw in the formulated substrate

x_2 = The amount of EFB in the formulated substrate

x_3 = The amount of PPF in the formulated substrate

x_4 = The amount of sawdust in the formulated substrate

x_5 = The amount of rice bran in the formulated substrate

x_6 = The amount of limestone in the formulated substrate

By referring to Table 1, the extension of the objective function can be stated as follows,

Minimize

$$Z = 0.5x_1 + 0.5x_2 + 0.8x_3 + 1.5x_4 + 0.8x_5 + 1.2x_6$$

Subject to:

1. Constraint (2) can be written as follow

$$x_1 + x_2 + x_3 + x_4 + x_5 + x_6 = 1$$

2. The expansion for Constraint (3) can be expressed as follows, using Tables 1 and Table 3 as references. Nitrogen minimum limits in the total substrate,

$$0.0065x_1 + 0.002x_2 + 0.014x_3 + 0.0032x_4 + 0.0073x_5 + 0.000x_6 \geq 0.0065$$

3. Nitrogen maximum limits in the total substrate,
- $$0.0065x_1 + 0.002x_2 + 0.014x_3 + 0.0032x_4 + 0.0073x_5 + 0.000x_6 \leq 0.0111$$

4. Carbon minimum limits in the total substrate,
- $$0.5176x_1 + 0.488x_2 + 0.472x_3 + 0.4604x_4 + 0.1487x_5 + 0.000x_6 \leq 0.47$$

5. Carbon maximum limits in the total substrate,
- $$0.5176x_1 + 0.488x_2 + 0.472x_3 + 0.4604x_4 + 0.1487x_5 + 0.000x_6 \leq 0.491$$

6. The expansion for Constraint (4) can be expressed as follows, using Table 2 as reference. Rice straw minimum inclusion rate

$$x_1 \geq 0.15 \sum_{n=1}^6 x_u$$

7. Rice straw maximum inclusion rate

$$x_1 \leq 0.6 \sum_{n=1}^6 x_u$$

8. EFB maximum inclusion rate

$$x_2 \leq 0.5 \sum_{n=1}^6 x_u$$

9. PPF maximum inclusion rate

$$x_3 \leq 0.5 \sum_{n=1}^6 x_u$$

10. Rice bran maximum inclusion rate

$$x_5 \leq 0.15 \sum_{n=1}^6 x_u$$

11. Limestone inclusion rate

$$x_6 = 0.02 \sum_{n=1}^6 x_u$$

12. The expansion for Constraint (5) can be expressed as follows,

$$x_u \geq 0, \text{ where } u = 1, 2, 3, \dots, 6$$

This model is then solved using Excel Solver Application.

Table 1. Type, Nutrient Contents and Approximate Costs (per kg)

| Ingredients | Type | N (%) | C (%) | Cost (RM/kg) |
|-------------|------------|-------|-------|--------------|
| Rice Straw | Main | 0.65 | 51.76 | 0.50 |
| EFB | Main | 0.2 | 48.8 | 0.50 |
| PDF | Main | 1.4 | 47.2 | 0.80 |
| Sawdust | Main | 0.32 | 46.04 | 1.50 |
| Rice bran | Supplement | 0.73 | 14.87 | 0.80 |
| Limestone | Supplement | - | - | 1.20 |

Source: [49,6,48,50] and market survey

Note: N-Nitrogen, C-Carbon, EFB-Empty Fruit Bunches, PPF-Palm press fibre

Table 2. Minimum and Maximum Rate of Each Ingredient in the Substrate

| Ingredients | Minimum Inclusion (%) | Maximum Inclusion (%) |
|-------------|-----------------------|-----------------------|
| Rice Straw | 15 | 60 |
| EFB | 0 | 50 |
| PDF | 0 | 50 |
| Sawdust | 0 | 100 |
| Rice bran | 0 | 15 |
| Limestone | 2 | 2 |

Note: EFB-Empty Fruit Bunches, PPF-Palm press fibre

Source:[13,6,10,26]

Table 3. Minimum and Maximum Rate of Each Ingredient in the Substrate

| Nutrient | Minimum Requirement (%) | Maximum Requirement (%) |
|----------|-------------------------|-------------------------|
| Nitrogen | 0.65 | 1.11 |
| Carbon | 47.0 | 49.1 |

Source: [37]

4. Proposed Formulation Substrate for Oyster Mushrooms

Six types of ingredients in the substrate were considered in formulating substrate for oyster mushrooms. The six ingredients were rice straw, EFB, PPF, sawdust, rice bran and limestone, denoted as x_1, x_2, x_3, x_4, x_5 and x_6 , respectively. They were chosen due to their accessibility and availability in Malaysia. One of the benefits of using this substrate is that it is readily available and cost-effective. The results displayed in this section were produced by using Microsoft Excel Solver.

Table 4 displays the optimal amount of ingredients and its total cost per kg. The total cost of the formulated substrate was obtained by 'sumproduct' the optimal amount of each ingredient in the substrate with the cost of ingredient per kilogram.

Based on the result, to achieve an optimal substrate for oyster mushrooms, the mixture must be composed of 0.6 kg of rice straw, 0.222 kg of EFB, 0.15 kg of PPF, 0.008 kg of rice bran and 0.02 kg of limestone. Rice straw has the most significant ratio in the substrate, probably due to its high nitrogen level and lower cost than other main substrates. Sawdust was not chosen probably due to its high cost, and the other lower-cost ingredients were able to provide sufficient nutrients for the substrate.

Table 4. Ingredient Amount in Formulated Substrate for Oyster Mushrooms

| Ingredients | Cost (RM/kg) | Amount (kg) |
|-------------|--------------|-------------|
| Rice straw | 0.5 | 0.600 |
| EFB | 0.5 | 0.222 |
| PDF | 0.8 | 0.150 |
| Sawdust | 1.5 | 0 |
| Rice bran | 0.8 | 0.008 |
| Limestone | 1.2 | 0.020 |
| Total | 0.5614 | 1 |

Table 5 shows the formulated substrate's nutrient content and the required nutrient limit that should be contained in the oyster mushroom substrate.

Table 5. Nutrient Content of the Formulated Substrate and the Required Nutrient Limit in Substrate

| Nutrients | Amount (kg) | Minimum Required | Maximum Required |
|-----------|-------------|------------------|------------------|
| Nitrogen | 0.0065 | 0.0065 | 0.0111 |
| Carbon | 0.491 | 0.47 | 0.491 |
| C/N ratio | 75.538 | | |

The formulated substrate has satisfied the minimum and maximum nutrient requirement for an oyster mushroom that was suggested in [37]. When formulating mushroom substrate, it is crucial to consider the ratio of

carbon-to-nitrogen (C/N) in the substrate. Miles and Chang [33] state that the optimal C/N ratio for oyster mushroom production is 32-150. The C/N ratio for the formulated substrate is 75.538, and it is within the considered range. Therefore, this formulated substrate is safe to be utilised for oyster mushroom cultivation as all the conditions needed are fulfilled.

5. Conclusions

The formulated substrate that consists of 0.6 kg of rice straw, 0.222 kg of EFB, 0.15 kg of PPF, 0.008 kg of rice bran and 0.02 kg of limestone was shown to have a nutrient content that is needed by mushroom optimally in order to grow. Compared to current practice for mushroom substrate, sawdust used is 50% and 50% supplemented ingredients. The optimal mixtures do not contain sawdust which is intelligent profit to the growers. The proposed optimal mixtures, too, have shown an affordable cost for mushroom growers, which estimated cost is RM0.56 per kg.

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