Faculty of Resource Science and Technology

Cultivation of *Pleurotus sajor-caju* (Fr.) Singer on *Metroxylon sagu* Rottb. Bark and Frond

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Cultivation of *Pleurotus sajor-caju* (Fr.) Singer on *Metroxylon sagu* Rottb. Bark and Frond

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Sawdust (SD) is a commercially used fruiting substrate component for the cultivation of *Pleurotus sajor-caju* (grey oyster mushroom) in Malaysia. However, due to the declining sawdust supply, this study was done to examine the possibility of using sago palm bark (SB) and sago palm frond (SF) as alternative base carbon sources in the fruiting substrate used as compared to sawdust. Nine substrate ratio mixtures were studied, including sawdust alone as a control and the combination of 25:75, 50:50, and 75:25 ratios between SD and SB or SF for mushroom cultivation of grey oyster mushroom. Mushroom cultivation techniques were conducted in the mushroom house. The lignocellulosic (lignin, cellulose, and hemicellulose) content for the substrate ratio mixtures was analysed alongside the pH value, extractives, ash, and moisture contents using standard methods. The singular substrate, 100SB, showed the highest hemicellulose content (60.7%), while the combination of 50SD:50SF substrate contained the highest lignin (40.7%). For cellulose, 75SD:25SF had a higher percentage at 46.2% compared to others. Singular substrate 100SB had the highest moisture content (11.7%). For extractives content, singular substrate 100SD had the lowest (2.9%). Ash content (1.4-5.2%) and pH value (4.7-6.1%) were significantly different from each other. For morphological characteristics, cap diameter (6.7-8.4 cm), stipe length (6.1-7.5 cm), and the number of effective fruiting bodies/bunch in different substrate ratio mixtures (1.7-2.2) were recorded in this study. The results showed a significant difference in the total colonization period, total sporocarp yield, and biological efficiency (BE) for singular substrate and substrate in combination. Singular substrate 100SB and 100SF gave a comparable performance as 100SD. For substrate in combination, 50SD:50SF is the most suitable ratio as a supplement to sawdust for the cultivation of *P. sajor-caju*. Singular substrate 100SD achieved a faster total colonization period (24.44 days) than other substrates

and obtained the fastest first harvest (50.33 days). The substrate in combination 50SD:50SF produced a higher total yield (88.09 g/bunch) and higher BE (17.62%) with a short total colonization period (26.45 days). The substrate containing SB and SF produced high values in cap diameter, stipe length, and effective fruiting bodies. Both singular and in combination tested, sago bark and frond showed good potential to be used as fruiting substrates for the cultivation of *P. sajor-caju* based on mycelial and sporocarp yield. These substrate ratio mixtures, as compared to sawdust, SB, and SF are as singularly and in combination with SD were comparable with SD and would be a good alternative for the growers of *P. sajor-caju* for mushroom cultivation.

Keywords: Mushroom cultivation, lignocellulosic, sago bark, sago frond, sawdust

***Penanaman Pleurotus sajor-caju (Fr.) Singer Pada Kulit Dan Pelepah Metroxylon sagu* Rottb.**

ABSTRAK

Habuk gergaji digunakan secara komersial sebagai komponen substrat untuk penanaman Pleurotus sajor-caju (cendawan tiram kelabu) di Malaysia. Oleh kerana penurunan bekalan habuk gergaji, kajian ini dilakukan untuk meneliti kemungkinan kulit sagu (SB) dan pelepah sagu (SF) terhadap pertumbuhan dan hasil cendawan tiram kelabu untuk digunakan sebagai alternatif kepada habuk gergaji. Sembilan campuran substrat dikaji, termasuk habuk gergaji (SD) sahaja sebagai kawalan dan kombinasi nisbah 25:75, 50:50, dan 75:25 antara SD dan SB atau SF untuk penanaman cendawan tiram kelabu. Penanaman cendawan dijalankan di dalam rumah cendawan. Kandungan lignoselulosa (lignin, selulosa dan hemiselulosa) untuk campuran substrat dianalisis bersama dengan nilai pH, kandungan abu dan kelembapan mengikut kaedah standard. Substrat tunggal, 100SB menunjukkan kandungan hemiselulosa tertinggi (60.7%), sementara gabungan substrat 50SD:50SF mengandungi lignin tertinggi (40.7%). Untuk selulosa, 75SD:25SF mempunyai peratusan yang tinggi pada 46.2% berbanding dengan yang lain. Kandungan lembapan bagi substrat tunggal 100SB adalah yang tertinggi (11.7%). Sementara untuk kandungan ekstrak, substrat tunggal 100SD mengandungi yang terendah (2.9%). Kandungan abu (1.4-5.2%) dan nilai pH (4.7-6.1%) juga dianalisis untuk kajian ini. Untuk ciri morfologi, diameter sporokarpa (6.7-8.4 cm), panjang batang (6.1-7.5 cm) dan bilangan badan/tandan buah matang dalam campuran substrat yang berbeza (1.7-2.2) dicatatkan dalam kajian ini. Hasil kajian menunjukkan perbezaan yang ketara dalam jumlah masa pengkolonian miselium, jumlah hasil sporokarpa, dan kecekapan biologi (BE) untuk substrat tunggal dengan gabungan. Substrat tunggal 100SB dan 100SF memberikan hasil yang sama dengan 100SD. Untuk gabungan

substrat, 50SD:50SF adalah yang paling sesuai sebagai tambahan pada habuk gergaji untuk penanaman P. sajor-caju. Substrat tunggal 100SD merekodkan tempoh pengkolonian miselium yang cepat (24.44 hari) berbanding dengan yang lain dan juga memperoleh penuaian pertama terpantas (50.33 hari). Substrat campuran 50SD:50SF menghasilkan jumlah hasil yang tinggi (88.09 g/tandan) dan BE yang tinggi (17.62%) dengan tempoh pengkolonian yang paling pendek (26.45 hari). Substrat yang mengandungi SB dan SF menghasilkan kepala cendawan dengan nilai diameter yang besar, panjang batang yang tinggi, dan sporokarpa yang matang. Kulit dan pelepah sagu, sama ada tunggal atau kombinasi yang diuji menunjukkan potensi yang baik untuk digunakan sebagai substrat untuk penanaman P. sajor-caju berdasarkan hasil miselium dan sporokarpa. Campuran substrat bagi SB dan SF, jika dibandingkan dengan habuk gergaji, mempunyai potensi yang sama sekiranya digunakan secara tunggal atau dalam kombinasi dengan SD. Hal ini akan menjadikan SB dan SF sebagai bahan alternatif yang baik untuk penanam P. sajor-caju.

Kata kunci: *Penanaman cendawan, lignoselulosa, kulit sagu, pelepah sagu, habuk gergaji*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BaCl	Barium Chloride
BE	Biological efficiency
dH^2O	Deionised water
FAMA	Federal Agricultural Marketing Authority
HSD	Honestly significant difference
H_2SO_4	Sulphuric Acid
IBM	International Business Machines
IDF	Insoluble dietary fibre
ISO	International Standardization Organization
MAFI	Ministry of Agriculture and Food Industries
NaOH	Sodium Hydroxide
PDA	Potato Dextrose Agar
PVC	Polyvinyl Chloride
SB	Sago Bark
SD	Sawdust
SDF	Soluble dietary fibre
SF	Sago frond
SPSS	Statistical Package for the Social Sciences
STN	Standard
TA	Thermal analysis
TAPPI	Technical Association of Pulp and Paper Industry
UNIMAS	Universiti Malaysia Sarawak

CHAPTER 1

INTRODUCTION

1.1 Study Background

Mushroom is a well-known fungus found growing in moist soil rich in organic substances, decaying materials, or on the damp rotten log of the wood trunk. Edible mushrooms are well-known food sources that highly nutritious (Oei, 2003; Bernaś et al., 2006; Çağlarirmak, 2011). Mushroom also contains a high content of essential amino acids for the human body, easily digested, and has no cholesterol content. In recent years, growing market demand for edible mushroom production can be seen all over the world.

According to Kalač (2013), over 200 mushrooms species were used as food sources worldwide, but only 35 species were commercially cultivated. *Pleurotus* sp. is among the most popular mushroom worldwide in terms of edible basidiomycetes. This species stands in third place in the yield of edible mushrooms, following *Agaricus* and *Lentinula* genus species (Cardosa et al., 2015). Oyster mushroom (*Pleurotus* sp.) was cultivated worldwide throughout the previous few decades (Royce, 2002). Due to white mycelium, *Pleurotus* sp. is associated with the white-rot basidiomycetes (Tsujiyama & Ueno, 2013). The common name ‘oyster’ comes from the white shell-like shape of the mushroom's fruiting body. *Pleurotus sajor-caju* (Fr.) is the most famous cultured species among these mushrooms and acknowledged for its deliciousness (Zhang et al., 2002; Valverde et al., 2015).

Mushroom cultivation has the potential to assist in the waste disposal and environmental waste management. Oyster mushroom cultivation plays a significant part in managing organic waste wherever dumping becomes difficult (Das & Mukherjee, 2007). An example of organic waste found in Malaysia, particularly in Sarawak, is sago palm tree waste. Palms fall under one of the oldest families of plants on earth (Ishizuka et al., 1995; Basu et al., 2014). One of them is sago palm, scientifically known as *Metroxylon sagu* Rottb. It is utilised as an essential crop in Southeast Asia due to the high quantity of starch within the trunk. Starch is one of the major dietary components for human populations. Sago palm is one of the most affordable and convenient food starch sources with the highest starch production per land area compared to different starch products (Rajyalakshmi, 2004).

1.2 Problem Statement

Sawdust used for mushroom cultivation is mainly obtained from forestry industries. However, forest harvesting can lead to deforestation, which will become the main problem for forest industry-practising countries (Shittu et al., 2019). Due to this, the supply of sawdust is diminishing and expensive. Alternative substitute to sawdust is much needed in mushroom cultivation industries in which sago bark and frond are considered as one potential substrate. In the process to obtain edible starch, the whole tree needs to be cut down to collect the inner trunk, and this results in a large amount of fibrous waste and trunk bark left in starch mills, which pollute the environment (Kuroda et al., 2001). Problems arise with the expansion of sago starch production in terms of the residues left from sago palm trees, such as trunk bark (cortex) and frond. According to Ngaini et al. (2014), around 15.6 tons of sago woody bark and 7.10 tons of fibrous pith waste from 600 logs of sago palm are generated in a day. The abundance of sago starch waste has created environmental problems as the waste accumulates in fields or is burned.

Therefore, comprehensive residue utilisation is needed to overcome this issue. It is reported that sago bark can be utilised as an animal feedstuff and sago frond in the pulp and paper industries (Flech, 1983; Alimon, 2009). Another way to utilize sago palm waste is to turn it into a substrate for mushroom cultivation. It contained lignocellulosic contents that facilitate the growth of mushrooms (Abd-Aziz, 2002). Currently, mill sawdust is widely used as a substrate for mushroom cultivation. However, there is a need to explore alternative base carbon sources for the mushroom fruiting substrate. With a large amount of sago fibrous and trunk bark, utilization of these carbon sources should be considered for usage as mushroom substrate. Therefore, this study was conducted to evaluate the performance of various substrate ratio mixtures consisting of sago bark (SB), sago frond (SF), and sawdust (SD) on the growth and yield of *P. sajor-caju*.

1.3 Objectives

The objectives of this study were:

i. to determine the chemical composition of sawdust, sago bark and frond; and

ii. to find out the effect of sago bark and frond as singular substrate and in combination with sawdust on mycelia growth and sporocarp yield of *P. sajor-caju.*

1.4 Chapter Summary

This chapter has provided the general introduction on grey oyster mushroom and sago palm with the problem faced on bark and frond residue management. This thesis aims to determine the suitability of sago bark and frond to be utilised as an alternative substrate to sawdust for the cultivation of grey oyster mushrooms.

CHAPTER 2

LITERATURE REVIEW

2.1 Mushroom

The most visible value of basidiomycetes to the human population is as a food source. Mushroom belongs to the class Basidiomycetes, order; Agaricales (Stanley et al., 2011). The fruiting bodies are big enough to be seen with naked eyes). Mushrooms are an excellent food source to reduce malnutrition in developing countries due to their nutritional value and high productivity, as well as their tasty flavour and nice texture (Eswaran & Ramabadran, 2000; Mowsurni & Chowdhury, 2010). It can be consumed as a substitute for meat. In general, edible mushrooms are rich in vitamin B and C, low in fat and calories, and contain higher protein than other plant origins and an excellent mineral nutrient source (Bahl, 1998; Ananbeh, 2003). Mushrooms are very nutritious products which rich in crude fibre and protein that can be generated from lignocellulosic waste materials (Chang, 2009; Singh, 2017).

According to Wasser (2014), mushrooms are thought to exert around 130 pharmacological benefits, i.e., antitumor, antioxidant, antihypertensive, antimicrobial, and antiviral activities. Approximately 700 of the known mushroom species are labelled to be safe with medicinal properties, where pharmacological effects have been shown for many traditionally used mushrooms, including species from genera *Pleurotus* (Stamets, 2002; Wasser, 2010). However, only around 25 mushrooms have been cultivated, and only ten are actively commercialised (de Mattos-Shipley et al., 2016), including *Pleurotus* sp. All the varieties or species of oyster mushrooms are edible except for poisonous *P. olearius* and *P. nidiformis* (Patar et al., 2018). According to Patel (2012), *Pleurotus* sp., like other edible and

medicinal mushrooms, is a good source of antimicrobial and antioxidant substances as well as a wide array of environmental and biotechnological applications.

2.1.1 Mycelia and Sporocarp of Mushroom

When the mycelium of a fungus reaches a particular growth stage, spores production begins either directly on the somatic hyphae or on special sponferous hyphae, where it may be loosely arranged or grouped into intricate structures, which are known as fruiting body or sporocarp. Mushroom mycelia can produce a group of complex extracellular enzymes that helps to degrade and utilizing lignocellulosic waste, which helps to reduce waste pollution (Chang, 2009). Because of this, mushroom mycelia had a significant role in the restoration of a damaged environment. Between 80% and 85% of all medicinal mushroom products are extracted from fruiting bodies, and 15% are derived from the extract from mycelia (Barros, 2007). Due to these, mushroom mycelia can be considered a good source of healthy compounds that may contribute to the formulation of medicinal, nutraceutical, and cosmetic products (Humberto et al., 2017).

2.1.2 Mushroom Cultivation

Mushroom cultivation is a useful method of environmental waste disposal and waste management where waste materials and crop residues can be converted into valuable food (Wood, 1985; Jebapriya et al., 2013). The general process of oyster mushroom cultivation is the same as other edible mushrooms, although various cultivation techniques are applied. It has three main steps in culturing mushrooms: isolating tissue culture from the fruiting body, preparing primary and secondary spawn, and culturing mushrooms from spawn to harvesting fruiting bodies (Dung, 2003). Pure culture or spawn is used to inoculate a suitable substrate. For *Pleurotus* sp., a wide range of lignocellulosic substrates can be used, including sawdust,

weed plants, molasses from the sugar industry, coconut shell, and cotton from the textile industry (de Mattos-Shipley et al., 2016).

Based on Sánchez (2010), many factors affect cultivation productivity, such as the strain, type of substrate, spawn, the moisture level, and different physicochemical conditions. As basidiomycetes are cultivated using lignocellulosic substrates and other organic matter, this industry can be considered as the most active industry that handles two big issues faced by a current growing population that is producing food with high nutritional value whilst decreasing waste and environmental pollution (de Mattos-Shipley et al., 2016).

2.1.3 Factor Affecting Oyster Mushroom Cultivation

Climatic and moisture condition with favourable temperature helps the production of mushroom fruiting bodies and yield of oyster mushroom (Van Peer et al., 2009). *Pleurotus* sp. requires a short growth time, and diseases and pest do not often attack their fruiting bodies. At the same time, they can be grown in simple and cheap ways with high yield, adapt to different temperature ranges, chemical tolerance and most importantly wider substrate utilisation (Bellettini, 2019). Mejia and Alberto (2013) mention that oyster mushrooms can grow at a moderate temperature from 18 °C to 30 °C. According to Oyetayo and Ariyo (2013), the substrate used for mushroom cultivation is important as it affects chemical, functional and sensorial characteristics of mushrooms as *Pleurotus* spp. are a saprophyte that extracts its nutrients from the substrate.

Water is one of the main factors that affect the success of mushroom growth as the nutrients are transported from the mycelium to the fruiting bodies by moisture flow (Van-Nieuwenhuijzen & Oei, 2005). According to Chang and Miles (2004), the optimum moisture content in the substrate should be in a range between 50% to 75% substrate to obtain a

successful growth of *Pleurotus* spp. Urben (2004) stated that each mushroom has its optimal pH range for development as it varies, for example, between pH 4.0 and pH 7.0 for mycelium growth and pH 3.5 to pH 5.0 for basidiocarp formation. Kalmis et al. (2008) stated that the optimum pH for mycelial growth and sporocarp formation is pH 6.5 to pH 7.0. The optimum humidity for most fungi to grow is between 20% to 70% (Pandey et al., 2001).

2.1.4 Lignocellulosic Component in the Mushroom Substrate

In every plant cell wall, there are three main lignocellulosic components: lignin, cellulose, and hemicellulose, which are the main components of agro-industrial wastes (Figure 2.1). Mushrooms produced a huge range of non-specific lignocellulosic enzymes, and because of that, they can be cultivated on a different agricultural waste (Zhang et al., 2002). Kholoud (2014) mentioned that mushroom grows on decayed organic matters high in lignocellulosic content and other complicated carbohydrates. According to Chang (2009), mushroom substrate can be defined as a kind of lignocellulosic material that promotes and supports mushroom growth. Rani et al. (2008) stated that mushrooms could successfully grow on the various agricultural waste that contains cellulose components. Because of their remarkable ability to successfully grow at various temperature ranges and different lignocellulosic wastes in a short time, the cultivation of *Pleurotus* sp. is important in the food industry (Chahal, 1989; Pala et al., 2012).

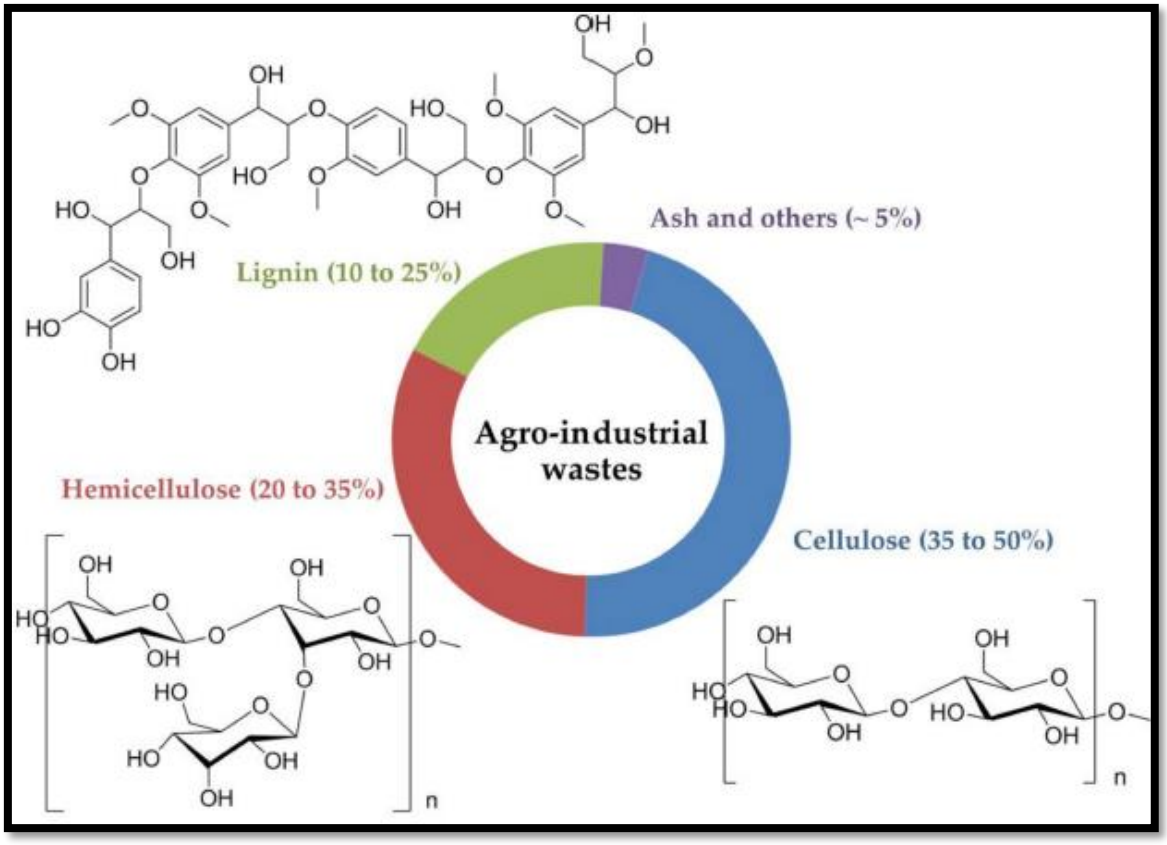

Figure 2.1: Main composition of agro-industrial wastes (Kumla et al., 2020)

Oyster mushrooms such as *P. sajor-caju* can grow to flourish on lignocellulosic substrates. Mushrooms can convert lignocellulosic waste materials into various products that benefit human beings, such as food, medicine, fertilizer, and most importantly, for protecting and regenerating the environment (Chang, 2009).

2.2 *Pleurotus sajur-caju* (Fr.) Singer

Oyster mushroom (*Pleurotus* sp.) is a type of edible fungus consumed by the human population worldwide. *Pleurotus* sp. can be found in tropical and subtropical rainforests and can be cultivated artificially (Bonatti, 2004). According to Cardosa et al. (2015), *Pleurotus* is among the most popular edible basidiomycetes. They ranked third in the production of edible mushrooms behind the genus *Agaricus* and *Lentinula*. Both fruiting bodies (sporocarp) and mycelia of *Pleurotus* sp. have been studied in terms of biological effector molecules besides the extensive study of their medicinal properties (Refaie, 2009).

In Malaysia, the grey oyster mushroom (*Pleurotus sajor-caju*) is the most common mushroom found in the market and popular as a food source due to easy cultivation with a wide range of temperature and a variety of agricultural wastes and natural resources (Asghar et al., 2007). *Pleurotus sajor-caju* is a mushroom widely cultivated in tropical and climatic zone countries due to its cheap and simple cultivation technique (Chang & Miles, 2004). *Pleurotus sajor-caju* is one of the major successful cultivated species among edible mushrooms and acknowledged for their deliciousness (Zhang et al., 2002; Miles & Chang, 2004). Various agricultural, forests and agro-industrial wastes can be used for *P. sajor-caju* cultivation since they can easily grow on wastes rich in cellulose and lignin (Pala et al., 2012).

Shnyreva et al. (2012) studied a strain of *P. sajor-caju* formed fleshy fruiting bodies on clear light stipes with grey pilei, and the spore prints were pale lilac. *Pleurotus sajor caju,* on a dry weight basis, has proteins content ranging from 11% to 42%, carbohydrates from 36% to 60%, and lipids from 0.2% to 8% (Khan & Tania, 2012). *Pleurotus sajor-caju* contains high nutrient content, as shown in Table 2.1.

Table 2.1: Nutritional compositions of *P. sajor-caju* (mean ± SD) (Han et al., 2016)

Nutrients	**Concentration (%)**
Protein	22.41 ± 0.65
Carbohydrate	60.47 ± 0.51
Calorific value (cal/g)	451.60 ± 2.70
Sucrose	0.19 ± 0.00
Total DF	56.99 ± 0.92
Soluble DF	8.21 ± 0.41
Insoluble DF	48.79 ± 0.90
β-glucan	3.32 ± 0.13

According to Wan Rosli and Solihah (2012), *P. sajor caju* can be considered an essential food in human diet. They are rich in dietary fibre, non-starchy carbohydrates, minerals, vitamin-B, low in fat content, and contains β-glucans (Ho et al., 2020). These are the component of soluble or insoluble dietary fibre (SDF, IDF) present in appreciable amounts in mushrooms and are connected to lower glycemic response and blood cholesterol levels. In terms of medicinal properties, *P. sajor-caju* contains various chemical constituents such as polysaccharides, triterpenoids, vitamin C, volatile compounds, ergosterol, and enzymes (Randhawa & Shri, 2017).

Environmental factor plays a vital role for the growth of mycelia (Khan & Chandra, 2017). The environmental factors which affect the fruiting body of *P. sajor-caju* are temperature, humidity, and pH of mushroom substrate. The optimum temperature for *P.*

sajor-caju mushroom cultivation was in a temperature range of 28 °C to 30 °C and humidity of 80% to 90% (Cikarge & Arifin, 2018). Urben (2004) mentioned that mushroom substrate has its optimum pH range for its growth, such as pH 4.0 to pH 7.0 for mycelium running and pH 3.5 to pH 5.0 for sporocarp formation of *P. sajor-caju*. A high concentration of hemicellulose (15-30%), lignin (27-34%), and cellulose (40-50%) in the substrate are needed to achieve a high yield in mushroom cultivation (Jeznabadi et al., 2016).

2.3 Industrial Benefits from Mushroom Cultivation

Mushroom production is important in the industrial economy, both in the food industry and biotechnological industry. According to Royse (2005), in 2004 alone, approximately 10 million tonnes were produced from edible and medicinal mushrooms in various countries. As for 2010 alone, 25 million tonnes of mushrooms were produced (Muhammad & Suleiman, 2015). Medicinal mushroom products help enhanced health and fitness, thus can be categorised as a dietary supplement (Chang & Buswell, 1996; Üstün et al., 2018). The *Pleurotus* sp. is high in medicinal values, and this can be proven with *Pleurotus sajor-caju* having a hypertensive effect through its active ingredients (Alam et al., 2008). Hence, based on the Ministry of Agriculture and Food Industries (MAFI) (2011), the consumption of mushrooms has increased at a relative rate from 1.0 kg/person in 2008 to 2.4 kg/person in 2020. This phenomenon is due to a greater concern for health and an increase in the population in Malaysia.

According to Chang (2009), mushroom cultivation can be labour-demanding agro-industrial activity. It will be a good economic and social impact by generating employment, thus increasing income for both women and youth, especially in rural areas in a developing country such as Malaysia. According to MAFI (2011), mushrooms are one of the seven

high-value crops cultivated assiduously in Malaysia, of which around 17 main types had been commercially cultivated. The most popular cultivated and commercialize in Malaysia is the grey oyster, followed by Shiitake and button mushroom (Mohd Tarmizi et al., 2013). It was reported that the total number of mushroom growers in Malaysia has increased every year, which most of the growers (about 80%) are on a small scale and produce below 50 kg per hectare of fresh mushrooms a day (MAFI, 2011).

Cultivation of mushrooms will play a significant role in increasing food protein, especially in developing countries since the growth substrates for mushrooms are mainly agricultural and industrial wastes that are inedible to humans (Chang & Miles, 1984; Mowsurni & Chowdhury, 2010). Mushroom cultivation is a fast-growing industry and is well known as a cash crop. The fruiting bodies yield can be sold in the local market as an additional income for a family or exported for an important source of foreign exchange that helps to improve the economic standard of the people. Chang (2009) stated that the oyster mushroom is a fast-growing organism and can be harvested in three to four weeks after spawning, which is suitable for short return agricultural nosiness and helps in improving the lives of the community.

2.4 Agricultural and Industrial Wastes as a Substrate for Mushroom Cultivation

Asia is the largest producer of agricultural wastes at 47%, followed by the United States (29%), Europe (16%), Africa (6%), and Oceania (2%) (Cherubin et al., 2018). Agro-industrial wastes (agricultural and industrial residue) have been used as substrates in mushroom cultivation (Kumla et al., 2020). In Malaysia, the wood industry is thriving, leading to mass production of sawdust and other wood residues in which most of the residues are left on the floor of the premises. These wood residues are later be used as a substrate for

mushroom cultivation. However, this raw material is relatively high cost and not readily available in many places. Therefore, searching for other more cost-effective substrates that are readily available is very much needed.

Furthermore, forest harvesting can lead to deforestation, which will become the main problem for forest industry-practising countries (Shittu et al., 2019). The harvested areas must be replanted to replace the extracted timber to avoid deforestation issues (Damette & Delacote, 2011). Malaysia had a high rate of deforestation as it lost an average of 96,000 ha or 0.43% per year of forest cover in a period from 1990 to 2010 (Raihan et al., 2018). In Sarawak alone, Jomo et al. (2004) revealed that Sarawak lost about 50% of its forest cover in the year 1971 until 1989, and as in 2012, Sarawak has lost 90% of its primary forest. As Raihan et al. (2018) studied, enormous reforestation programs have been implemented to minimize the damage done by timber harvesting and restore deforested and degraded forest land. Woon and Norini (2002) mentioned that Malaysia has committed to maintaining at least 50% of the land area under forest cover to achieve Sustainable Forest Management. Due to this effort, less sawdust and other wood residues produced by the timber industry lead to other alternative sources being used as a substrate for mushroom cultivation.

According to Chinda and Chinda (2007), many agricultural and industrial by-products can be used in some way for mushroom production. The example of industrial waste as a substrate for mushroom production include cocoa shell wastes, cottonseed bulb, cotton wastes from the textile industry, cassava peelings, wheat straw, rice straw from and corncobs (Stanley et al., 2011). Other than that, paddy straw mixed with fruits and vegetable peels such as sweet lime, watermelon, banana, pomegranate, and onion can be used as substrates for mushroom cultivation (Lalithadevy & Many, 2014).

Agro-industrial wastes in large quantities that are produced globally contribute to environmental and health problems. Chang (2009) stated that improperly disposed of waste by dumping or burning in the surrounding environment leads to environmental pollution and health hazard. A massive amount of lignocellulosic and other organic waste residues was generated annually through various activities such as agricultural and food processing. Mushroom cultivation is a useful method of environmental waste disposal and waste management where waste materials and crop residues can be converted into valuable food (Wood, 1985; Nicolcioiu et al., 2016). Mane et al. (2007) mentioned that agro-waste could also be accustomed to producing exceptionally nutritious food and of high industrial worth due to its straightforward cultivation technique. Sago palm industrial residue has the potential as a substrate for mushroom production.

2.5 Sago Palm Tree

Sago palm (*Metroxylon sagu*) falls under the families of Arecaceae and can be found in Malaysia, Indonesia, Thailand, the Philippines, Brunei, Papua New Guinea, and South Pacific region (Table 2.2). The word 'sago' means starch-containing palm pith in Javanese. Yamamoto et al. (2003) stated that sago palm is a rare crop that can grow on tropical peat soil with the absence of drainage of groundwater and produces a large amount of starch.

Table 2.2: The species of *Metroxylon* and their distribution (Rauwerdink, 1986)

Taxa	**Distribution**
1. *M. sagu* Rottb.	Malay islands
2. *M. warburgii* Heim.	New Hebrides
3. *M. vitiense Benth* et	Hook Fiji
4. *M. amicarum* Becc.	Carolines
4.1. var. commune Becc.	
4.2. var. maius Becc.	
5. *M. salomonense* Becc.	Solomon Islands

Sago palm is hapaxanthic, producing flowers and fruits at the end of its life cycle, and soboliferous means sago palm has the ability to produce sucker for propagation (Flach, 1997; Howell et al., 2015). Sago palm is immune to floods, drought, strong wind, fire, and an extensive fibrous root system traps silt loads and removes heavy metals, faecal contaminants, and pollutants (Singhal et al., 2008). Sago forest is an excellent carbon sink for carbon sequestration, thereby remedy the greenhouse effect and global warming caused by the release of carbon dioxide into the atmosphere from industrialisation and motorised vehicles (Stanton, 1991; Syafinie & Ainuddin, 2013). These properties were later supported by Flach (1997) and Wulan (2018), where sago palms are environmentally friendly, economically acceptable, and promote a social balance agroforestry system.

Sago palm is considered the 'starch crop of the 21st century by the community of scientists (Jong, 1995; Singhal et al., 2008). Sago palm is exploited as a staple in Southeast Asia due to the great amount of starch content (150-250 kg/trunk) in the trunk (Kuroda et al., 2001). In Malaysia, 15% to 20% increase every year in the production of sago starch and export value which was reported as the fifth agricultural income after pepper, palm oil, cocoa, and rubber (Awg-adeni et al., 2009). The state of Sarawak is the main sago starch exporter based on data from the Agricultural Statistic of Sarawak (2011). Sago starch products are not limited to food. The derivations of starch can be used in the paper industry, pharmaceutical applications, fuel, and other industrial uses (Flach, 1983; Singhal et al., 2008).

2.5.1 Chemical Composition of Sago Palm Tree

Sago pith is where the starch is accumulated. The starch content of the pith from commercially harvested trunks could range from 18.8% and 38.8% for fresh weight (Wina et al., 1986; Rashid et al., 2020). According to Singhal et al. (2008), the amount of phenolic compound is less than 1%, while the lignin content is between 9% to 22%. In contrast, lignin is associated with hemicelluloses in the pith cell walls. Sago starch contains 27% amylose and 73% amylopectin (Ito et al., 1979; Noomhorm & Tokiwa, 2006; Tongdang et al., 2008). According to Abd-Aziz (2002), sago '*hampas*' composition consists of 65.7% starch, 14.8% crude fibre, 1% crude protein, no fat content, and 59.1% moisture.

2.5.2 Palm Industrial Waste Utilization and Environmental Problems

Sago palm industrial wastes consist of sago '*hampas*', bark and fronds. Sago '*hampas*' is the fibrous residue left behind after the starch was extracted out. This industrial waste can be used for another application instead of being thrown away. Flach (1983), listed the application of sago palm wastes (Figure 2.2).

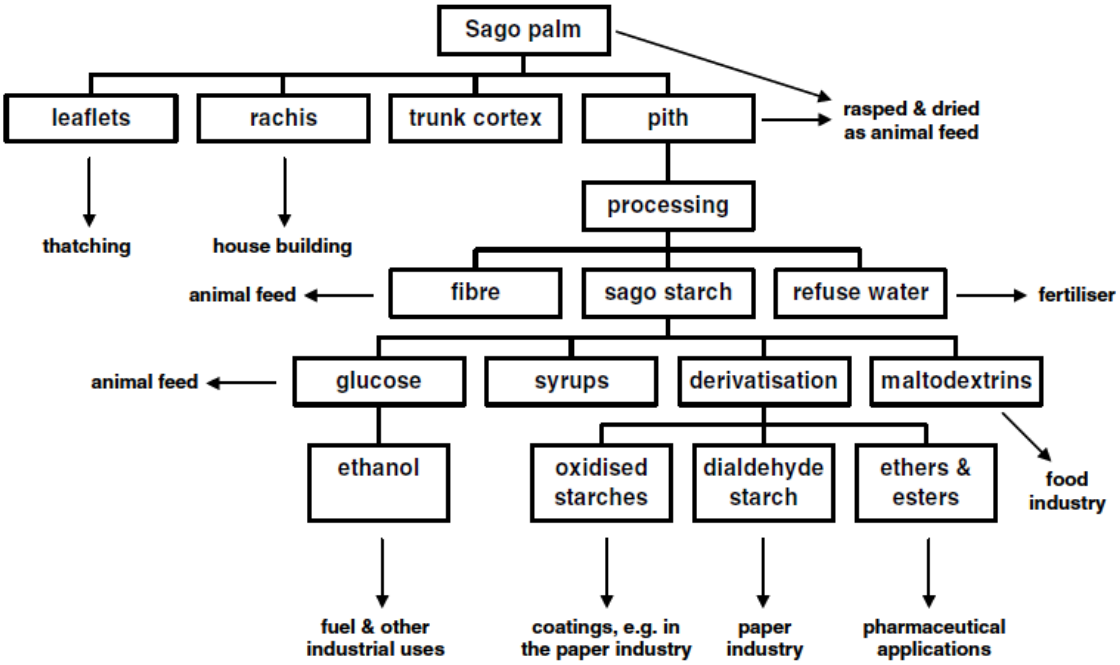

Figure 2.2: Application of Sago (Flech, 1983)

Zadrazil (1992) and Rasol (2012) also supported the statement by saying the non-pith parts of the palm are excellent to be utilised as a building material for local or urban houses or sheds. However, modern communities nowadays do not use palm fronds as the roof, which leads to the fronds being discarded after the tree was cut down in the field and caused environmental and health pollution.

The most common environmental problems caused by sago palm industries are sago fibrous residue (*hampas*) and wastewater. Phang et al. (2000) stated that the wastewater is discharged into the rivers where statistically, around 10 to 22 tons of wastewater per day is produced by each factory. The claim was supported by Haryanto and Siswari (2004), stated that large-scale processing of sago could build-up residual sago starch in rivers that could lead to pollution. Due to the increasing awareness of the environmental impact of heavy metals, a regulation for purification of industrial wastewater before discharged into natural water is implemented with strict legislation to control water pollution as stated in Environmental Quality (Schedules Wastes) Regulation, 1989 in Malaysia (Yeoh & Chong, 1991). With the ascending production of sago palm, huge amounts of fibrous residues and trunk bark are leftover in the starch mills, which pollute the environment (Kuroda et al., 2001; Haryanto & Siswara, 2004).

Besides Sago '*hampas*' and bark, sago frond also caused an environmental problem as 20% of the waste from sago starch production in Malaysia is sago frond. Awg-Adeni et al. (2009), stated that a large amount of sago trunk cortex had caused an environmental problem as it is stacked in the fields or burnt. The open burning of agricultural waste to remove the frond and bark wastes is no longer reasonable. Effective utilisation of waste is very much needed. Agricultural waste can be avoided or minimised if the waste is reused for agricultural activity, such as mushroom cultivation.

2.6 Sago Bark and Frond as a Substrate for Mushroom Cultivation

Studies have been conducted to determine whether the sago palm fibres (*hampas*) can act as substrates for oyster mushroom cultivation as Phang et al. (2000) and Awg-Adeni et al. (2010) stated that sago fibrous residue could be used as compost for mushroom cultures. In this study, *P. sajor-caju* cultivation was conducted using sago bark and fronds

as substrates, and the chemical composition of the substrates was determined. The cellulose, hemicellulose, lignin, extractives, pH, moisture content, and ash were determined according to the method presented by Yang et al. (2006) and revised by Mansor et al. (2019), STN ISO (1993), and TAPPI (2002; 2013) standard methods. Therefore, this study is essential to enhance the utilisation of sago waste.

2.7 Chapter Summary

This chapter summarised the previous studies in the respective field related to this thesis on sago palm trees and oyster mushrooms. With Malaysia having a suitable climatic condition for growing grey oyster mushrooms, this type of species will be tested in this study for mushroom cultivation. Mushroom cultivation is a useful method of environmental waste disposal and managing sago palm tree, especially bark and frond residue.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Sawdust, Sago Bark and Sago Frond for Major Chemical Composition Test

The substrates studied were rubberwood sawdust (SD), sago bark (SB), and sago frond (SF) obtained from a local sago grower in Lundu. Approximately five kilograms of each sample were collected. Singular and combinations of substrates were tested (Table 3.1) to determine the suitable chemical composition and substrates ratio mixtures for the cultivation of *P. sajor-caju*. Each substrate, SB, and SF was tested singularly (100%) with SD as a control (Figure 3.1). The samples were prepared according to the Lap-Preparation of the sample for composition analysis (Hames et al., 2004). The samples were sundried for a week and ground using laboratory disintegrator ST-24B to a smaller size and passed through 35 meshes (500 microns) sieve pan.

Table 3.1: Substrates ratio mixtures (on a dry weight basis) used for major chemical composition determination for mushroom cultivation

Substrates Ratio Mixtures	Symbol
100% Mill sawdust	100SD
100% Sago bark	100SB
100% Sago frond	100SF
75% Mill sawdust:25% Sago bark	75SD:25SB
50% Mill sawdust:50% Sago bark	50SD:50SB
25% Mill sawdust:75% Sago bark	25SD:75SB

Table 3.1 continued

75% Mill sawdust:25% Sago frond	75SD:25SF
50% Mill sawdust:50% Sago frond	50SD:50SF
25% Mill sawdust:75% Sago frond	25SD:75SF

3.2 Major Chemical Composition Determination

Determination of pH values in room temperature of substrate ratio mixtures extracts was conducted referring to the standard method STN ISO 6588 (50 0381) (2009) for cold extraction. TAPPI T211 (2002) standard method in determining ash for wood was used as a guideline for this experiment. For the moisture content determination test, TAPPI T550 (2013) standard method was referred. Lignocellulosic (cellulose, hemicellulose and lignin) and extractives percentages were determined by using the biomass solvent extraction standard method provided by Yang et al. (2006) and revised by Mansor et al. (2019). Three replicates for each substrate were prepared and tested.

3.2.1 pH Value

A total of 2.0 g of the substrate sample was mixed with 100 ml of distilled water. The sample was left for 1 hour at room temperature (25 °C), and the pH of the samples was measured using a standard pH meter. Three replicates of measurements were carried out for each sample.

3.2.2 Ash Content

A total of 5 g of moisture-free substrate sample were placed in a crucible and ignited in a muffle furnace at 525±25 °C for 30 minutes to 60 minutes. The percentage of ash content can be calculated (Equation 3.1) using the formula;

$$\text{Ash, \%} = \frac{\text{Weight of Ash (g)} \times 100}{\text{Initial weight of moisture free sample}} \quad \text{Equation 3.1}$$

3.2.3 Moisture Content

For this test, 5 g of substrate ratio mixtures sample weight using electric balance and placed inside the oven at 105 °C until a constant weight is achieved. The moisture content percentage can be determined (Equation 3.2) using the MC formula below;

$$\text{MC} = \frac{\text{Initial Weight} - \text{Oven-dry Weight}}{\text{Oven-dry Weight}} \times 100 \quad \text{Equation 3.2}$$

3.2.4 Extractives Content

A total of 1 g of each substrate ratio mixture (A) is prepared and added with 60 ml of Acetone. The substrate sample was heated on a hot plate for 2 hours under 90 °C constant temperature. After heating, the sample was dried in an oven at 105 °C until a constant weight was obtained (B). The extractives were identified using Equation 3.3:

$$(A - B) = \text{Extractives in sample (g)} \quad \text{Equation 3.3}$$

3.2.5 Hemicellulose Content

Continuation from Equation 3.3, the extractives-free (B) substrate sample was added with 150 ml of Sodium Hydroxide (NaOH) (0.5 mol/L) solution. The sample was heated for 3.5 hours using a hot plate under a controlled temperature at 80 °C. The sample was filtered and washed using deionised water (dH^2O) to remove Na^+. pH paper was used to detect the presence of Na^+ in the sample where the reading should indicate neutral close to pH 7. The Na^+ free sample was dried in the oven at 105 °C until a constant weight was achieved (C). The amount of hemicellulose was calculated using Equation 3.4 as below;

(B-C) = Amount of Hemicellulose in sample (g) Equation 3.4

3.2.6 Lignin Content

The sample (C) was added with 30 mL of 98% Sulphuric Acid (H_2SO_4) and left for 24 hours at ambient temperature (25 °C). Later, the sample was boiled at a constant temperature of 100 °C for 1 hour using a hot plate. Next, the sample was filtered and washed using deionised water (dH^2O) to remove sulphate ion left by H_2SO_4. Detection of sulphate ion present in the sample was done by titration process with 10% Barium Chloride (BaCl) solution. The residue from the sample will turn opaque white and deposit white powder when sulphate ion was present. The sample free of sulphate ion was dried in an oven at 105 °C until a constant weight was obtained (D). The final weight of the sample was calculated as lignin content as indicated in Equation 3.5 as below;

(D) = Amount of Lignin in sample (g) Equation 3.5

3.2.7 Cellulose Content

Assuming the total lignocellulosic component inside the substrate ratio mixtures sample is equal to 1 g as shown in Equation 3.6, 1 g is referred to as the total amount of substrate sample used for this test. The differences between the initial weight of the sample (1 g) with three other component weights (extractives, hemicellulose, and lignin) from the experimental flow were calculated to determine the content of cellulose (E).

$$(A - B) + (B - C) + D + E = 1 \text{ g} \qquad \text{Equation 3.6}$$

The biomass solvent extraction standard method for the determination of lignocellulosic (Section 3.2.4 until Section 3.2.7) was performed on three replicates per sample.

3.3 *Pleurotus sajor-caju* Culture

Grey oyster mushroom was collected from a local market and identified as *P. sajor-caju* using a molecular identification technique sequencing of ITS4 and ITS5 region (Appendix 10; Appendix 11). *Pleurotus sajor-caju* culture used in this study was maintained on the Potato Dextrose Agar (PDA) slant and sterile water at 4 °C for at most three months. The stock cultures were deposited at Mycology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak.

3.4 *Pleurotus sajor-caju* Spawn Preparation

Paddy grains were used for spawn preparation. The spawns were prepared in 850 ml polypropylene plastic bags, and PVC tubing was used to close the bags' mouths. The bags were autoclaved at 121 °C for 15 minutes and left to cool at room temperature. Sterilized

paddy grains were inoculated with viable mycelia of *P. sajor-caju* from the PDA medium and incubated at 28 °C until the mycelia fully colonised the bags.

3.5 Substrate Preparation and Spawn Inoculation

The substrates used in this study were SD, SB, and SF. The SD was obtained from a local wood factory. All the sago substrates were obtained from a local sago grower in Lundu, Sarawak, Malaysia. Approximately 5 kg of SB and SF were sundried for a week and then ground by a grinding machine into 0.5 cm to 1.5 cm length pellets (particle size).

To determine appropriate substrates and ratio mixtures towards grey oyster mushroom cultivation, nine substrate ratio mixtures in comparison and combination of SD, SB, and SF were studied (Table 3.1). 100% SD substrate was utilised as the control treatment. Each substrate (SD, SB, and SF) was tested singularly (100%). In addition, there were six combinations of each pair of substrates at ratios of 3:1 (75:25), 1:1 (50:50), and 1:3 (25:75). Three replicates for each subtrate ratio mixture singularly and in combination were prepared for three batches in this test.

After mixing the materials at these ratios, they were prepared using a 100:1:1 ratio for the sample (500 g):rice bran:chalk ($CaCO_3$). Each substrate ratio mixture was supplemented with 1% rice bran obtained from a local rice mill and 1% chalk. The substrates and supplements were mixed thoroughly. Next, the substrates were added with tap water, and about 60% moisture was adjusted by adding water to the substrate for the final mixture. Each substrate was packed into polyethylene plastic bags and sterilised in an autoclave for 15 minutes at 121 °C and left to cool at room temperature. Next, each bag was inoculated with 10 g spawn per bag, and three culture bags were prepared for each substrate ratio mixture.

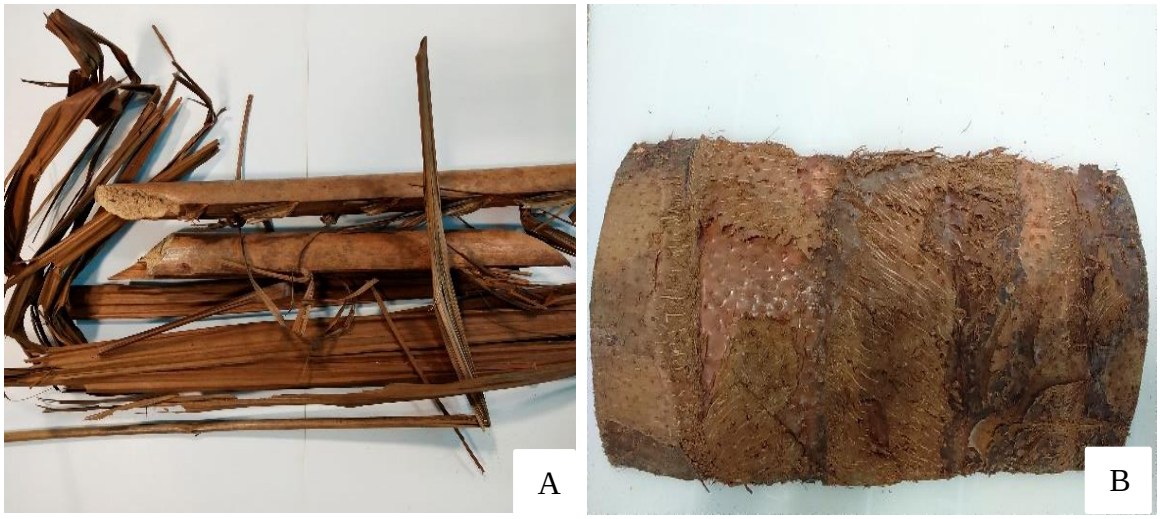

Figure 3.1: The raw sample used for the test, Sago frond (A) and Sago bark (B)

Figure 3.2: Ground substrate (Sawdust (A), Sago bark (B) and Sago frond (C))

3.6 Substrate Incubation and Sporocarp Harvesting

The inoculated substrate bags were maintained inside the incubation room at 28 °C and around 60% to 70% relative humidity. The bags were placed on a mushroom shelf outside the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS) once the substrate bags were entirely covered with mycelium. Three mushroom flushes were harvested from every bag for each substrate, and the total days taken from inoculation until the first harvest was inspected and documented. The bag was examined to detect any fungal contaminants by observing the presence of green moulds grown on the bag surface. Temperature (30 °C) and relative humidity (80%) of mushroom shelf were monitored regularly. The mycelia density was represented by the symbol '-', which means poor, '+', which means moderate, and '++', which means abundant (Shrestha et al., 2006). The weight (g/bunch) of an individual bunch of mushrooms per bag and the proportion of flushes (stipe length and cap diameter) of the harvested fruiting bodies were measured and recorded. The amount of effective fruiting bodies per bag at the first, second, and third flushes and the total means were recorded per bag (g/bag). The collected data were applied to calculate the total yield and biological efficiency (BE) after the harvesting period ended. Biological efficiency was calculated using the following equation (Equation 3.7), and the results were recorded;

$$\text{BE, \%} = \frac{Grams\ of\ fresh\ sporophore\ produced}{Grams\ of\ dry\ substrate\ used} \times 100\% \qquad \text{Equation 3.7}$$

3.7 Experimental Design and Data Analysis

The research was performed in the Mycology Laboratory, Faculty of Resource Science and Technology, UNIMAS in Malaysia, from January 2018 until September 2019. The experiment was arranged in a randomised block design one-way ANOVA in three batches of test and three cultured bags were prepared per treatment in each batch. The collected data were analyzed using IBM SPSS Statistics 20. The differences between the substrates were examined using a one-way ANOVA. The test of significance with a *p*-value < 0.05, which is considered significant, was done using the Tukey HSD multiple range test. All data were expressed as mean ± standard deviation.

3.8 Chapter Summary

This chapter described the methods and materials used in the experiment to determine the major chemical composition, lignocellulosic contents. Mushroom cultivation techniques and harvesting methods were also explained in this chapter. Three replicates for each substrate ratio mixtures were prepared for three test batches to determine the major chemical composition present in the substrate, which may or may not affect the cultivation of mushrooms. Grey oyster mushroom cultivation technique and harvesting methods were also explained in this chapter. Three replicates for each substrate ratio mixtures were prepared for three test batches to determine mycelia growth and sporocarp yield of grey oyster mushroom.

CHAPTER 4

RESULTS

4.1 Major Chemical Compositions Analysis and Lignocellulosic Contents

4.1.1 Major Chemical Composition

Chemical composition of the substrate such as pH value, ash, moisture, and extractives content are important factors for mushroom cultivation for mycelia growth and development of fruiting body. Overall, the pH value for all singular substrates and in combination ranged from pH 4.6 to pH 6.1. The pH value in singular substrate 100SD was the highest (6.1), while 100SF has pH 4.8 and singular substrate 100SB recorded a lower pH of 4.6. However, there is no significant difference with substrate in combination, 75SD:25SB, which recorded a higher pH value at 5.3, while 25SD:75SB recorded lower pH at 4.7 (Table 4.1).

For ash content, singular substrate 100SD has the lowest (1.4 wt%) while 100SF has the highest at 5.2 wt%. Substrate combination of SD and SF (1.7-4.5 wt%) have no significant difference for ash content. The ash content percentages for this study, both singular and in the combination of substrate ratio mixtures, were between a range of 1.4% to 5.2%. For moisture content, the percentages were shown by singular substrate 100SB (11.7 wt%) while singular substrate 100SF contained 9.7 wt% and 100SD at 9.3 wt%. For substrate in combination, 50SD:50SF recorded moisture content (7.9 wt%), while other substrates (8.7-9.7 wt%) have no significant difference from each other. Singular substrate 100SD (2.9 wt%) showed the lowest extractives content, followed by 100SB (5.8%), while 100SF (9.2 wt%) had the highest extractives content. Substrate combination of SD and SB combination (7.0-8.8 wt%) showed no significant difference for extractives content.

Table 4.1: Mean pH, ash, moisture content and extractives content values of substrates (in wt%)

Substrate Ratio Mixture	pH	Ash (wt %)	Moisture (wt %)	Extractives (wt %)
100SD	6.1 ± 0.1^{a}	1.4 ± 0.1^{a}	9.3 ± 0.3^{a}	2.9 ± 0.9^{a}
100SB	4.6 ± 0.11^{c}	3.9 ± 0.1^{c}	11.7 ± 0.3^{b}	5.8 ± 0.2^{c}
100SF	4.8 ± 0.10^{bc}	5.2 ± 0.1^{c}	9.7 ± 0.2^{a}	9.2 ± 1.3^{b}
75SD:25SB	5.3 ± 0.04^{b}	1.7 ± 0.1^{b}	8.8 ± 0.4^{ac}	8.7 ± 0.5^{b}
50SD:50SB	5.1 ± 0.17^{b}	2.2 ± 0.2^{b}	9.7 ± 0.3^{a}	8.8 ± 0.2^{b}
25SD:75SB	4.7 ± 0.06^{c}	2.6 ± 0.1^{c}	9.7 ± 0.3^{a}	8.1 ± 0.6^{b}
75SD:25SF	5.2 ± 0.04^{b}	2.5 ± 0.1^{b}	8.7 ± 0.3^{ac}	7.0 ± 0.6^{c}
50SD:50SF	5.1 ± 0.19^{b}	3.3 ± 0.1^{b}	7.9 ± 0.3^{c}	7.7 ± 0.8^{bc}
25SD:75SF	4.9 ± 0.06^{bc}	4.5 ± 0.1^{bc}	9.4 ± 0.5^{a}	8.8 ± 0.2^{b}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters within column are not significantly different according to Tukey HSD's mean multiple comparisons test. Standard Deviation is represented in ±.

4.1.2 Lignocellulosic Contents

Lignocellulosic content (hemicellulose, cellulose and lignin), is essential in fruiting body development. The lignocellulosic contents of sago bark, sago frond and sawdust mushroom substrates were calculated based on weight loss percentage (%). Singular substrate, 100SB (60.7%), had the highest percentage of hemicellulose content (Figure 4.1).

In comparison, sago bark has significantly higher hemicellulose content compared to sago frond (46.1%). Both 100SB and 100SF have significantly higher hemicellulose content compared to 100SD (26.5%). For substrate in combination, 25SD:75SB had the highest hemicellulose (45.6%). However, there was no significant difference for a lower hemicellulose content among the substrates 75SD:25SF (24.1%) 75SD:25SB (24.1%) and 50SD:50SF (26.0%).

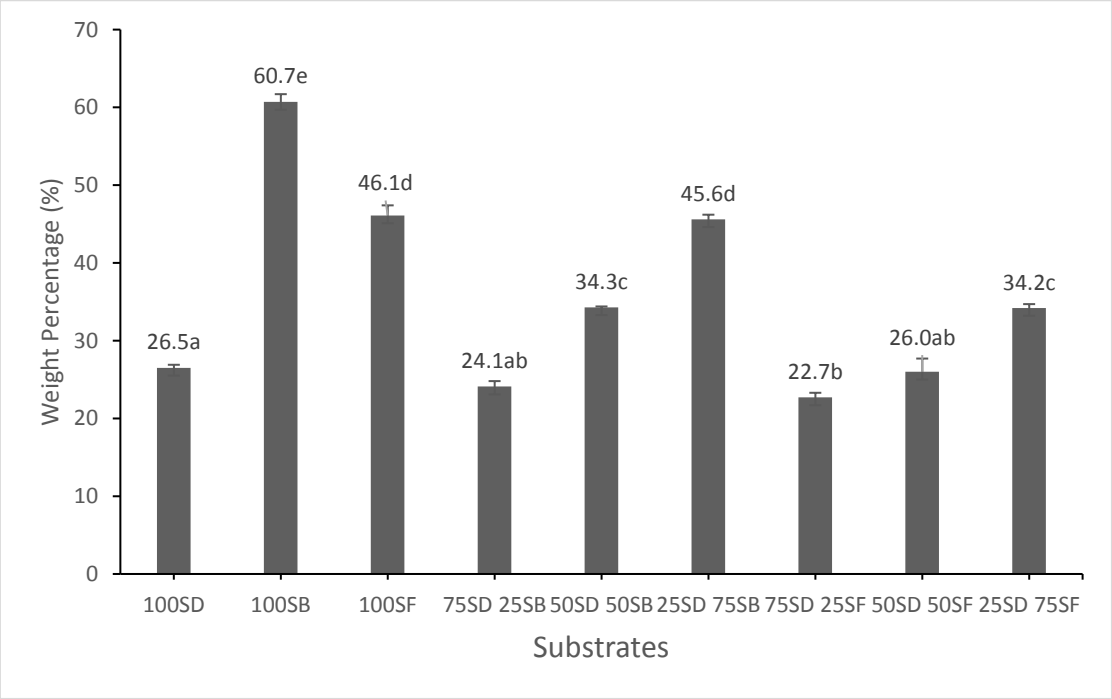

Figure 4.1: Hemicellulose content (%) in different substrate (Error bars represent standard deviation)

By comparing the substrates based on the percentage of lignin, singular substrate 100SB had the lowest lignin content (9.9%) significantly compared to other singular substrates where 100SD had the highest lignin content (25.9%) followed by 100SF (17.1%). For substrate in combination, 50SD:50SF (40.7%) had the highest lignin content compared

to the rest of the substrates (Figure 4.2). Both 25SD:75SB (25.8%) and 75SD:25SF (24.1%) contained lower lignin content compared to the other substrates. From the results, it can be concluded that a substrate combination between sawdust and sago frond has higher lignin content.

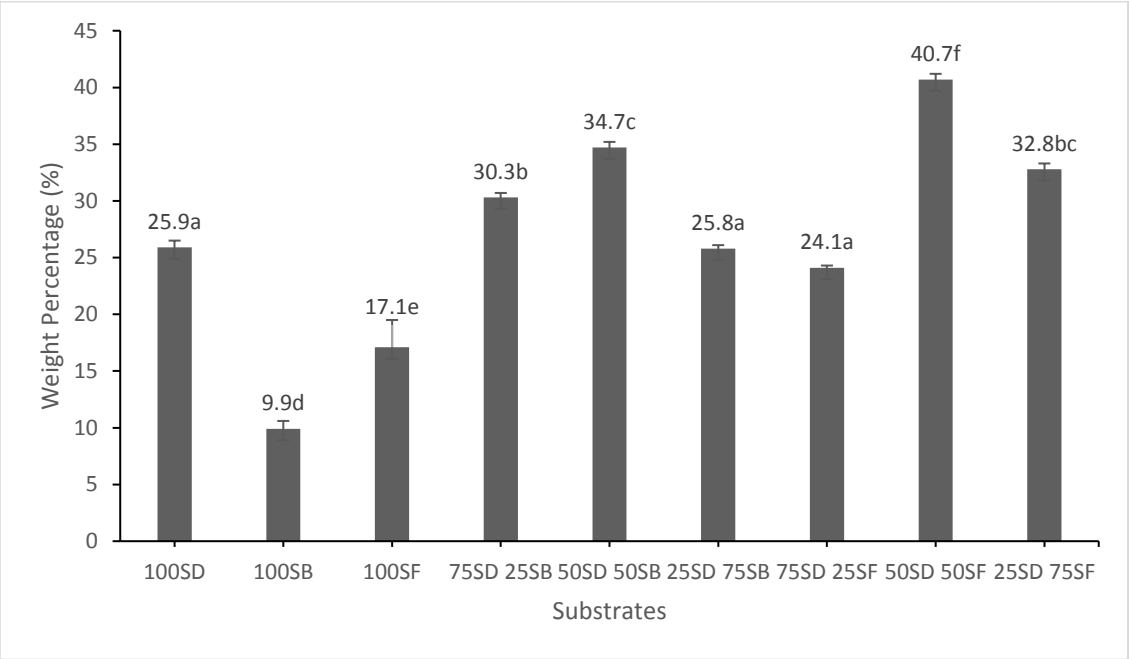

Figure 4.2: Lignin content (%) in different substrate (Error bars represent standard deviation)

For cellulose content, singular substrate, 100SD had the highest cellulose (44.7%), followed by 100SF (27.6%) and 100SB with the least cellulose content 23.6%) (Figure 4.3). For substrate in combination, the highest cellulose content was shown by 75SD:25SF (46.2%). Both 25SD:75SB (20.5%) and 50SD:50SB (22.2%) recorded low cellulose content. Based on the result of this study, it can be concluded that the higher the ratio of sawdust in the substrate when combined with sago bark or sago frond, the higher the cellulose content.

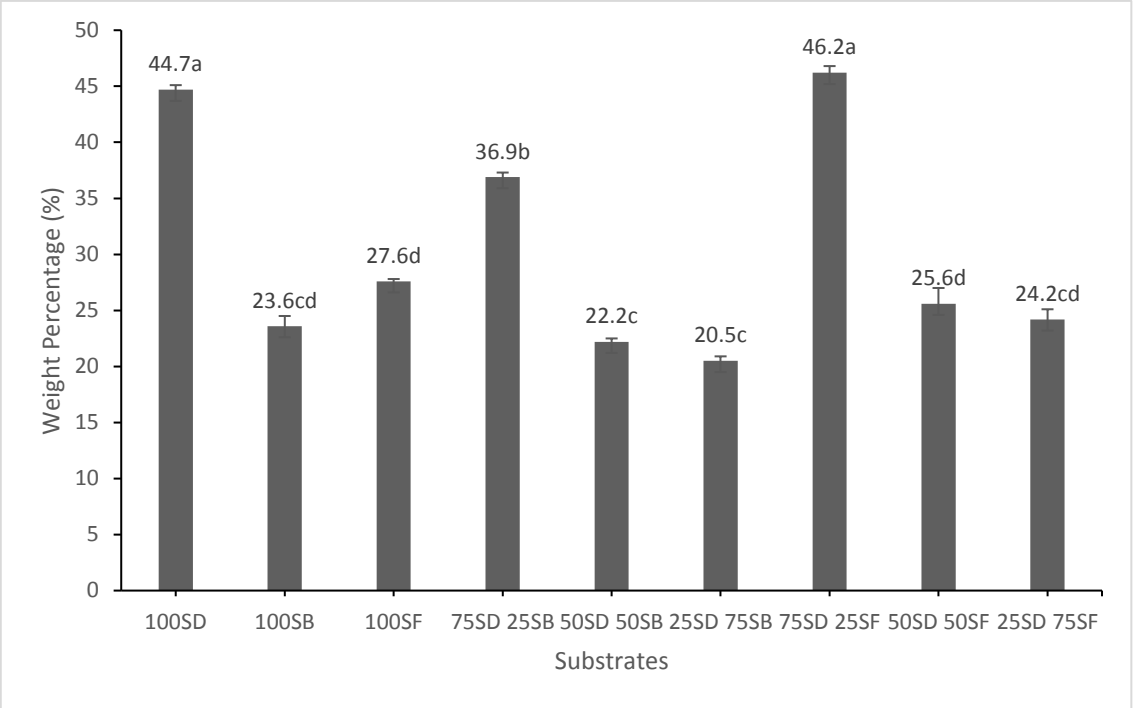

Figure 4.3: Cellulose content (%) in different substrate (Error bars represent standard deviation)

4.2 Pure Culture of *P. sajor-caju*

Fresh fruiting bodies of *Pleurotus sajor-caju* were collected from the local market and confirmed as *P. sajor-caju* by using molecular identification technique. The pure cultures were prepared by using the tissue culture technique on a PDA medium. The pure cultures showed white mycelium on the petri dish (Figure 4.4).

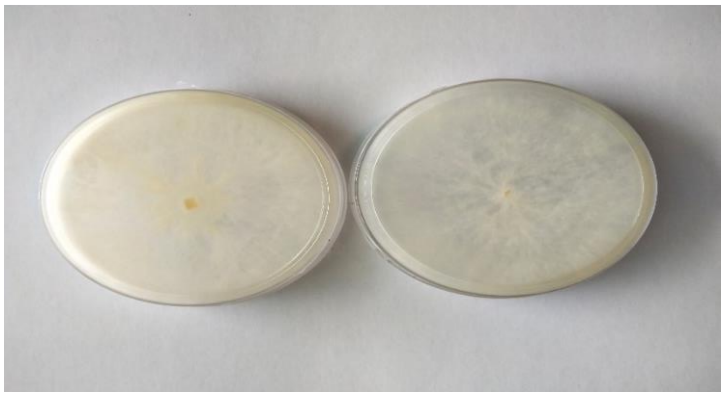

Figure 4.4: Pure culture of *P. sajor-caju*

4.3 Spawn

Spawn was prepared in five bottles containing paddy grains. Twenty-one days after pure culture was inoculated, all bottles have been successfully grown with *P. sajor-caju* mycelium. The mycelium was observed growing after five to six days after inoculation. On day 16 to 17, the mycelium fully colonized the paddy grains in the spawn bottle (Figure 4.5).

Figure 4.5: Spawn (paddy) containing mycelia of *P. sajor-caju*

4.4 Mycelia Growth

Throughout this study, there was no fungal contamination detected on the surface of the bags for all substrate ratio mixtures. The surface of the bags was not contaminated with

black or green moulds. For the mycelial growth of *P. sajor-caju* for the mycelia density, the duration for mycelia to fully covered the bag and the duration for the first pinhead formation were recorded. The *P. sajor-caju* was cultivated in three singular substrates, and seven combinations of substrates were recorded and presented in Table 4.2 and Table 4.3.

4.4.1 Mycelia Density

The results of mycelial density were based on Figure 4.6, where A is Dense (++), B Medium Dense (+), and C Thin (-). The results are presented in Table 4.2. Singular substrate 100SD recorded denser mycelia density (++) for Batch 1 and 2 while having a moderate mycelia density (+) for Batch 3 compared to the other substrates. Meanwhile, singular substrates 100SF and 100SB recorded a moderate mycelia density (+) for all three mushroom cultivation batches. For substrate in combination, 75SD:25SB, 50SD:50SB, and 25SD:75SB showed medium mycelia density (+) for all three batches. In contrast, 75SD:25SF, 50SD:50SF, and 25SD:75SF showed medium mycelia density (+) in the first two batches while less density mycelia (-) in Batch 3.

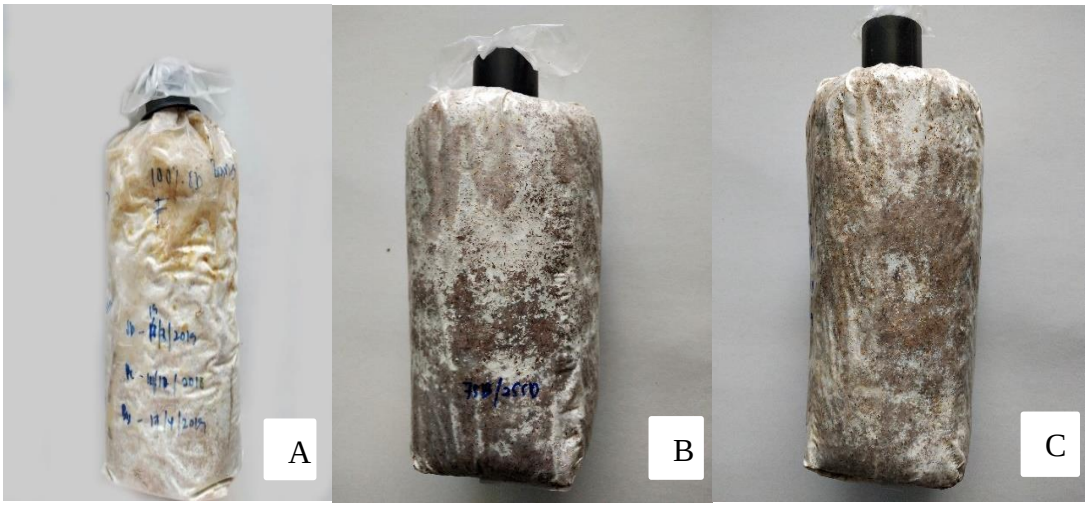

Figure 4.6: Mycelia density of *P. sajor-caju*; Dense (A), Medium Dense (B), Thin (C)

Table 4.2: Mycelia density in substrate mixtures of sawdust, sago bark and sago frond

Substrate	Batch 1	Batch 2	Batch 3
100SD	++	++	+
100SB	+	+	+
100SF	+	+	+
75SD:25SB	+	+	+
50SD:50SB	+	+	+
25SD:75SB	+	+	+
75SD:25SF	+	+	-
50SD:50SF	+	+	-
25SD:75SF	+	+	-

Dense (++), Medium Density (+), Thin (-). SD, sawdust; SB, Sago bark; SF, Sago frond.

4.4.2 Mycelia Growth and Pinhead Formation

Nine substrate ratio mixtures were tested to determine the duration of mycelia growth and pinhead formation of *P. sajor-caju*. The results shown in Table 4.3 show significant differences in the morphological parameters of *P. sajor-caju* on singular substrate 100SD, 100SB with substrate in combination 50SD:50SB, 25SD:75SB, 50SD:50SF, and 25SD:75SF substrates ratio mixture.

Table 4.3: Effect of different substrate ratio mixtures on morphological parameter and characteristics of the sporocarp of oyster mushroom

Substrate ratio Mixtures	Total colonisation period (day)	First harvest (day)	Cap diameter (cm)	Stipe length (cm)	No. of effective fruiting bodies/ bunch	Sporocarp weight (g/bunch)
100SD	24.44 ± 0.84^{a}	50.33 ± 3.35^{a}	7.30 ± 1.10^{a}	6.57 ± 0.71^{a}	2.13 ± 0.42^{a}	26.00 ± 3.75^{ab}
100SB	28.78 ± 2.14^{b}	71.86 ± 5.17^{b}	8.37 ± 0.55^{b}	7.47 ± 0.81^{a}	1.83 ± 0.50^{a}	21.66 ± 1.75^{ab}
100SF	27.56 ± 0.77^{ab}	67.33 ± 2.03^{bc}	7.30 ± 2.23^{a}	6.67 ± 1.51^{a}	1.77 ± 0.40^{a}	22.55 ± 1.36^{ab}
75SD:25SB	25.00 ± 0.89^{a}	59.46 ± 1.08^{ac}	7.87 ± 0.57^{a}	6.40 ± 0.44^{a}	1.70 ± 0.20^{a}	19.17 ± 2.74^{b}
50SD:50SB	27.22 ± 1.71^{ab}	53.90 ± 4.01^{a}	7.53 ± 1.11^{a}	6.87 ± 1.27^{a}	1.70 ± 0.56^{a}	19.99 ± 3.39^{b}
25SD:75SB	27.33 ± 1.34^{ab}	63.10 ± 0.35^{bc}	7.70 ± 0.69^{a}	6.77 ± 0.50^{a}	1.67 ± 0.12^{a}	19.70 ± 0.59^{b}
75SD:25SF	24.78 ± 0.19^{a}	67.43 ± 1.63^{c}	6.70 ± 0.46^{a}	6.13 ± 0.76^{a}	2.16 ± 0.57^{a}	23.18 ± 3.37^{ab}
50SD:50SF	26.45 ± 1.35^{ab}	59.00 ± 7.37^{ac}	8.07 ± 1.00^{a}	6.60 ± 0.40^{a}	2.13 ± 0.70^{a}	29.36 ± 5.19^{a}
25SD:75SF	26.67 ± 0.67^{ab}	69.00 ± 1.30^{bc}	6.87 ± 0.65^{a}	6.07 ± 0.32^{a}	1.93 ± 0.64^{a}	20.39 ± 3.23^{b}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters within column are not significantly different according to Tukey HSD's multiple comparison of means test. SD, sawdust; SB, Sago bark; SF, Sago frond. Standard Deviation is represented in ±.

The results showed significant differences in the total colonisation period of *P. sajor-caju* grown on substrates ratio mixtures containing SD, SB, and SF. Colonization of *P. sajor-caju* was completed between 24.44 days to 28.78 days after spawn inoculation in this study for three singular substrates and seven substrate ratio mixtures. Based on Table 4.3, the mycelia of *P. sajor-caju* took shorter time to fully colonised the singular substrate of 100SD (24.44 days) while 100SB (28.78 days) took longer time compared to other substrate ratio mixtures. In comparison, substrate ratio mixture containing singular or in the combination of SB or SF took a longer time (24.78-28.78 days, respectively) to completely colonized the substrates. The result showed that substrate combinations containing a mixture of sawdust (SD) have shorter time of mycelial colonisation than 100 per cent of the substrate containing sago bark and sago frond.

The first harvest of *P. sajor-caju* cultivation was collected between 50.33 days to 71.86 days for singular substrate or in combination substrate of SD, SB, and SF. Based on Table 4.3, faster first harvest (50.3 days) of *P. sajor-caju* was recorded from singular substrate 100SD. Singular substrate 100SB recorded slower first harvest (71.9 days) compared to other substrate ratio mixtures.

There were no significant differences between singular substrate 100SD and substrate ratio mixture 50SD:50SB (53.9 days). The same goes for singular substrate 100SB and substrate ratio mixture 25SD:75SB (69.0 days). There was a significant difference between singular substrate 100SD, 100SB and 75SD:25SF (67.43 days). The results showed substrates combination containing SD and SB or SD and SF took similar time for the first harvest of sporocarp compared to 100 per cent SD.

4.4.3 Cap Diameter, Stipe Length and Number of Effective Fruiting Body

There were significant differences in cap diameter and no significant differences in stipe length of *P. sajor-caju* grown on different substrate ratio mixtures (Table 4.3). Singular substrate 100SB recorded the widest cap diameter at 8.37 cm and a longer stipe length (7.47 cm). Singular substrates 100SD and 100SF showed no significant difference in cap diameter and stipe length. Meanwhile, substrate in combinations showed no significant difference in both cap diameter (6.70-8.07 cm) and stipe length (6.13-6.87 cm). Based on the Federal Agricultural Marketing Authority (FAMA) (2012) standard for the size classification of cap diameter (refer to Appendix 9), seven substrate ratio mixtures (6.70-7.87 cm) were labelled as size code 2 (M). In contrast, singular substrate 100SB (8.37 cm) and 50SD:50SF (8.07 cm) were labelled as size code 3 (L). From this test result, it can be concluded that all the substrate ratio mixtures have comparable cap diameter and stipe length for *P. sajor-caju*.

The effective fruiting body (sporocarp) is the edible part of the mushroom. The mean number of effective fruiting bodies per bag of *P. sajor-caju* shown no significant difference among singular substrate and different substrate ratio mixtures consisting of SD, SB, and SF (Table 4.3). Based on the data recorded, the substrate ratio mixtures containing 75SD:25SF (2.2) had a slightly higher mean number of effective fruiting bodies of *P. sajor-caju* per bag. Substrate in combination of 75SD:25SB, 50SD:50SB, and 25SD:75SB recorded no significant differences in the mean number of effective fruiting bodies per bag (1.7) of *P. sajor-caju*, lower compared to the rest of the substrate ratio mixtures. Substrate ratio mixture containing sago frond has similar number of the fruiting body of *P. sajor-caju* compared to a substrate ratio mixture containing sago bark.

4.4.4 Sporocarp Yield (in Individual Flush) Per Bag

The sporocarp weight (in individual flush) per bag of *P. sajor-caju* depended on cap diameter, the length of the stipe, and the number of effective fruiting bodies per flush. Mushroom weight is closely related to total fruiting bodies yield and BE. For singular substrates, 100SD (26.00 g), 100SB (21.66 g), and 100SF (22.55 g) have no significant differences in terms of sporocarp weight. Meanwhile, for substrate in combination, there was a significant difference (Table 4.3) between the highest (50SD:50SF) with the four lowest substrate ratio mixtures mean yield per bag which were 75SD:25SB (19.17 g), followed by 25SD:75SB (19.70 g), 50SD:50SB (19.99 g), and 25SD:75SB (20.39 g). Based on the sporocarp of *P. sajor-caju* (individual flush), 50SD:50SF substrate ratio mixtures recorded a higher mean yield of the sporocarp per bag (individual flush) at 29.36 g, followed by singular substrate 100SD with 26.0 g per bag compared to other substrate ratio mixtures (Table 4.3). While the combination of 75D:25SB has a lower yield per bag of *P. sajor-caju* recorded at 19.17 g. The results showed substrate ratio mixtures containing sago frond have a better mean yield of sporocarp per bag of *P. sajor-caju* compared to substrate ratio mixtures containing sago bark.

4.5 Sporocarp Yield and Biological Efficiency (BE) of *P. sajor-caju*

The main purpose of mushroom cultivation is for yield. For this study, the sporocarp of *P. sajor-caju* (in three successive flushes) were gathered from substrate bags (500 g). *Pleurotus sajor-caju* grown on various substrates ratio mixtures showed a significant difference in mushroom harvest. The weight of the mushroom yield per flush, along with total sporocarp yield and biological efficiency (BE), are shown in Table 4.4.

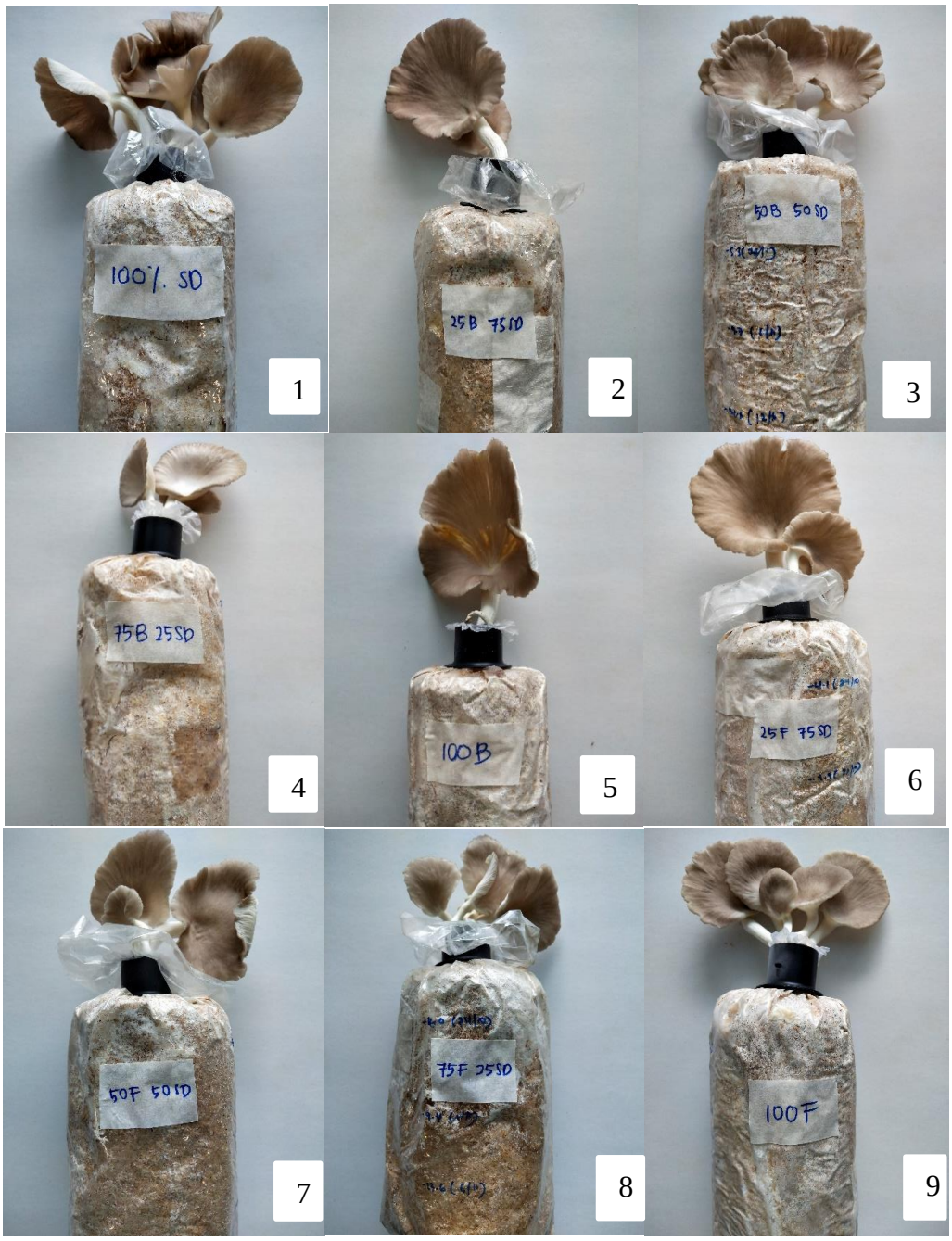

Figure 4.7: Example of sporocarp yield for each substrate; 100SD (1), 75SD:25SB (2), 50SD:50SB (3), 25SD:75SB (4), 100SB (5), 75SD:25SF (6), 50SD:50SF (7), 25SD:75SF (8) and 100SF (9). SD, sawdust; SB, Sago bark; SF, Sago frond

Table 4.4: Effect of different substrate ratio mixtures on sporocarp yield and Biological Efficiency of *P. sajor-caju*

Substrate Ratio Mixtures	**1st flush (g/bag)**	**2nd flush (g/bag)**	**3rd flush (g/bag)**	**Total yield (g/bag)**	**BE (%)**
100SD	25.82 ± 11.01^{a}	21.69 ± 4.40^{ab}	30.48 ± 4.08^{a}	77.99 ± 11.23^{ab}	15.60 ± 2.24^{ab}
100SB	24.19 ± 1.46^{ab}	19.90 ± 6.59^{ab}	20.91 ± 6.61^{a}	65.00 ± 5.27^{ab}	13.00 ± 1.05^{ab}
100SF	21.06 ± 5.52^{ab}	23.00 ± 7.08^{ab}	23.60 ± 7.40^{a}	67.66 ± 4.08^{ab}	13.53 ± 0.82^{ab}
75SD:25SB	21.27 ± 4.98^{ab}	18.01 ± 2.39^{a}	18.21 ± 4.16^{a}	57.49 ± 8.20^{a}	11.50 ± 1.64^{b}
50SD:50SB	21.08 ± 5.40^{ab}	21.84 ± 4.60^{ab}	18.14 ± 3.17^{a}	61.06 ± 12.11^{ab}	12.21 ± 2.42^{b}
25SD:75SB	20.07 ± 3.00^{ab}	16.77 ± 2.13^{a}	22.26 ± 3.22^{a}	59.10 ± 1.75^{a}	11.82 ± 0.35^{b}
75SD:25SF	21.75 ± 7.38^{ab}	22.26 ± 4.90^{ab}	25.55 ± 9.38^{a}	69.56 ± 10.12^{ab}	13.91 ± 2.02^{ab}
50SD:50SF	38.91 ± 10.38^{a}	32.32 ± 4.24^{b}	16.86 ± 1.98^{a}	88.09 ± 15.57^{b}	17.62 ± 3.11^{a}
25SD:75SF	18.21 ± 2.13^{b}	15.89 ± 2.73^{a}	27.05 ± 10.70^{a}	61.15 ± 9.68^{ab}	12.23 ± 1.93^{b}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters within column are not significantly different according to Tukey HSD's multiple comparison of means test. SD, Sawdust; SB, Sago bark; SF, Sago frond. Standard Deviation is represented in ±.

There was no specific pattern (consistence) in terms of mushroom yield (Table 4.4) from the first flush, second flush, and third flush of *P. sajor-caju* for all singular and in a combination of substates ratio mixture of SD, SB, and SF. *Pleurotus sajor-caju* grown on singular and in the combination of different substrates ratio mixtures showed a significant difference in sporocarp yield and BE. The total yield of mushrooms ranged from 57.49 g/bag to 88.09 g/bag. For singular substrate, 100SD (77.99 g/bag), 100SB (65.00 g/bag), and 100SF (67.66 g/bag) showed no significant differences in total yield. For substrate in combination (Table 4.4), 50SD:50SF recorded a higher total yield harvested per bag (3-time harvest) with 88.09 g/bag. Substrate 75SD:25SB gave a lower sporocarp yield (57.49 g/bag), though it was no significant difference from substrate ratio mixture 25SD:75SB (59.10 g/bag). There was no significant difference between all the substrate for the total yield (g/bag) except for the highest (50SD:50SF) with the lowest yield (75SD:25SB & 25SD:75SB).

Biological efficiency (BE) is an excellent criterion parameter to work out the potency of substrate transformation within the fruiting body. Most of the time, substrates that give higher yield will give higher BE. For singular substrates, 100SD (15.60%), 100SB (13.00%), and 100SF (13.53%) have no significant differences in terms of BE. As for substrate in combination (Table 4.4), the substrates ratio mixture of 75SD:25SB showed a lower BE (11.50%) while 50SD:50SF showed higher BE (17.62%) compared to others. However, there is no significant different of BE between the 75SD:25SB with 25SD:75SB (11.82%), 50SD:50SB (12.21%) and 25SD:75SF (12.23%). From the result, it can be concluded that substrate ratio mixtures containing sago bark have similar biological efficiency with sago fronds.

4.6 Chapter Summary

This chapter has recorded the results obtained for the chemical composition of sawdust, sago bark and sago frond. The chemical composition of pH value, ash, moisture, and extractives are similar for every substrate ratio mixtures. Lignocellulosic content of substrates ratio mixture significantly different from each other. Mycelia growth of substrates ratio mixtures is not significantly different from each other. Substrate ratio mixtures containing sago bark have similar sporocarp yield and biological efficiency with sago fronds.

CHAPTER 5

DISCUSSION

5.1 Major Chemical Composition of Different Substrate Ratio Mixtures

The pH value influences the proper growth and development of mushrooms under different substrates. According to Chang (2007), mushrooms prefer slightly acidic (pH 3.5) to slightly basic (pH 6.5) pH of substrates. The pH has a great influence on the morphological development of mushrooms (Chang, 1988; Urben, 2004). For commercial production, the pH of substrate ratio mixtures was adjusted to 6.00±0.15 using Calcium hydroxide ($CaOH_2$) to achieve a suitable pH condition for mushroom cultivation. In this study, the initial pH value of all substrate ratio mixture, the substrates were prepared without the addition of Calcium hydroxide ($CaOH_2$).

From this study, the pH value ranged from pH 4.6 to pH 6.1 for all substrates, indicating suitable pH conditions for mushroom cultivation. These values are in range for optimum pH for mushroom substrate with what is reported by Urben (2004), which stated that mushroom substrate has its optimum pH range for growth at pH 4.0 to pH 7.0 for mycelium running and pH 3.5 to pH 5.0 for sporocarp formation. This statement is supported by the report done by Hong et al. (1983) and Kalmis et al. (2008), which reported that the optimum range of pH for mycelium growth is about pH 5.5 to pH 7.0. It can be concluded that based on pH value by referring to the previous study done on mushroom substrate cultivation, SB (pH 4.6-5.3) and SF (pH 4.8-5.2) have suitable pH conditions as a substrate for *P. sajor-caju* cultivation. To ensure the pH is in optimum value, it is recommended to add $CaOH_2$ in the substrate and adjusted to optimum pH in future studies.

The percentage of ash content in both singular and in combination of substrates ranged from 1.4% to 5.2%. The analysis of ash content in the sample can be explained as removing organic content by burning, which only leaves inorganic minerals (McClements, 2003). Ash content determines the amount and type of minerals in the sample tested. The amount of minerals indicates the sample's physiochemical properties, which in this study, mushroom substrate and inhibit the growth of microorganisms (Dairy Foods, 2010). The ash content of wood can be considered low as it was between 0.2% and 1% dry weight (Browing, 1963; Klašnja et al., 2013). As for this study, low ash contents was obtained from substrates ratio mixtures (sago bark, sago frond and sawdust). Due to low percentage of ash content recorded, it can be concluded that sago bark and frond ash contents are suitable for mushroom substrate as it does not affect the performance of the substrates.

Moisture content in a substrate is important for mushroom cultivation because it influences yield and mushroom production (Wang et al., 2001). The residues of sago starch left on the sago bark might contributing to the high initial moisture content of singular substrate 100SB. This statement is supported by Mustafa Kamal et al. (2019), which showed that sago starch has a high moisture content (up to 38.8 wt%). The results from this study are similar to a study by Carll et al. (2009), which stated that on average, the moisture content of wood is between 7% to 19% by weight depending on the surrounding relative humidity. As the initial moisture content for all substrate ratio mixtures was in the range for average wood moisture content, the amount of water added in the substrate ratio mixtures for mushroom cultivation was essentially within the same range (60% water content). As for this, SB (9.7-11.7%) and SF (7.9-9.7%) are suitable as substrate for *P. sajor-caju* cultivation.

The extractives are responsible for the substrate's colour, smell, and durability (Rowell, 2012). Extractives consists of a group of cell wall chemicals such as fatty acids, phenols, resin acid, waxes, and other minor organic compounds (Mansora et al., 2019). In this study, extractives content for all substrates ranged from 2.9% to 9.2%. The values are fall on the range similar to the study by Croan (2004), where the percentage for wood waste extractives content for mushroom substrate was between 3.05% to 9.02%, which were comparable with the percentage of extractive content in this study. For mushroom cultivation substrate, any species of wood (soft or hardwood) except pine wood can be used as the medium as long as it does not contain any extractive substances that potentially inhibit the growth of fungi (Santoso, 1992). The higher the extractives content, the more durable the substrate from fungal contamination (mould and wood-rotting fungi) as fungal growth was flourished by removing the extractives from the wood. However, fungi removed 70 to 99.9 per cent of the extractives (Croan, 2002). Their metabolic activity during its growth substantially reduced wood extractives (Blanchette et al., 1996). Therefore, low extractives content did not affect the growth of mushrooms. For this study, the extractive contents of all substrate ratio mixtures were low and, in the range, which does not affect mushroom cultivation. Based on extractives contents, SB (5.3-8.8%) and SF (7.0-9.2%) are suitable for a substrate for *P. sajor-caju* cultivation.

5.2 Lignocellulosic Composition of Different Substrate Ratio Mixtures

Lignocellulosic content in mushroom substrate is vital in supporting the growth, maturity, and fruiting of mushroom (Chang & Miles, 1988; Salami et al., 2017). Cellulose is the most biodegraded component of the lignocellulose by fungi for the production of the fruiting body (Thomas et al., 1998; Andlar et al., 2018). Salmones et al. (2005) stated that lignin is the least and hemicellulose is less profusion compared to cellulose. Cellulose is the

most abundant component, followed by hemicellulose and lignin in agro-industry wastes such as oil palm empty fruit bunch and sugarcane bagasse (Kumla et al., 2020). According to Menon and Rao (2012), agricultural lignocellulosic biomass consists of 40% to 50% cellulose, 25% to 30% hemicellulose and 15% to 20% lignin. Adebayo and Martinez-Carrera (2015) stated that agro-waste materials such as paddy straw, which mainly constituted of cellulose (46.2%), lignin (26.2%), and hemicellulose (27.6%), acted as a major source of carbon and energy for *Pleurotus* species cultivation as *Pleurotus* sp. are primary wood rot fungi in which are able to colonize different types of agricultural wastes as substrates thus, they are cultivated on various lignocellulosic wastes.

According to Badu et al. (2011), lignocellulosic contents of sawdust (various wood species) were cellulose (44.79-46.76%), hemicellulose (15.32-16.29%) and lignin (27.55-34.08%). For sawdust, results from this study showed that singular substrate 100SD contained 44.7% cellulose, 26.5% hemicellulose, and 25.9% lignin. Saldarriaga et al. (2012) reported that sawdust contains 21.94% hemicellulose, 35.67% cellulose and 33.07% lignin. The lignocellulosic contents may vary slightly from the other study as the preparation process and method used for analysis are different and may affect the results. In this study, lignocellulosic percentages were determined by biomass solvent extraction standard method provided by Yang et al. (2006) and revised by Mansora et al. (2019), while for Saldarriaga et al. (2012), the samples were analysed by TGA Q500IF of TA Instrument.

Cellulose is the most important lignocellulosic content for mushroom cultivation as it is the major structural component of the cell wall responsible for mechanical strength (Adebayo & Martinez-Carrera, 2015). Higher cellulose percentages recorded for 75SD:25SF (46.2%) but had no significant difference with singular substrate 100SD (44.7%), while

lower content was recorded for 25SD:75SB (20.5%). These results were similar to the study by Atilia (2019) for cellulose content (20.7-41.9%). Hemicellulose acts as intermediates in the biosynthesis of cellulose (Vercoe et al., 2005). For hemicellulose, singular substrate 100SB recorded the highest (60.7%) while 75SD:25SF (22.7%) recorded the lowest content. The results recorded in this study were higher than the finding by Badu et al. (2011), which obtained 15.32% to 16.29% for hemicellulose on sawdust.

Pleurotus sp. is one of the most productive lignin-degrading organisms (Adebayo et al., 2015). Lignin plays a central role in carbon cycling, and its heterogeneous structure gave organisms their structural rigidity and serve as protector for cellulose and hemicellulose from degradation (Vetayasupom, 2006). In this study, 50SD:50SF recorded the highest lignin (40.7%) while 100SB (9.9%) recorded the lowest. The results in this study were similar to Atilia (2019) finding on lignin (4.8%-26.2%).

Cellulose (20.5%-46.2%), hemicellulose (22.7%-60.7%), and Lignin (9.9%-40.7%) contents of sago bark and frond are similar to sawdust. By comparing the previous works by Atilia (2019) and Badu et al. (2011), it can be concluded that the lignocellulosic contents of sago bark and sago frond combined with sawdust, or singularly are suitable as a substrate for *P. sajor-caju* cultivation.

5.3 Effect of Different Substrates Ratio Mixtures on Morphological Parameters of *P. sajor-caju*

The total colonization period and fruiting body formation were affected by the substrate combination. However, the mycelia density, mushroom cap diameter, stipe length, and the number of sporocarps were not significantly affected by substrate combinations of SD, SB, and SF. The results from this study show that all substrates' ratio mixtures have a moderate surface mycelia density. One of the factors that contribute to mycelial density is the nutrition available in the substrates. Donini et al. (2009) reported that mycelia tend to grow rapidly in poor nutritional substrates to reimburse its nutritional requirements, thus becoming less dense. Based on this statement, substrates SD, SB and SF both singularly and in combination used in this study are poor nutritional as the mycelia observed were less dense in three batches. Other than that, environmental factor such as climatic and moisture condition with temperature affect mycelia growth (Van peer et al., 2009) in which affecting the density of mycelia as shown in this study. Thus, there was no specific pattern of surface mycelia density that strong enough to assert that one substrate ratio mixture is better than the other as all substrates showed similar mycelial density.

Mycelia growth is the first step in providing suitable internal conditions for fruiting. The fastest mycelia growth becomes a vital factor in mushroom cultivation (Pokhrel et al., 2009). In this study, the colonisation of *P. sajor-caju* mycelia on the substrate was completed between 24.44 and 28.78 days after incubation. Emiru et al. (2016), reported that oyster mushroom mycelium took 2 to 3 weeks to colonise SD substrate after complete inoculation. Mycelia completely colonised the substrate containing 100% SD in shorter time (24.44 days), while 100% SB took a longer time (28.78 days) than other substrate ratio mixtures.

This study showed that SB and SF as a singular substrate had a longer period for mycelia growth than substrate in combination with SD.

Based on mycelia growth, a substrate containing SB (25.00-28.78 days) took longer period to fully colonise the substrates than SD (24.44 days) alone. However, SF (24.78-27.56 days) took a similar period as SD. Similar results are also reported by Shah et al. (2004), where a substrate (wheat straw and leaves) with a higher ratio of sawdust have the fastest growth of mycelia to colonize the substrates completely. Therefore, SB and SF incorporated with SD enhanced the growth rate of mycelia compared to substrates containing only SB and SF.

One of the factors that makes sawdust an excellent substrate is that sawdust contains cellulose for decomposition that promotes fungal growth (World Export, 2019). However, substrates having high lignin contents take longer time for mycelia growth and fruiting body formation (Oei, 2003). The results obtained in this study showed that 100SD, having a lower lignin content (25.9%) compared to another substrate ratio mixture, resulting in faster mycelial growth. It was also observed that different ratio mixtures have different lengths of days taken for mycelia growth. This may occur due to different conditions of the substrate, such as surface area and pore size. Tinoco et al. (2001) reported that the substrates' larger surface area and pore encouraged faster mycelium growth rate.

Mushroom cultivation required several essential environmental conditions for growth, such as temperature. An optimum temperature between 28 °C to 30 °C is needed to ensure better growth of mycelia. The temperature is reported to play a vital role in mycelia's growth (Khan & Chandra, 2017). Rajak (2011), in his study, reported that the optimum temperature for mycelial growth of *P. sajor-caju* was between 20 °C to 25 °C. Okwulehie

and Okwujiako (2008), reported the best condition for growth of mushroom mycelium was between 20 °C to 30 °C, but declined below 15 °C or above 35 °C.

However, the formation of hyphae and the primordia may not be restricted to a particular temperature, even though developments of both structures are generally preferred at definite temperatures (Scrase & Elliott, 1998). In this study, the surrounding temperature during mycelial growth and fruiting was at 30 °C on average. The mushroom shelves' temperature was monitored regularly to maintain optimum temperature (30 °C) for mycelial growth and fruiting development.

Birhane (2016) stated that pinhead formation (fruiting body) started after mycelium growth was completed. The fruiting body is the harvestable part of mushroom cultivation. It takes approximately 4 to 5 days for the pinhead to emerge from the day the cap opens. The formation of the fruiting body of the oyster mushroom is developed 3 to 5 days after pin-head emergence. The harvesting procedure required pulling out the mushroom fruiting bodies from the substrate bag. According to FAMA (2012), the edible mushroom part of mature grey oyster mushroom, known as the effective fruiting body, can be identified when the cap is grey, expanded, and has a thin flattened surface, while the gills are white with wider gaps.

Days for the colonisation period of mycelia and the first harvest of *P. sajor-caju* were shorter for 100SD, whereas 100SB took longer time compared to other substrate ratio mixtures. Mycelia completely colonised 100SD in 24.44 days, while 100SB took 28.78 days. However, there is no significant difference between 100SD and other substrates, except for 100SB in the total colonisation period. The results (100SD) were similar to the study by Fasehah and Shah (2017) for *P. sajor-caju* to colonised sawdust (26 days) as substrate (500

g). Mycelium growth in this research was much slower than the study by Emiru et al. (2016) in which oyster mushroom mycelium took 2 to 3 weeks to colonise SD substrate after complete inoculation. This may be because of the inconsistency of humidity and temperature due to ever-changing weather in Malaysia that affects mycelia's growth in substrate bags that slow it down. It was observed in the present study, based on the colonisation period alone, substrate containing SB (25.00-28.78 days) and SF (24.78-27.56 days) took a similar period of growth as compared to SD (24.44 days) alone. It can be concluded that SB and SF incorporated with SD performed similarly as SB and SF alone in term of colonization period.

The results obtained from this study (50.33 days) for the first harvest of singular substrate 100SD are in agreement with a study by Ibrahim et al. (2015) of which the first harvest on sawdust was obtained on 59.2 days. Singular substrate 100SB took longer period for the first harvest collected (71.86 days). In the present study, based on the first harvest period, substrate containing SB (53.90-71.86 days) and SF (59-69 days) took longer period of growth compare to SD (50.33 days). The substrate containing sago frond was recorded to have slow pinhead emergence (first harvest) compared to sago bark and sawdust, which takes around 59 days to 69 days to emerge. This study also indicated that SB (71.86 days) and SF (67.33 days) as a singular substrate took longer period of the first harvest compared to substrate in combination with SD except for 75SD:25SF (67.43 days) and 25SD:75SF (69 days).

In this study, the duration of pinhead formation was between 19 to 26 days after the mycelia fully covered the substrate. Ibrahim et al. (2015) stated that grey oyster mushroom cultivated on 500 g sawdust took 37 days to 57 days for pinhead emergence after inoculation. Pinhead usually starts to emerge four to seven days after opening the cap. The results in the

present study were in agreement with Bugarski et al. (1994), who stated that the first fruiting body formed on different days, depending on the substrates. It can be concluded that SB and SF incorporated with SD enhanced the pinhead formation (first harvest) compared to SB and SF alone.

This study indicated that the substrate combination of SB, SF and SD affected the colonization period and the first harvest of *P. sajor-caju*. The period for first pinhead emergence (first harvest) on different substrate ratio mixtures differed. This may vary due to different substrates ratio mixtures that affect pinhead emergence, thus leading to a longer first harvest. The possible reasons for this variation may be due to nutrient availability of substrates (nitrogen, sugar, fats, protein starch, and lignin) and temperature. These variations might cause the difference in the number of days taken to complete pinhead formation of oyster mushrooms on different substrates (Bhatti et al., 1987).

In addition, temperature and relative humidity also play a significant role in the number of days taken for pinhead formation, as mentioned by Dunkwal and Jood (2009). Optimum pH value (pH 3.5-5.0) also needed for pinhead formation (Urben, 2004). A longer period needed for fruiting body emergence might be due to low humidity (82%) in the mushroom house. One of the reasons that slowed the growth of mycelia may be due to the autoclave sterilized substrates bags compressed by high pressure for 80 minutes and the air was discharged, leading to the shortage of oxygen (Yang et al., 2013).

5.4 Effects of Various Substrate Ratio Mixtures on *P. Sajor-caju* Sporocarp Characteristics

The mean number of effective fruiting bodies (sporocarp) per bunch was recorded. The number of sporocarp per bag had no significant differences between the substrate ratio mixtures for *P. sajor-caju*. The results showed that the maximum effective fruiting body of *P. sajor-caju* ranged between 1.70 to 2.16 bodies per bunch for all substrate ratio mixtures. In a study by Onuha (2007), wood sawdust had a low number of fruiting bodies production. This finding agrees with the results of this study, where 100SD only recorded an average of 2.1 number of fruiting bodies which is similar to substrate 50SD:50SF. Sago bark substrate ratio mixtures have lower number of fruiting bodies (1.7-1.8) than sago frond substrate ratio mixtures (1.8-2.2). However, there are no significant differences between all the substrate ratio mixtures for the number of fruiting bodies produced. This can be concluded that sago bark and sago frond produce the same amount of fruiting body as sawdust. The same goes with the combination of sawdust with sago bark and sago frond as substrates, where these substrate ratio mixtures produced the same amount of fruiting body of *P. sajor-caju*. It was observed in the present study that based on the mean number of effective fruiting bodies per bunch alone, substrate containing SB and SF have the same ability as SD, indicating that SB and SF have the potential as a substrate for *P. sajor-caju*.

This sudy observed the similarity in mushroom cap diameter and stipe length of *P. sajor-caju* in all singular and in combination substrate ratio mixtures. The mushroom cap diameter and stipe length development were not significantly affected by substrate types and ratio mixtures. There were no significant differences in the cap diameter and stipe length of *P. sajor-caju* grown on nine different substrate ratio mixtures. The widest cap diameter (8.37 cm) was recorded for the substrate ratio 100SB, while no significant differences were

recorded for other substrate ratio mixtures. The diameter of the mushroom cap shows no significant difference between SB (7.53-8.37 cm), SF (6.70-8.07 cm), and SD (7.30 cm).

Referring to FAMA (2012), on the marketable quality requirement for fresh grey oyster mushroom (MS 2515:2012), all mushrooms must be uniform in size and maturity with fresh, clean, and free from any deform and damage. The mushroom harvested in this study for all singular and in combination with substrate ratio mixtures showed marketable quality. Based on the stipe length and mushroom cap diameter of *P. sajor-caju*, most of the SB and SF substrate combination with SD has better morphological properties than SD alone. The results showed that SB and SF are suitable as a substrate for mushroom cultivation.

The present investigation showed that the substrate combination of SB, SF, and SD does not affect mushroom cap diameter, stipe length, and the number of effective fruiting bodies/bunch. For *P. sajor-caju* cultivation in this study, the temperature recorded in the mushroom shelf was around 30 °C with humidity around 82%, which was favourable as the optimum temperature for grey oyster mushroom cultivation was in the temperature range 28 °C to 30 °C and humidity 80% to 90% (Cikarge & Arifin, 2018). For a tropical country like Malaysia, which is suitable for the cultivation of oyster mushrooms, this condition explained the reason for the marketable quality obtained for *P. sajor-caju*. The quality of oyster mushroom harvested can be improved by supplementing substrates with various additives, including nitrogen sources, which have been reported to improve the growth, yield, and quality of mushrooms (Royes, 2002; Mane et al., 2007; Onyango et al., 2011).

5.5 Effect of Different Substrate Ratio Mixtures on Sporocarp Yield and Biological Efficiency of *P. sajor-caju*

The sporocarp weight (g/bunch) of *P. sajor-caju* depends on the cap diameter, stipe length, and total effective fruiting bodies per bunch. The sporocarp weight is also associated with the yield and BE of mushrooms. For singular substrate SD, SB and SF, the sporocarp weight was not significantly different from each other. This shown that SB and SF have the potential as an alternative substrate to SD. The sporocarp weight grown on substrate in combination with SD, SB and SF were significantly different. A higher sporocarp weight of *P. sajor-caju* (29.36 g/bunch) was obtained from 50SD:50SF substrate. Meanwhile, a lower sporocarp weight was recorded for 75SD:25SB substrate (19.17 g/bunch). The sporocarp yield from this study consisting of SD, SB, and SF were lower compared to other studies, Fasehah and Shah (2017) obtained a mean yield of 42.83 g/bunch while Haastrup and Aina (2019) harvested 45.15 g/bunch of *P. sajor-caju* on sawdust as a substrate.

This study produced lower mean yields for SD (26.0 g/bunch) which may be due to different environmental factors such as temperature and humidity when conducting the study. Sago frond combined with sawdust gave a high mean yield (20.39-29.36 g/bunch) compared to sago bark (19.17-21.66 g/bunch). As a singular substrate, SB and SF performance were similar to SD in which sago bark and frond residue are suitable as an alternative substrate to sawdust in terms of sporocarp yield per bag of *P. sajor-caju*. For substrate in combination, substrate ratio mixtures containing sago frond have a higher mean yield per bag than sago bark, which shows sago frond is suitable as a supplement to sawdust for mushroom cultivation.

The main purpose of mushroom cultivators is their yield. For this study, the sporocarp of *P. sajor-caju* (in three successive flushes) were collected from substrate bags (500 g). *Pleurotus sajor-caju* grown on various substrate ratio mixtures showed significant differences in the mushrooms harvested. There was no specific pattern in the mushroom yield from first, second, and third flushes. For singular substrate, SB and SF performance were comparable with SD in terms of total sporocarp yield. This showed that SB and SF have the potential as an alternative substrate to SD in term of total sporocarp yield. For mushroom cultivation using sawdust as a substrate, the total yield in this study is lower compared to the study by Ahmed et al. (2016), recorded a maximum yield of 199.70 g/bag (500 g substrate bag) for three harvests through the same cultivation technique which was similar with the present study.

Substrate ratio mixture containing singular or combined with sawdust of sago bark (56.60-64.99 g/bag) and sago frond (61.15-88.09 g/bag) also showed the same low pattern in mean yield for three consecutive harvests per bag. However, based on this study, substrate in combination with SD, SF has a higher potential than SB in fruiting bodies yield. This may be due to the chemical composition, which recorded higher lignin and cellulose content percentages in the substrate of singular and combination of SF compared to SB. As a singular substrate, SB and SF performance was similar to SD in which sago bark and frond residue are suitable as an alternative substrate to sawdust for *P. sajor-caju* mushroom cultivation. For substrate in combination, substrate ratio mixtures containing sago frond and sawdust have a higher total sporocarp yield per bag than sago bark, which shows sago frond is suitable as a supplement to sawdust for mushroom cultivation.

Biological efficiency is a way to calculate the effectiveness of a mushroom strain and substrate combination when growing mushrooms. Most of the time, substrates with higher yields produced higher BE. The higher the BE, the higher the substrate's applicability for mushroom cultivation as the substrate's BE indicates their applicability to support the growth of mushroom strain (Megersa et al., 2013). In general, BE of *P. sajor-caju* in the present research is much lower than the study by Ibrahim et al. (2015), which obtained 36.41% BE for grey oyster mushroom on SD using a similar technique. These results differed from the study by Pathmashini et al. (2008), in which the best biological yield for oyster mushroom was achieved for the substrate containing SD.

The variations in the yield and BE of *P. sajor-caju* fully grown on different substrate ratio mixtures are due to variations of the substrate's physical and chemical composition. From the results, it can be assumed that the substrate composition hugely influences the biological efficiency values obtained in mushroom production (Chang & Miles, 1982). As singular substrate, SB and SF performance were similar to SD in which it showed that sago bark and frond residue are suitable as an alternative substrate to sawdust in terms of sporocarp yield of *P. sajor-caju*. For substrate in combination, substrate ratio mixtures containing sago frond and sawdust have higher total sporocarp yield and BE per bag than sago bark, which shows sago frond is suitable as a supplement to sawdust for mushroom cultivation. Based on total yield and BE from this study, sago bark and frond residue have the potential as a substrate for grey oyster mushroom cultivation.

As the total yield and BE were lower than expected, several factors might contribute to the results. The substrate particle size may be a major factor affecting oyster mushrooms' growth in this study as the substrates used (sawdust, sago bark and frond) may have different

size and texture. Bellettini et al. (2015) stated that small particles (less than 0.5 cm) would compress the substrate, which will interfere with the aeration system for the utilization of microorganisms. Zhang et al. (2002) also found that oyster mushrooms' yield will decline when the straw ground into smaller particles. The low mean yields of *P. sajor-caju* observed in this study might be due to the small particle size of sago bark and frond used and mixed with larger particles size of sawdust, which disturbed the aeration of mycelium. Pandey et al. (2001) stated that the humidity in the mushroom house should be in the range of 70% to 90% to get maximum yield.

The humidity applied during the spawn running and mycelium running should be between 60% to 75% and 85% to 87% (Chang & Miles, 1992; Li et al., 2016). Ahmed et al. (2013) mentioned that the humidity of the mushroom house could be maintained by spraying water three times per day. However, in this study, even though the mushroom house was sprayed according to the suggested method, the humidity could not be maintained due to climate conditions, resulting in several mushroom bags producing minimal sporocarp yield for three harvests. The present study indicated that SB, SF and in combination with SD not affecting fruiting bodies yield and BE of *P. sajor-caju*. Further improvement on the particle's sizes of sago bark and sago frond residue to make it larger in size, alongside cultivation technique or environmental condition in the future study, can be made to show the potential of sago bark and frond residue as a substrate for *P. sajor-caju* cultivation.

5.6 Effect of Chemical Composition on Mycelia Growth and Sporocarp Yield of *P. sajor-caju*

Many factors affect cultivation productivity, such as the strain, type of substrate, type of spawn, moisture level, and different physicochemical conditions (Sánchez, 2010). Cellulose is the most important lignocellulosic content for mushroom cultivation (Adebayo & Martinez-Carrera, 2015). A high cellulose concentration in the substrate is needed to achieve a high yield of mushroom cultivation (Jeznabadi et al., 2016). Kholoud (2014) mentioned that mushroom grows on organic matters high in lignocellulosic content. A wide range of lignocellulosic substrates can be used, including sawdust, weed plants, molasses from the sugar industry, coconut shell, and cotton from the textile industry (de Mattos-Shipley et al., 2016).

Based on this study with comparison from previous study, sago bark and sago frond have an appropriate amount of lignocellulosic content. Sago bark and frond contained hemicellulose (22.7-60.7 wt%), lignin (9.9-40.7 wt%), and cellulose (20.5-46.2 wt%). Lignocellulosic composition is a major factor in selecting the substrates as the total yield and biological efficiency of sago bark and sago frond as singular substrate and in combination with sawdust were not significantly different from substrate contain only sawdust. The present finding indicated that SB and SF as cellulosic biowastes can be used as effective substrates singularly and when combined with SD for the cultivation of *P. sajor-caju* thus can be considered as an alternative substrate to sawdust for mushroom cultivation.

5.7 Mycelia Growth and Sporocarp Yield of *P. sajor-caju* Using Sago Bark and Frond as a Substrate

There was no specific pattern in mycelia texture, density, growth, pinhead formation duration, and the number of fruiting bodies that strong enough to indicated that one substrate ratio mixture is better than the other. However, significant differences were observed in the sporocarp yield and BE of *P. sajor-caju*. Studies by Alananbeh et al. (2014) showed that substrate made from a mixture of different ratio mixtures of lignocellulosic wastes provides advantages that affect several properties such as yield of the sporocarp and biological efficiency.

This study showed that the substrates and mixing ratio mixtures affected the mushroom's yield, biological efficiency, and morphological properties. For example, a substrate in a combination of 50SD:50SF increased yield and BE and resulted in improved morphological properties of *P. sajor-caju* compared to the singular substrate. For singular substrate, SB and SF are similar to SD in total yield and BE. As for the substrate combination, SF has a higher potential than SB, which can be seen in 75SD:25SF, 50SD:50SF, and 25SD:75SF as these substrates produced higher sporocarp yield and BE compared to SB substrate combination. It is also shown that when SD in combination with SF produced more yield than SD, which supports the above statement that 50SD:50SF gave better results, which is suitable for oyster mushroom cultivation. This shows that *P. sajor-caju* preferred sago frond as a substrate more than sago bark with sawdust combination. As a singular substrate, SB and SF performance in mycelia growth and sporocarp yield were similar with SD in which sago bark and frond are suitable as an alternative substrate to sawdust for *P. sajor-caju* cultivation. For substrate in combination, substrate ratio mixtures containing sago frond and sawdust have better performance than sago bark, which shows

sago frond is suitable as a supplement to sawdust for grey oyster mushroom cultivation. The findings indicated that sago bark and frond could be an alternative substrate to sawdust for grey oyster mushroom cultivation.

5.8 Chapter Summary

The results obtained in this chapter prove that SB and SF, in terms of mycelia growth and sporocarp yield, can be an alternative substrate to sawdust for grey oyster mushroom cultivation as the performance of substrate (sago bark and frond) in singular or combination were similar with sawdust. The present finding indicated that SB and SF as cellulosic biowastes could be used as effective substrates singularly and in combination with SD for the cultivation of *P. sajor-caju* thus can be considered an alternative substrate to sawdust for mushroom cultivation.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has successfully revealed the major chemical and lignocellulosic content of sago bark and sago frond, which were suitable for the cultivation of grey oyster mushrooms. The values are in the range of lignocellulosic needed for substrate in *P. sajor-caju* mushroom cultivation. The high cellulose content in all the substrates will ensure the mycelia of *P. sajor-caju* grow successfully and obtain the essential nutrients for its growth. Therefore, utilising these lignocellulosic wastes as substrates for mushroom production would reduce the detrimental environmental effects of these waste products.

Based on the results, singular substrate SB and SF have comparable performance to SD as a control. As for substrate combination, the substrate mixtures of SD with SF have better mycelia growth and sporocarp yield of *P. sajor-caju* grown compared to only SD and SB. The evaluation of SB and SF at different ratio mixtures give positive results, where the ratio mixture with a high amount of SB and SF can effectively produce sporocarp. Sago bark (100SB) and frond (100SF) in singular substrate have the potential to be utilized as one of the substitutional substrates to replace sawdust in the cultivation of *P. sajor-caju*. For substrate combination, 50SD:50SF is suitable as a supplement to sawdust for *P. sajor-caju* mushroom cultivation The present study also indicated that SB and SF could be used and further developed for local growers' mushroom cultivation. In addition, these residues as a substrate can be used as alternatives to wood sawdust which would help to reduce the adverse environmental effects of these products. Furthermore, an economical strategy for turning waste products into a beneficial food source is represented in this study. Hence, the ability

of mushrooms such as *P. sajor-caju* as a high potential converter of cheap celluloses wastes like sago bark and frond into valuable protein should be taken advantage of in search of minimizing environmental pollution.

6.2 Recommendations

Some improvements are recommended for future studies to achieve consistency and more desirable results. The mushroom house for oyster mushroom cultivation needs to be placed in an area with less light source as the weather condition in Malaysia is hot and humid. In addition, a misting system should be installed in the mushroom house to give a constant and fixed humidity for the mushroom.

It is recommended that further study is needed to analyse the nutritional content of fruiting bodies produced from these substrates since the nutrient composition may depend on the fruiting substrate composition. The analysis of heavy metals and toxic chemicals could also be done to ensure that the sago residues are safe to be utilized as fruiting substrates.

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APPENDICES

Appendix 1: Hemicellulose, lignin and cellulose in Substrate ratio mixtures of sawdust, sago bark and sago frond (in wt%)

Substrate Ratio	Hemicellulose (wt %)	Lignin (wt %)	Cellulose (wt %)
100SD	26.5 ± 0.4^{a}	25.9 ± 0.6^{a}	44.7 ± 0.4^{a}
100SB	60.7 ± 1.0^{e}	9.9 ± 0.7^{d}	23.6 ± 0.9^{cd}
100SF	46.1 ± 1.3^{d}	17.1 ± 2.4^{e}	27.6 ± 0.2^{d}
75SD 25SB	24.1 ± 0.7^{ab}	30.3 ± 0.4^{b}	36.9 ± 0.4^{b}
50SD 50SB	34.3 ± 0.1^{c}	34.7 ± 0.5^{c}	22.2 ± 0.3^{c}
25SD 75SB	45.6 ± 0.6^{d}	25.8 ± 0.3^{a}	20.5 ± 0.4^{c}
75SD 25SF	22.7 ± 0.6^{b}	24.1 ± 0.2^{a}	46.2 ± 0.6^{a}
50SD 50SF	26.0 ± 1.7^{ab}	40.7 ± 0.5^{f}	25.6 ± 1.4^{d}
25SD 75SF	34.2 ± 0.5^{c}	32.8 ± 0.5^{bc}	24.2 ± 0.9^{cd}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Tukey HSD's mean separation test.

Appendix 2: Total colonization period in substrate ratio mixtures per batch (in days)

Substrate Ratio	Batch 1	Batch 2	Batch 3	Mean
100SD	23.67	25.33	24.33	24.44 ± 0.84^{a}
100SB	30.33	29.67	26.33	28.78 ± 2.14^{b}
100SF	28.00	28.00	26.67	27.56 ± 0.77^{ab}
75SD 25SB	24.67	26.00	24.33	25.00 ± 0.89^{a}
50SD 50SB	27.67	25.33	28.67	27.22 ± 1.71^{ab}
25SD 75SB	26.00	28.67	27.33	27.33 ± 1.34^{ab}
75SD 25SF	24.67	24.67	25.00	24.78 ± 0.19^{a}
50SD 50SF	25.00	27.67	26.67	26.45 ± 1.35^{ab}
25SD 75SF	26.00	27.33	26.67	26.67 ± 0.67^{ab}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Tukey HSD's mean separation test.

Appendix 3: Duration of first harvest in substrate ratio mixtures per batch (in days)

Substrate Ratio	Batch 1	Batch 2	Batch 3	Mean
100SD	51.00	53.30	46.70	50.33 ± 3.35^{a}
100SB	67.00	71.30	77.30	71.86 ± 5.17^{b}
100SF	65.00	68.70	68.30	67.33 ± 2.03^{bc}
75SD 25SB	58.70	60.70	59.00	59.46 ± 1.08^{ac}
50SD 50SB	54.30	57.70	49.70	53.90 ± 4.01^{a}
25SD 75SB	63.30	62.70	63.30	63.10 ± 0.35^{bc}
75SD 25SF	66.70	69.30	66.30	67.43 ± 1.63^{c}
50SD 50SF	59.70	66.00	51.30	59.00 ± 7.37^{ac}
25SD 75SF	67.70	69.00	70.30	69.00 ± 1.30^{bc}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Tukey HSD's mean separation test.

Appendix 4: Mean cap diameter of fruiting bodies yield per batch (in cm)

Substrate Ratio	Batch 1	Batch 2	Batch 3	Mean
100SD	7.3	8.4	6.2	7.3 ± 1.10^{a}
100SB	8.1	9.0	8.0	8.4 ± 0.55^{b}
100SF	5.5	9.5	6.9	7.3 ± 2.23^{a}
75SD 25SB	7.7	8.5	7.4	7.9 ± 0.57^{a}
50SD 50SB	8.7	6.5	7.4	7.5 ± 1.11^{a}
25SD 75SB	8.5	7.3	7.3	7.7 ± 0.69^{a}
75SD 25SF	6.2	6.8	7.1	6.7 ± 0.46^{a}
50SD 50SF	9.1	8.0	7.1	8.1 ± 1.00^{a}
25SD 75SF	6.9	6.2	7.5	6.9 ± 0.65^{a}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Tukey HSD's mean separation test.

Appendix 5: Mean stipe length of fruiting bodies yield per batch (in cm)

Substrate Ratio	Batch 1	Batch 2	Batch 3	Mean
100SD	6.7	7.2	5.8	6.6 ± 0.71^{a}
100SB	6.6	8.2	7.6	7.5 ± 0.81^{a}
100SF	6.0	8.4	5.6	6.7 ± 1.51^{a}
75SD 25SB	6.6	5.9	6.7	6.4 ± 0.44^{a}
50SD 50SB	8.3	5.9	6.4	6.9 ± 1.27^{a}
25SD 75SB	7.3	6.3	6.7	6.8 ± 0.50^{a}
75SD 25SF	5.3	6.3	6.8	6.1 ± 0.76^{a}
50SD 50SF	6.6	7.0	6.2	6.6 ± 0.40^{a}
25SD 75SF	6.2	5.7	6.3	6.1 ± 0.32^{a}

Significant at 0.05 level in ANOVA; mean values with the same lower-case letters are not significantly different according to Tukey HSD's mean separation test.

Appendix 6: Mean fruiting bodies yield per batch (in individual flush and in three harvests)

Appendix 6.1: 100SD

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body (g)	19.18	20.70	30.62	33.54	52.49	27.40	20.66	16.84	10.99
Second Harvest /Total fruiting body (g)	12.77	48.31	19.17	18.42	19.86	17.93	20.51	19.25	18.99
Third Harvest/Total fruiting body (g)	44.41	14.04	19.07	43.42	20.66	36.47	18.36	42.43	35.48
Average weight	25.45	27.68	22.95	31.79	31.00	27.27	19.84	26.17	21.82
Total Weight	76.36	83.05	68.86	95.38	93.01	81.80	59.53	78.52	65.46
Total Mean Weight	76.09			90.06			67.84		
Mean weight	25.36			30.02			22.61		

Appendix 6.2: 100SB

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	15.27	24.31	37.89	34.10	23.44	13.70	34.66	16.49	17.85
Second Harvest /Total fruiting body	12.40	16.74	12.28	19.46	40.36	20.85	17.45	16.71	22.80
Third Harvest/Total fruiting body	22.68	24.60	38.34	19.37	17.09	15.34	21.00	14.18	15.59
Average weight	16.78	21.88	29.50	24.31	26.96	16.63	24.37	15.79	18.75
Total Weight	50.35	65.65	88.51	72.93	80.89	49.89	73.11	47.38	56.24
Total Mean Weight	68.17			67.90			58.91		
Mean weight	22.72			22.63			19.64		

Appendix 6.3: 100SF

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	35.89	14.93	31.29	18.40	13.95	19.03	10.93	21.85	23.28
Second Harvest /Total fruiting body	14.08	28.44	10.64	17.22	17.09	26.37	42.56	29.61	20.94
Third Harvest/Total fruiting body	14.89	17.33	21.53	19.26	48.50	28.16	14.20	34.33	14.20
Average weight	21.62	20.23	21.15	18.29	26.51	24.52	22.56	28.60	19.47
Total Weight	64.86	60.70	63.46	54.88	79.54	73.56	67.69	85.79	58.42
Total Mean Weight	63.01			69.33			70.63		
Mean weight	21.00			23.11			23.54		

Appendix 6.4: 75SD 25SB

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	10.30	32.48	21.12	40.57	20.37	17.76	12.96	18.41	17.44
Second Harvest /Total fruiting body	40.25	11.76	10.24	13.94	18.02	18.82	17.75	13.82	17.45
Third Harvest/Total fruiting body	19.67	29.91	19.42	19.34	10.66	16.43	10.88	24.95	12.65
Average weight	23.41	24.72	16.93	24.62	16.35	17.67	13.86	19.06	15.85
Total Weight	70.22	74.15	50.78	73.85	49.05	53.01	41.59	57.18	47.54
Total Mean Weight	65.05			58.64			48.77		
Mean weight	21.69			19.55			16.26		

Appendix 6.5: 50SD 50SB

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	13.76	26.03	18.09	28.36	11.44	10.59	44.96	21.15	15.33
Second Harvest /Total fruiting body	19.56	19.25	31.74	18.86	12.05	18.99	48.49	10.66	16.92
Third Harvest/Total fruiting body	14.32	17.30	15.54	13.93	14.35	22.65	28.77	16.77	19.65
Average weight	16.94	18.28	23.64	16.40	13.20	20.82	38.63	13.72	18.29
Total Weight	47.64	62.58	65.37	61.15	37.84	52.23	122.22	48.58	51.90
Total Mean Weight	58.53			50.41			74.23		
Mean weight	19.62			16.81			23.55		

Appendix 6.6: 25SD 75SB

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	25.05	23.50	21.77	19.94	15.29	22.10	17.88	16.32	18.81
Second Harvest /Total fruiting body	17.30	10.61	17.25	21.08	15.12	12.10	12.45	26.45	18.55
Third Harvest/Total fruiting body	19.18	16.82	19.81	29.81	13.94	30.18	23.81	29.29	17.51
Average weight	20.51	16.98	19.61	23.61	14.78	21.46	18.05	24.02	18.29
Total Weight	61.53	50.93	58.83	70.83	44.35	64.38	54.14	72.06	54.87
Total Mean Weight	57.10			59.85			60.36		
Mean weight	19.03			19.95			20.12		

Appendix 6.7: 75SD 25SF

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	16.37	32.45	16.54	37.94	23.67	25.70	13.35	15.97	13.72
Second Harvest /Total fruiting body	14.32	26.63	11.56	16.64	29.39	19.96	27.38	22.63	31.82
Third Harvest/Total fruiting body	27.28	43.16	34.76	39.33	17.21	19.28	16.45	18.69	13.78
Average weight	19.32	34.08	20.95	31.30	23.42	21.65	19.06	19.10	19.77
Total Weight	57.97	102.24	62.86	93.91	70.27	64.94	57.18	57.29	59.32
Total Mean Weight	74.36			76.37			57.93		
Mean weight	24.78			25.46			19.31		

Appendix 6.8: 50SD 50SF

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	28.58	32.22	20.35	28.89	54.73	46.33	38.30	44.76	56.00
Second Harvest /Total fruiting body	12.40	53.38	17.09	32.05	35.57	39.99	41.41	32.25	26.73
Third Harvest/Total fruiting body	19.28	12.18	15.01	19.05	20.74	17.59	16.27	17.87	13.78
Average weight	20.09	32.59	17.48	26.66	37.01	34.64	31.99	31.63	32.17
Total Weight	60.26	97.78	52.45	79.99	111.04	103.91	95.98	94.88	96.51
Total Mean Weight	70.16			98.31			95.79		
Mean weight	23.39			32.77			31.93		

Appendix 6.9: 25SD 75SF

Bag	Trial 1			Trial 2			Trial 3		
First Harvest/Total fruiting body	19.09	13.96	17.72	17.72	14.79	18.62	37.04	12.19	12.79
Second Harvest /Total fruiting body	17.64	10.39	22.74	10.90	11.57	15.89	13.11	21.72	19.03
Third Harvest/Total fruiting body	34.72	26.44	54.26	40.82	16.21	19.27	26.59	10.22	14.94
Average weight	23.82	16.93	31.57	23.15	14.19	17.93	25.58	14.71	15.59
Total Weight	71.45	50.79	94.72	69.44	42.57	53.78	76.74	44.13	46.76
Total Mean Weight	72.32			55.26			55.88		
Mean weight	24.11			18.42			18.63		

Appendix 7: Biological Efficiency (BE) per batch in each substrate ratio mixtures

Ratio	Trial 1			Trial 2			Trial 3			BE
Yield	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	
100SD	76.36	83.05	68.86	95.38	93.01	81.80	59.53	78.52	65.46	15.60
BE %	15.27	16.61	13.77	19.08	18.60	16.36	11.91	15.70	13.09	
75SD 25SB	70.22	74.15	50.78	73.85	49.05	53.01	41.59	57.18	47.54	11.50
BE%	14.04	14.83	10.16	14.77	9.81	10.60	8.32	11.44	9.51	
50SD 50SB	47.64	62.58	65.37	61.15	37.84	52.23	122.22	48.58	51.90	12.21
BE	9.53	12.52	13.07	12.23	7.57	10.45	24.44	9.72	10.38	
25SD 75SB	61.53	50.93	58.83	70.83	44.35	64.38	54.14	72.06	54.87	11.82
BE%	12.31	10.19	11.77	14.17	8.87	12.88	10.83	14.41	10.97	
100SB	50.35	65.65	88.51	72.93	80.89	49.89	73.11	47.38	56.24	13.00
BE%	10.07	13.13	17.70	14.59	16.18	9.98	14.62	9.48	11.25	
75SD 25SF	57.97	102.24	62.86	93.91	70.27	64.94	57.18	57.29	59.32	13.91
BE%	11.59	20.45	12.57	18.78	14.05	12.99	11.44	11.46	11.86	
50SD 50SF	60.26	97.78	52.45	79.99	111.04	103.91	95.98	94.88	96.51	17.62
BE%	12.05	19.56	10.49	16.00	22.21	20.78	19.20	18.98	19.30	
25SD 75SF	71.45	50.79	94.72	69.44	42.57	53.78	76.74	44.13	46.76	12.23
BE%	14.29	10.16	18.94	13.89	8.51	10.76	15.35	8.83	9.35	
100SF	64.86	60.70	63.46	54.88	79.54	73.56	67.69	85.79	58.42	13.53
BE%	12.97	12.14	12.69	10.98	15.91	14.71	13.54	17.16	11.68	

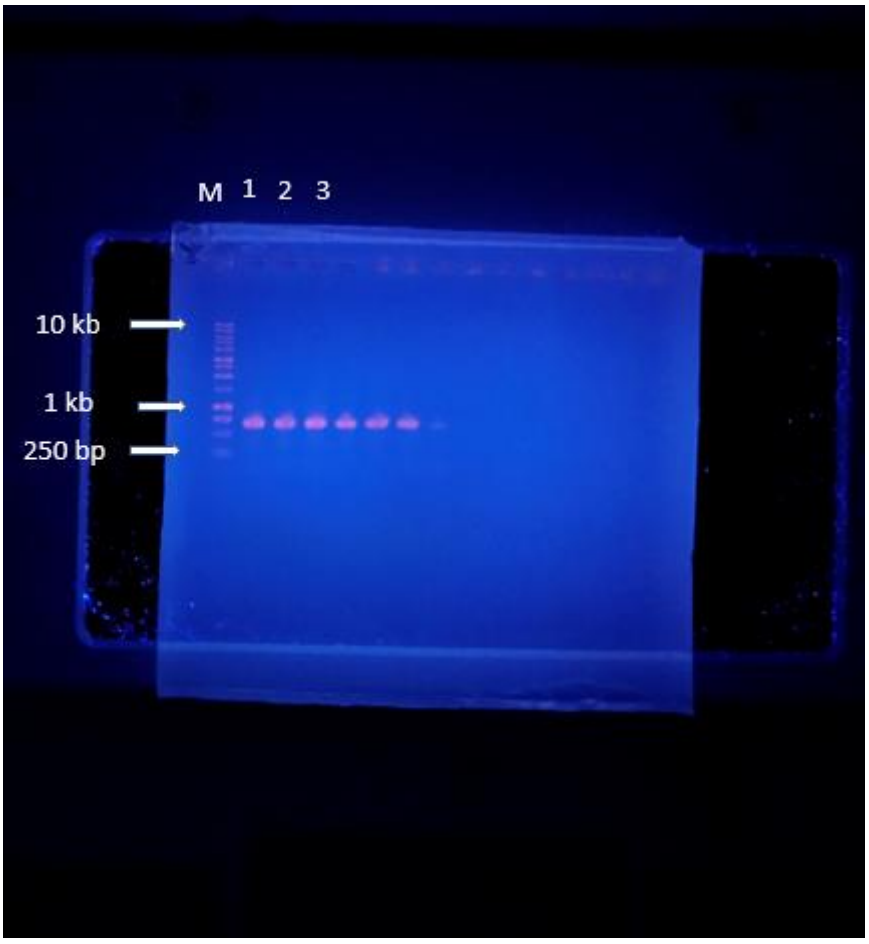
Appendix 8: Grade requirements for fresh oyster mushroom

Grade	Requirements	Torelances
Premium	Oyster mushroom in this class shall be uniform in size and maturity, fresh, clean and free from any deform and damage. Oyster mushroom of this grade shall meet those of Grade 1.	Fresh ≤ 5 % Uniform size ≤ 5 % Uniform maturity ≤ 5 % Deformity ≤ 5 % Damage ≤ 3 % Maximum 5 % by number or weight shall be allowed in each package of produce.
1	Oyster mushroom in this class shall be uniform in size and maturity, fresh, clean and practically free from any deform and damage. Oyster mushroom of this grade shall meet those of Grade 2.	Fresh ≤ 5 % Uniform size ≤ 10 % Uniform maturity ≤ 5 % Deformity ≤ 10 % Damage ≤ 5 % Maximum 15 % by number or weight shall be allowed in each package of produce.
2	Oyster mushroom in this class shall be uniform in size and maturity, fresh, clean and reasonably free from any deform and damage.	Fresh ≤ 10 % Uniform size ≤ 20 % Uniform maturity ≤ 10 % Deformity ≤ 10 % Damage ≤ 5 % Maximum 25 % by number or weight shall be allowed in each package of produce.

Appendix 9: Size classification for fresh oyster mushroom

Size code		Diameter (mm)
1	S	< 40
2	M	40 to 80
3	L	> 80

Appendix 10: Gel viewing of molecular identification



Appendix 11: Molecular Identification were identified by the basic local alignment search tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Pleurotus sajor-caju strain S048 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: AY540328.1 Length: 694 Number of Matches: 1

See 1 more title(s) See all Identical Proteins(IPG)

Range 1: 1 to 655 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1199 bits(649)	0.0	654/656(99%)	2/656(0%)	Plus/Minus

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Query  7    CTACCTGATTTGAGGTCAA-TTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTC  65
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  655  CTACCTGATTTGAGGTCAAATTGTCAAATTGTCCTTGCGGACGATTAGAGAGCTGGACTC  596

Query  66   TATTCATGCGTGCTATTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAG  125
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  595  TATTCATGCGTGCTATTGATGAGTGATAATTATCACATCATGCGCAGAGGCAATGAGAAG  536

Query  126  TCCTGCTAATGCATTTAAGAGGAGCCGACTTGTTACAGCCAGCAACCCCCAACAATCCAA  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  535  TCCTGCTAATGCATTTAAGAGGAGCCGACTTGTTACAGCCAGCAACCCCCAACAATCCAA  476

Query  186  ACATCACAAATAAATGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGG  245
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Sbjct  475  ACATCACAAATAAATGTGAGTTTGAGAATTTAATGACACTCAAACAGGCATGCCCCTCGG  416

Query  246  AATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA  305
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Sbjct  415  AATACCAAGGGGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCA  356

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Sbjct  355  CATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTT  296

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Sbjct  295  GAAAGTTGTATTATGGTTTATAGGCACAAGGCCCATTAAATGACATTCGTAGACATACAT  236

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Sbjct  235  TTGGGGTGTGTTAAGTAAATAGACTGCGTTGTCACACCGAGACGTTTAAATCCCAGCAAA  176

Query  486  CCAAGTCTGACGACTTGAAGGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGA  545
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Sbjct  175  CCAAGTCTGACGACTTGAAGGACGACTTCACAGATCTATCAAAAGTTCACAGGTGGTTGA  116

Query  546  AAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCAT  605
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Sbjct  115  AAGACTAGTGAAGCGTGCACATGCCCCTAGAGGCCAGCAACAACTCCATAGTGAATTCAT  56

Query  606  TAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTTTACTTCCA  661
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Sbjct  55   TAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGACTTTT-ACTTCCA  1
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