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Potential Enhancement in The Nutritional Value of Local Agro-Waste Through Cultivation of Pink Oyster Mushroom (*Pleurotus djamor*)

A. L. R. Hutabarat, W. M. Lestari*, A. Kuswoyo, A. M. Ali, and I. Sari

Feed Technology Study Program, Politeknik Negeri Tanah Laut,
Jalan A. Yani KM. 6.0, Desa Panggung, Kec. Pelaihari, Kab. Tanah Laut,
Provinsi Kalimantan Selatan, Indonesia 70815
*Corresponding Author: wennimeika@politala.ac.id

ABSTRACT

The present study aimed to examine the effect of locally available agro-industrial wastes on the growth and productivity of pink oyster mushrooms (*Pleurotus djamor*) and the potential use of substrates as animal feeding based on its compositional change following mushroom growth. A total of three substrate formulations labeled OPF (80% oil palm frond), CS (80% corn straw), and OPFCS (40% oil palm frond + 40% corn straw) were prepared. Aside from the earliest mycelium completion, pinhead formation, and fruiting bodies maturation, CS and OPFCS exhibit the best total yield, dry weight, and biological Efficiency. On the contrary, *P. djamor* grown in OPF resulted in the minimum in most of the parameters measured. Therefore, CS, single and in combination with OPF could be utilized as an alternative growing media for the cultivation of *P. djamor*. The analysis of chemical compositions showed that the growth of *P. djamor* significantly reduced the crude fiber (CF) and increased the crude protein (CP) content of the mycelium-treated substrate. In contrast, THE CP content of the spent is unchanged or relatively lower than that of the initial substrate. It can be concluded that compared to the spent, mycelium-treated substrate had higher values of being used for animal feeding.

Keywords: *Pleurotus djamor*, oil palm frond, corn straw, chemical composition

INTRODUCTION

Among edible mushrooms, oyster mushrooms are one of the most commercially produced in the world due to their nutritional benefit and ability to grow on a large variety of agricultural and forestry residues (Sanli and Peksena, 2020). These advantages provide growers with various local agroforestry residues to use as substrates. Previous studies have shown the feasibility of oil palm fronds and corn straws to support the growth of oyster mushrooms (Ali et al., 2018; Ibrahim et al., 2015). In South Kalimantan, oil palm and corn are among the most important agricultural commodities. However, recycling biomass as the growth media for mushroom cultivation is still not well recognized by the local community, and most parts of the plant are left to decompose in its habitat.

As well as providing plant-based protein, mushroom production generates a large amount of spent substrate. The spent substrate can be utilized for ruminant feeding due to the improvement of the nutritional value of the fungal-treated substrate (Fazaeli et al., 2004; Mhlongo et al., 2021; Tuyen et al., 2013). Mycelium secretes three types of cell wall-degrading enzymes, such as cellulolytic and lignin-degrading enzymes, during the vegetative and generative growth of the mushroom (Kucharska et al., 2018). These enzymes break complex compounds, resulting in lower substrates' crude fiber content (Akinfemi and Ogunwole, 2012; Mhlongo et al., 2021). In addition, research indicates that mushroom cultivation may increase the protein content of agricultural wastes (El-Rahman et al., 2014; Shrivastava et al., 2011) because of mycelia enzymes secreted into the substrate during fermentation (Sallam et al., 2007).

The purpose of our work was to evaluate the growth and productivity of *P. djamor* in on local agriculture waste biomass (oil palm frond, corn straw, and the mixture of oil palm frond and corn straw) as well as the potential use of substrates as feedstuff based on its compositional change following mushroom growth. We hypothesize that i) these locally available agricultural waste is suitable for *P. djamor* production, ii) *P. djamor* cultivation improve the feeding value of low-quality substrates by increasing the protein concentration and reducing the crude fiber content of the agricultural waste. To test this, we measured the effect of the substrate on the growth and productivity of *P djamor* and evaluated the chemical composition of substrates during mushroom growth.

MATERIALS AND METHODS

Substrate preparation, mushroom cultivation, and experimental design

Pleurotus djamor spawn was used for this study obtained online from a mushroom farmer unit in Sumedang, West Java. The experiments conducted in the Laboratory of Feed Technology Politeknik Negeri Tanah Laut started from August to October 2022. Oil palm frond (OPF) and corn straw (CS) were used as basal substrates obtained from Bajuin District. Three treatments of growing substrates used in this experiment: OPF (80% oil palm frond), CS (80% corn straw), and OPFCS (40% oil palm frond + 40% corn straw). The basal substrates were soaked in water for moisture absorption. Then the excess water was drained to 60-70% of moisture. Each formulation was enriched with 18% rice bran and 2% limestone (Table 1). A 500 g of each formulation was then packed and compressed in 18 cm x 35 cm polyethylene bags. Ten bags were prepared as replicates for each treatment. The bags filled with the media mixtures were taken to the steamer and pasteurized at 100°C for 3 hours to avoid microbial contamination. After sterilization, the bags were left to cool for 24 h and inoculated with about 5 g of spawn under aseptic conditions. The inoculated bags were transferred into the incubation room, with indirect sunlight incubated at 27-30°C and 80-85% relative humidity for mycelium running. During the fruiting stage, the openings of the bags were opened when the mycelia had covered all the substrate. The harvesting was done based on the maturation of the fruiting bodies.

Chemical analysis of substrate

The dried samples of the initial substrate, mycelium-treated substrate, and spent substrates were analyzed for dry matter (DM), organic matter (OM), crude fiber (CF), crude protein (CP), and ether extract (EE) according to AOAC (1990) standard method. The change of chemical composition in the substrate during the mushroom growth was expressed as "loss (%)" (Figure 1). The loss of nutrients in mycelium-treated substrates (MS) was determined concerning initial substrates. The loss of nutrients in spent substrates (MS) determined concerning initial substrates

Assessment of cultivation parameters

The growth and productivity performance of the cultivated *P. djamor* was determined using the following parameters: Mycelium completion period: number of days from inoculation until complete mycelium colonization on the substrate; Pinhead formation period: days to first sprouting of mushrooms; Fruiting bodies maturation period: the time elapsed between the day of inoculation and the day of the first harvest; Total fresh weight: the accumulation of fruiting bodies weights; Total dry fruiting bodies weight: the weight of oven-dried fruiting bodies; Biological Efficiency (BE) = (Total fresh weight of mushroom(g) × 100)/ Dry weight substrate (g).

Statistical analysis

The growth, productivity, and nutrient content of substrates parameters were arranged as a complete randomized design. The results were subjected to analysis of variance (ANOVA) followed by Duncan's t-test at a 5% probability. Statistical data analysis was carried out with SAS statistical program for Windows (version 9.0, SAS Institute Inc., Cary, NC, USA).

Table 1. Substrate formulation based on local agricultural waste

Code	Oil Palm Frond (%)	Corn Straw (%)	Rice Bran (%)	Limestone (%)	Total
OPF	80	0	18	2	100
CS	0	80	18	2	100
OPFCS	40	40	18	2	100

OPF: Oil Palm Frond-based substrate; CS: corn straw-based substrate; OPFCS: Mixture of oil palm frond and corn straw-based substrate

RESULT AND DISCUSSION

Growth of *P. djamor*

Mushroom cultivation involves three important stages: the completion of the mycelium, the formation of pinheads, and the maturation of the fruiting bodies (Raymond et al., 2013). Table 2 shows that these stages were completed within 27.96 days, 39.03 days, and 43.33 days, respectively.

The substrate formulations significantly ($P<0.05$) affected the mycelium completion, pinhead initiation, fruiting bodies maturation, and the total cultivation period of *P. djamor* (Table 2). Duncan's Multiple Range Test (DMRT) showed that the F3 and F2 significantly ($P<0.05$) took a shorter period (24.4 and 26.2 days, respectively) compared to F1 (33.2 days). Corresponds to mycelium completion, the F3 media also took the shortest time for pinhead formation (35.00 days), and fruiting bodies maturation (43.33 days), although not significantly different compared to F2. F1 media took the longest ($P<0.05$) period on mycelium completion, pinhead initiation, and fruiting bodies maturation (Table 2).

The growth of mycelium on OPF media in this study was slower than those recorded from a 500 g mixture of oil palm frond and rice bran that took 31.60 and 40.80 days for mycelium completion and fruiting bodies maturation, respectively (Ibrahim et al., 2015). Compared to OPF, the combination of OPF and MS in the present study significantly increased the oyster mushroom growth by 26%. Zárate-Salazar et al. (2020a) reported that compared to rice straw, corn stubble accelerated the mycelium growth and fruiting bodies maturation by 18% and 12%, respectively. Previous research showed an increase in mycelium run when two or more substrates were combined (Baysal et al., 2003; Mane et al., 2007; Owaid et al., 2016). The growth rate of mycelium is affected by substrate chemical composition, plant structure, origin, particle size, and the presence of polyphenols (Jonathan et al., 2012; Zárate-Salazar et al., 2020).

Productivity of *P. djamor*

Mature fruiting bodies were collected and weighed immediately after harvest. As shown in table 3, the media formulation significantly ($P<0.05$) affected the fresh and dry weight of the fruiting bodies, as well as the biological Efficiency.

Table 2. Effect of different media formulations on the growth of *P. djamor*

Code	Mycelium Completion (Days)	Pinhead Formation (Days)	Fruiting Bodies Maturation (Days)
OPF	$33,2 \pm 1.75^{a}$	$45,2 \pm 2.15^{a}$	49.6 ± 2.06^{a}
CS	$26,2 \pm 2.78^{b}$	36.8 ± 2.47^{b}	41.1 ± 2.51^{b}
OPFCS	24.4 ± 1.89^{b}	35.00 ± 2.14^{b}	39.3 ± 2.36^{b}
Average	27.96	39.03	43,33
SEM	4.80	4.86	5.38
p Value	<.0001	<.0001	<.0001

Means on the same row with different superscripts are significantly varied ($P < 0.05$). SEM = standard error of the mean. OPF: Oil Palm Frond-based substrate; CS: corn straw-based substrate; OPFCS: Mixture of oil palm frond and corn straw-based substrate

Table 3. The effect of different media formulations on the productivity of *P. djamor*

Code	Fresh Weight of Fruiting Bodies (g)	Dry Weight of Fruiting Bodies (g)	Biological Efficiency (%)
OPF	36.27 ± 2.44^{c}	3.56 ± 0.24^{b}	13.49 ± 0.77^{b}
CS	47.94 ± 1.23^{a}	5.42 ± 0.35^{a}	15.16 ± 1.17^{a}
OPFCS	44.89 ± 2.23^{b}	5.23 ± 0.40^{a}	15.86 ± 0.99^{a}
Rata-rata	43.04	4.73	14.84
SEM	4.17	0,11	0.98
p Value	<.0001	<.0001	<.0001

Means on the same row with different superscripts are significantly varied ($P < 0.05$). SEM = standard error of the mean. OPF: Oil Palm Frond-based substrate; CS: corn straw-based substrate; OPFCS: Mixture of oil palm frond and corn straw-based substrate.

The highest productivity of *P. djamor* was obtained from CS and OPFCS (Table 3) with 15.16 – 15.86% of BE. As per the findings of this study, the cultivated oyster mushroom was designed to only be harvested once. Thus, the bags from all treatments have not reached their maximum number of flushes. According to Elenwo and Okere (2007), a short production cycle could reduce the BE. Cultivation of *P. ostreatus* on corn cobs resulted in 31.7% BE from three mushroom flushes (Koutrotsios et al., 2014). The BE of *Pleurotus spp* on different oil palm biomass has been reported by previous studies. The combination of OPF and rubber tree sawdust resulted in 11.42 – 20.89% BE (Ali et al., 2018), compared to 4.6 – 11.3% on a combination of oil palm empty fruit bunch (EFB) and rubber tree sawdust (Mohd Tabi et al., 2008).

Chemical composition of the substrate

As shown in table 4, the nutrient content varied among all three substrates during mushroom growth. Cultivation of *P. djamor* affected the DM and OM content of the substrates significantly (P<0.05). The DM and OM contents decreased throughout the cultivation of *P. djamor* due to the utilization of soluble and structural carbohydrates in the substrate by fungal cells, which was also reflected in the reduction of cell wall constituents (Li et al., 2001). However, an experiment by Montañez-Valdez et al. (2015) did not find significant changes in DM after 53 days of cultivation of *P. djamor* on corn stover.

Mushroom treatments have been shown to reduce fiber constituents in substrates (Koutrotsios et al., 2014; Shrivastava et al., 2014). Incubation of *P. djamor* significantly (P<0.05) reduces the CF content of OPF, CS, and OPFCS by 13%, 18%, and 15% during the mycelium run, and another 21%, 31%, and 26% loss (P<0.05) during the fruiting bodies maturation (Fig 1). According to Li et al. (2001), cellulose degradation rates were faster during the fruiting bodies' maturation than the mycelium run due to the rapid development of mushroom fruiting bodies, which led to a greater requirement for energy and carbon sources within a short period. Solubilization of plant cell walls during mushroom growth improved the soluble-insoluble carbohydrate ratio (Fazaeli et al., 2004; Yilkal, 2015), thereby enhancing rumen microbes' ability to absorb nutrients Khattab et al. (2013).

Compared to the initial substrate, a significant (P<0.05) increase of 114% and 139% in CP content was observed on mycelium-treated OPF and OPFCS, respectively (Fig 1C). However, their spent CP values appeared to be relatively lower (P>0.05) than those obtained from the initial substrate due to 56% and 72% loss of CP content after mushroom production (Fig 1C). Similarly, the production of *P. pulmonarius* (Fr.) Quél. Reduce CP content of cotton waste, *Cymbopogan citratus*, and leaves of *Pannicum maximum* by 12%, 46%, and 22%, respectively (Silva et al., 2002).

Table 4. Effects of mushroom growth on the nutritional content of substrates

Code	Type of Substrate	Parameter (%)				
		DM	OM	CF	CP	EE
OPF	IS	90,10 ± 0,39^{a}	80.03 ± 1.31^{a}	52.49 ± 1.77^{a}	1.11 ± 0.27^{a}	1.61± 0.27
	MS	89,58 ± 0,38^{a}	80.19 ± 0.83^{a}	45.66 ± 1.57^{b}	2.39 ± 0.53^{b}	5.89 ± 0.32
	SS	87.68 ± 0,84^{b}	77.54 ± 1.68^{b}	35.86 ± 1.06^{c}	1.05 ± 0.16^{a}	0,88± 0.15
	P value	<.0001	0.0341	<.0001	<.0001	<.0001
	SEM	0.23	1.75	2.25	0.13	0.068
CS	IS	90.77 ± 0.78^{a}	82.06 ± 1.98^{a}	52.15 ± 2.54^{a}	0.89 ± 0.08^{a}	1.30 ± 0.14
	MS	86.75 ± 3.38^{b}	77.29 ± 2.07^{b}	42.70 ± 1.54^{b}	1.50 ± 0,56^{a}	5.67 ± 0.15
	SS	85.45 ± 0.83^{b}	75.00 ± 1.05^{b}	29.17 ± 1.60^{c}	1.15 ± 0,30^{a}	0.75 ± 0.35
	P value	<.0001	0.0009	<.0001	0.1227	<.0001
	SEM	4.24	3.31	3.80	0.14	0.053
OPFCS	IS	90.48 ± 0.74^{a}	81.29 ± 0.82^{a}	55.59 ± 2.23^{a}	0.10 ± 0.21^{a}	0.72± 0.26
	MS	90.13 ± 0.18^{a}	81.10 ± 0.37^{a}	47.09 ± 2.11^{b}	2.39 ± 0.55^{b}	4.25± 0.16
	SS	84.04 ± 0.32^{b}	75.69 ± 0.57^{b}	34.42 ± 0.49^{c}	0.66 ± 0.51^{a}	0.77 ± 0.34
	P value	<.0001	<.0001	<.0001	0.0010	<.0001
	SEM	0.34	0.38	3.23	0.20	0.070

Means on the same row with different superscripts are significantly different (P < 0.05). OPF: Oil palm frond-based substrate; CS: Corn stover-based substrate; OPFMZ: Combination of oil palm frond and corn stover-based substrate. IS : Initial Substrate; MS: Mycelium treated substrate; SS : Spent Substrate

Montañez-Valdez et al. (2015) observed no statistically significant difference between the initial and spent substrate of *P. djamor*-treated corn straw. This result contradicts the finding of many studies (Begna et al., 2019; Fazaeli et al., 2014; Mhlongo et al., 2021), including a study using OPF and CS (Tuyen et al., 2013).

The conflicting results are likely due to the different strains and substrates used between studies. The protein content of OPF and CS in the present study is much lower (1.11%; 0.89%) (Table 4) than that of Tuyen et al. (2013) (39%; 46%). A substantial portion of nitrogen might be used during the formation of fruiting bodies, which results in the present study's depletion of spent substrate CP content. Nitrate, a form of nitrogen, is important for synthesizing proteins, major components of mushroom cell walls (Bellettini et al., 2019). According to Manson et al. (1989), during the generative stage, *P. ostreatus* degraded lignocellulosic material, followed by severe nitrogen and carbon depletion, leading to a reduction in the weight of the wastes used as substrates. Koutrotsios et al. (2014) evaluated the chemical composition of various agro-industrial and forestry by-products after *P. ostretus* production. Among the nine residues tested, almond and walnut shells, beech sawdust, and corn cobs presented lower protein content, while the opposite result was obtained from the other six materials.

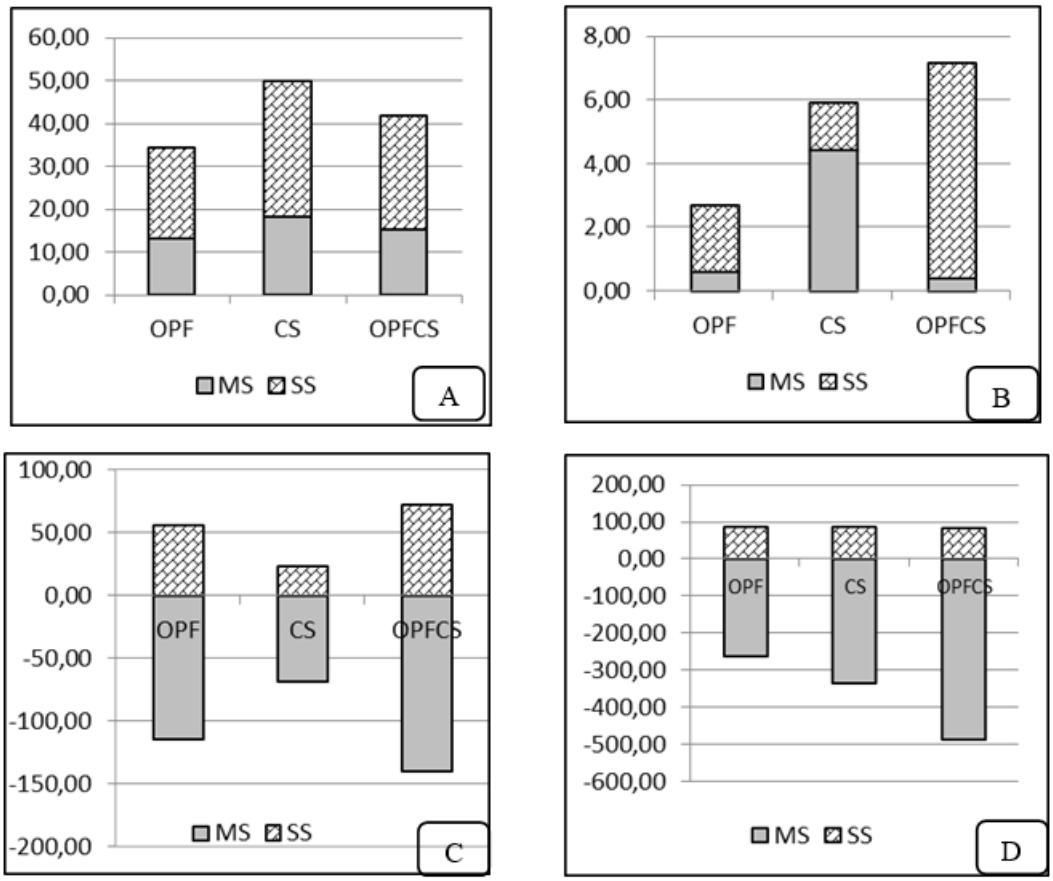

Figure 1. Losses of dry matter (A), crude fiber (B), crude protein (C), and ether extract (D) in mycelium-treated (MS) and spent substrate (SS) of OPF, CS, and OPFCS

Besides CP, fluctuation in EE content was observed during *P. djamor* growth. The EE content significantly increased by 264-488% in mycelium-treated substrates, although reduced by 81-86% after the harvest of fruiting bodies (Fig 1D). Khattab et al. (2013) reported a 68% increase in EE of *P. ostreatus*-treated rice straw after 21 days of incubation. The same length of *P. ostreatus* incubation in cowpea shells reduced the EE content by 15% (Kinfemi et al., 2009). A study by Koutrotsios et al. (2014) observed that compared to the initial substrate, most spent substrates contained lower crude fat contents.

Ideally, the spent substrate from harvesting the mushrooms should be fed to cattle. However, as a result of the present study,

desirable chemical changes such as improved CP and reduced CF were more pronounced in mycelium-treated substrates. The post-harvested substrate had no change or a relatively lower concentration of CP and EE than the initial substrate. A Similar undesirable result was also found in 53 days of cultivation of *P. djamor* on CS (Montañez-Valdez et al**.,** 2015), where the initial substrate consistently showed a higher CP content and *in Sacco* digestibility compared to the spent substrate. On the contrary, Jafari et al. (2007) reported a 29% and 3.8% increase in CP content and *in vitro* digestibility of *P. djamor*-treated rice straw after 18 days of incubation. 21 days incubation of *P. ostreatus* also improved the CP content of purple corn straw by 69% (Khonkhaeng and Cherdthong, 2020). These results suggest that length of incubation, the species of mushroom, and types of substrates are among the important factors affecting the utilization of fungal-treated agro waste as animal feeding (Yilkal, 2015)

CONCLUSION

This experiment presents a promising alternative utilization of the underutilized local agro-industrial waste, such as oil palm frond and corn straw, in *Pleurotus djamor* production. Corn straw, single and in combination with an oil palm frond, took the shortest duration in mycelium completion, formation of a pinhead, and fruiting bodies maturation, as well as gave the highest yield and biological efficiencies, thus suitable in the cultivation of *Pleurotus djamor.*

Cultivation *of Pleurotus djamor* on an oil palm frond and corn straw resulted in a reduction of crude fiber and enhancement in crude protein content of the mycelium-treated substrate. However, this desirable effect did not occur in all substrate formulations after mushroom production, which consequently will limit the practical application of *P. djamor* spent substrate utilization as ruminant feeding. Further studies, therefore, are necessary to investigate an effective pretreatment process that considers the characteristics of lignocellulosic residues to improve the utilization of spent mushroom substrate as ruminant feeding.

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