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Research Article

# Assessment of Palm Press Fibre and Sawdust-Based Substrate Formulas for Efficient Carpophore Production of *Lentinus squarrosulus* (Mont.) Singer

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**Abstract** Development of efficient substrate formulas to improve yield and shorten production time is one of the prerequisites for commercial cultivation of edible mushrooms. In this study, fifteen substrate formulas consisting of varying ratios of palm press fibre (PPF), mahogany sawdust (MS), *Gmelina* sawdust, wheat bran (WB), and fixed proportions of 1% calcium carbonate ($CaCO_3$) and 1% sucrose were assessed for efficient *Lentinus squarrosulus* production. Proximate compositions of mushrooms produced on the different substrate formulas were also analysed and compared. Substrate formulations containing 85% PPF, 13% WB, 1% $CaCO_3$, and 1% sucrose were found to produce the highest carpophore yield, biological efficiency and size (206.5 g/kg, 61.96%, and 7.26 g, respectively). Days to production (first harvest) tended to increase with an increase in the amount of WB in the substrate formulas, except for PPF based formulas. The addition of WB in amounts equivalent to 8~18% in substrate formulas containing 80~90% PPF resulted in a decrease in the time to first harvest by an average of 17.7 days compared to 80~90% MS with similar treatment. Nutritional content of mushrooms was affected by the different substrate formulas. Protein content was high for mushrooms produced on formulas containing PPF as the basal substrate. Thus, formulas comprising PPF, WB, $CaCO_3$, and sucrose at 85% : 13% : 1% : 1%) respectively could be explored as starter basal ingredients for efficient large scale production of *L. squarrosulus*.

**Keywords** Efficient production, *Lentinus squarrosulus*, Proximate compositions, Substrate formulas, Yield

Mushroom production can be a lucrative cottage industry for low-income rural households in developing countries. However, mushroom production in Nigeria is still under-developed which is in part due to un-reliable production technology and lack of consumer knowledge of the nutritional benefits of mushrooms as essential dietary component rather than as meat substitutes for low-income earners. Thus, lower prices are received for fresh mushrooms compared to maize, millet and sorghum, which make production un-attractive to prospective growers. Extensive research has been conducted on the most efficient production technology and substrate formulations for *Pleurotus* spp. [1-3] which are predominantly cultivated in Nigeria. However, there are not many reference texts available for use to improve yield, quality and shorten the time to first harvest of *Lentinus squarrosulus* commonly known as 'shiitake with tilted scales' which could present growers with a new alternative crop for selection.

*L. squarrosulus* is widely distributed across sub-Saharan Africa and many parts of Asia and is currently attracting interests due to its rapid mycelia growth and potential for use in food and biodegradation [4]. The fungus is highly appreciated for its meaty taste and texture [5]. *L. squarrosulus* has been proposed to have high potentials for commercial cultivation due to its abundant nutritional content, considerable amounts of flavour nucleotides and monosodium glutamate-like amino acids [6]. Although the cultivation of *L. squarrosulus* on sawdust, leaves and bark of fruit trees in Nigeria has been reported [7, 8], yield and biological efficiency obtained in these studies were relatively low and the influence of substrate mixtures on the chemical composition of the mushroom was not evaluated. If edible mushrooms are to be cultivated on new substrate formulations, it is proper in addition to yield, to ascertain the effects of these formulas on the chemistry of the harvested mushrooms.

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Cost-effective production of edible mushrooms depends on the reliability, availability and cost of substrate ingredients [9]. Until now, sawdust has not found other competitive uses in many parts of Nigeria except for domestic cooking and as bedding materials in poultry farms. On the other hand, crude palm oil is basically the final product of oil palm processing and the other components including palm press fibre (PPF) are discarded. PPF is a form of recovered fibrous residue from palm fruit during palm oil extraction and accounts for about 11% of the fresh fruit bunch [10]. These palm oil wastes are heterogeneous water insoluble materials consisting of cellulose, hemicelluloses, and lignin and to a lesser extent pectin, starch and other polysaccharides [11]. The environmental concerns associated with the disposal of these organic wastes rich in lignocelluloses make these a viable option for mushroom production. This study aims to assess various substrate formulations of sawdust, PPF and wheat bran (WB) for efficient production of *L. squarrosulus* which could serve as an incentive to local farmers to venture into mushroom production.

## MATERIALS AND METHODS

**Culture media and spawn preparation.** *L. squarrosulus* culture obtained from Forestry Research Institute, Ibadan, Nigeria was selected for this study because it is a local cultivar used by growers in South-West Nigeria. Subcultures of the mother mycelia were grown on Petri dishes containing 39 g/L of potato dextrose agar powder (Difco, Sparks, MD, USA) and 1.5 g/L yeast extract (Difco) (PDYA medium). Mycelia incubation took place at 28°C for 7 days and was subsequently used for spawn preparation using sorghum grain as spawn base. One kilogram of grains were first soaked in water overnight and thereafter parboiled for 15 min. After draining off excess water, grains were put on a rubber kitchen sieve and mixed at intervals for 1 hr to help evaporation to dryness. After drying, 4 g calcium sulphate was added and properly mixed into the grain to reduce grain adhesion. The pre-treated grains were filled in sterilised 500 mL jars (200 g per jar), covered with aluminium foil, fastened with rubber rings and sterilised in an autoclave at 121°C and 103 kPa pressures for 1 hr. Once cooled, each jar was inoculated with 1~2 mycelia wedges taken from 7-day-old cultures grown on PDYA. The inoculated spawn jars were thoroughly shaken to distribute the mycelium within the grains, and were incubated at room temperature (28~30°C). Grains were fully colonized by mycelium after 9 days and thereafter were used as planting spawn.

**Substrates and preparation.** The characteristics of organic basal substrates; PPF, mahogany sawdust (MS), *Gmelina* sawdust (GS), and WB used in the experiment are as shown in Table 1. Several substrate formulations comprising varying ratios of basal substrates and WB with fixed proportions of calcium carbonate ($CaCO_3$) and sucrose of 1% were prepared as shown in Table 2. Formulations without WB added were also tested as substrates. WB (a by-product of wheat flour production) was supplied by Nigerian Eagle Flour Mills, Ibadan, Nigeria. Sawdust (MS and GS) was obtained from a local sawmill in Nsukka, Nigeria. The sawdust passed through a US standard sieve size 10 (< 2 mm). Dried PPF was obtained from a communal palm oil processing site in Nsukka, Nigeria. PPF was soaked in tap water overnight and thereafter, excess water was drained off on a locally made woven basket until dripping stopped. Homogenous substrate formulas were prepared by the mixing of component ingredients according to specific percentages. Sufficient water was added (by weight) to increase substrate moisture content to 70%. Moistened substrates (500 g) were filled into heat-resistant polyethylene bags (17.5 × 15 cm) and sterilised in an autoclave at 121°C and 103 kPa pressures for 1.5 hr. Dry substrate weights were determined by drying 100 g substrate mixtures in an oven for 48 hr at 80°C.

**Table 1.** Chemical composition of some organic substrates used in the study

| Chemical composition | Substrates | | | |
|---|---|---|---|---|
| | PPF | MS | GS | WB |
| C (%)[a] | 45.61 | 47.19 | 47.63 | 46.33 |
| N (%)[a] | 1.36 | 0.20 | 0.30 | 2.58 |
| C/N | 33.54 | 235.95 | 158.77 | 17.76 |
| Cellulose : lignin ratio | 5.21 | 2.68 | 1.55 | 2.22 |

PPF, palm press fibre; MS, mahogany sawdust; GS, *Gmelina* sawdust; WB, wheat bran.
[a]Dry weight basis.

**Cultivation conditions.** The sterilised substrates were cooled to below 30°C, after which they were inoculated with 6 teaspoons of grain planting spawn under aseptic conditions in a laminar air flow cabinet. Inoculated bags were incubated at room temperature (28~30°C) in the dark to allow spawn run. After mycelium colonization was completed in the substrates, the bags were moved to a fruiting room with air temperature 29~32°C. The fruiting room roof was made of asbestos with cornice underneath, walls made of creosote-treated wood and floor made of plastered cement concrete. Sufficient air changes were provided by an electric standing fan and open windows of the fruiting room. Once pin heads were observed, the top of each bag was cut open to expose the developing primordia. During this fruiting period, relative humidity was maintained at about 70~85% by water bathing the fruiting room two times a day.

Mushrooms were harvested from the substrates the same time each day, counted and weighed. Total yield (g/kg substrate) was obtained from two flushes in a harvest period of 28 days. Days to production was calculated as the time elapsed between the day of inoculation and the day of first harvest. Biological efficiency (BE) was determined as the ratio of the weight of fresh mushrooms per 100 g dry

substrate (including additives) and expressed as a percentage. Carpophore size was determined as the ratio of total weight of fresh mushrooms harvested to total number of mushrooms harvested (per replicate).

**Analytical methods.** Chemical properties of the substrate samples were determined before mushroom cultivation on an air-dry weight basis and are as shown in Table 1. Carbon (C) was determined after oxidizing the samples in a digestion tube at 120°C by the addition of potassium dichromate solution and sulfuric acid [12]. Nitrogen (N) content was determined using Kjeldahl standard digestion procedure [12] and C : N ratios were calculated. Lignin and cellulose were analysed according to the method described by Goering and Van Soest [13] for dietary fibre and cellulose : lignin ratios calculated.

Mushrooms harvested from the different substrate formulas were dried prior to proximate analysis. The crude protein, fat, ash and fibre were analysed following the method of Association of Official Analytical Chemists [14]. The factor 4.38 was used to calculate the crude protein [15]. Carbohydrate content was calculated by difference as total carbohydrates: 100 − (% Moisture + % Crude protein + % Crude fat + % Crude fibre + % Ash) [16]. Energy values (EVs) were estimated on the basis of the crude protein, fat and total carbohydrates contents of harvested mushrooms using the factors 2.62, 8.37, and 3.48 kcal/100 g respectively [15].

**Experimental design.** The experimental design was a completely randomized design with five replications per substrate formula. At least triplicate data from two independent experiments were subjected to analysis of variance using Minitab 17 Statistical Software (Minitab Inc., State College, PA, USA). Mean separations were compared using Fisher's least significant difference at $p = 0.05$.

## RESULTS

**Yield and BE.** Results for carpophore yield and BE of substrate formulas are shown in Table 2. Substrates containing 85% PPF supplemented with 13% WB, sucrose, and $CaCO_3$ of 1% (treatment 4) gave the highest total carpophore yield of 206.50 g/kg. This is followed by 90% MS supplemented with 8% WB, sucrose, and $CaCO_3$ of 1% (treatment 8) with 162.00 g/kg total yield. In general, as supplement levels increased up to 13% for WB, yields also increased. However, as supplement levels increased further to 18%, yield and BE decreased. In addition, substrate formulations of 80~85% GS supplemented with 13~18% WB (treatments 14~15) failed to produce carpophores despite extensive mycelia colonization and primordia formation prior to opening the top of the substrate bags for carpophore induction. Thus, carpophore yield was not always proportional to increasing levels of nutrient. Yields were 3.6-fold higher for substrates containing 90% MS supplemented with 8% WB (treatment 8) compared to 98%

**Table 2.** Yield (g/kg), biological efficiency (% BE), size (g/mushroom), cap diameter (cm), stipe length (cm), and days to production (from time of spawning) for *Lentinus squarrosulus* carpophores as influenced by substrate formulae

| No. | Substrate formulae (% dry wt)[a] | Substrate type | Yield (g/kg)[b] | BE (%)[c] | Size (g)[b] | Cap diameter (cm)[b] | Stipe length (cm)[b] | Days to production[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 98 : 0 : 1 : 1 | PPF | 73.20 def* | 21.97 | 2.56 ± 1.06 fg* | 4.69 ± 1.80 d* | 4.76 ± 1.28 a* | 43.00 ± 2.94 bcd* |
| 2 | 95 : 3 : 1 : 1 | PPF + WB | 99.10 b-f | 19.82 | 4.39 ± 1.07 cde | 5.49 ± 2.34 cd | 4.31 ± 0.59 a | 41.25 ± 2.87 cde |
| 3 | 90 : 8 : 1 : 1 | PPF + WB | 157.90 abc | 47.37 | 5.73 ± 0.78 a-d | 5.95 ± 1.58 a-d | 4.56 ± 0.89 a | 36.00 ± 1.41 ef |
| 4 | 85 : 13 : 1 : 1 | PPF + WB | 206.50 a | 61.96 | 7.26 ± 1.15 a | 5.50 ± 1.61 cd | 4.90 ± 1.63 a | 35.67 ± 1.89 ef |
| 5 | 80 : 18 : 1 : 1 | PPF + WB | 151.00 a-d | 30.2 | 4.53 ± 0.76 cde | 5.53 ± 1.52 cd | 4.93 ± 0.96 a | 34.33 ± 1.7 f |
| 6 | 98 : 0 : 1 : 1 | MS | 44.80 f | 8.96 | 4.48 ± 1.94 cde | 5.66 ± 1.45 bcd | 2.95 ± 0.87 b | 36.00 ± 2.45 ef |
| 7 | 95 : 3 : 1 : 1 | MS + WB | 79.53 c-f | 15.91 | 4.53 ± 0.11 cde | 5.91 ± 1.52 a-d | 3.03 ± 0.86 b | 39.00 ± 0.82 def |
| 8 | 90 : 8 : 1 : 1 | MS + WB | 162.00 ab | 58.94 | 6.08 ± 1.06 abc | 6.91 ± 1.59 ab | 4.38 ± 0.73 a | 48.00 ± 5.00 b |
| 9 | 85 : 13 : 1 : 1 | MS + WB | 132.70 a-e | 45.47 | 5.49 ± 1.83 bcd | 5.98 ± 1.79 abc | 5.11 ± 1.52 a | 55.25 ± 8.17 a |
| 10 | 80 : 18 : 1 : 1 | MS + WB | 67.00 ef | 13.39 | 4.02 ± 1.47 def | 5.07 ± 1.77 cd | 4.25 ± 1.55 a | 56.00 ± 10.61 a |
| 11 | 98 : 0 : 1 : 1 | GS | 44.15 f | 8.83 | 1.98 ± 0.10 g | 4.36 ± 0.60 cd | 2.32 ± 0.33 b | 37.50 ± 3.67 def |
| 12 | 95 : 3 : 1 : 1 | GS + WB | 66.50 ef | 13.29 | 3.69 ± 1.62 efg | 5.18 ± 1.37 cd | 2.90 ± 0.84 b | 40.00 ± 2.45 def |
| 13 | 90 : 8 : 1 : 1 | GS + WB | 142.00 a-e | 42.58 | 6.80 ± 2.17 ab | 7.11 ± 1.50 a | 4.31 ± 0.96 a | 47.00 ± 4.08 bc |
| 14 | 85 : 13 : 1 : 1 | GS + WB | NT[d] | NT | NT | NT | NT | NT |
| 15 | 80 : 18 : 1 : 1 | GS + WB | NT | NT | NT | NT | NT | NT |

PPF, palm press fibre; MS, mahogany sawdust; GS, *Gmelina* sawdust.

*Means followed by the same letter in the same column are not significantly different at $p = 0.05$ level according to Fisher's least significant difference.

[a]Percentage dry weight ratio of constituents in the substrate formulas; organic substrate : wheat bran (WB) : $CaCO_3$ : sucrose.

[b]Each value is expressed as mean or mean ± SD of at least three replicates. ANOVA analysis were performed using Minitab 17 Statistical Software.

[c]% BE = (g fresh carpophores/g dry substrate) × 100 (includes additives weight).

[d]Not tested (see text).

MS non-supplemented with WB (treatment 6). Similarly, yield was 3.2-fold higher for 90% GS supplemented with 8% WB (treatment 13) compared to 98% GS non-supplemented with WB (treatment 11). Thus, it appears that 8% WB addition may be optimal for *L. squarrosulus* carpophore yield on sawdust-based formulas.

Comparison of the BEs of the several substrate formulas used in the present study showed that, in general, increasing the supplement levels increased the BE, although this was not always the case. For example, BEs for treatments 1 and 8 were higher than those for treatments 2 and 9, respectively (Table 2).

**Carpophore size and quality contributing parameters.** Significant variations ($p = 0.05$) were found among substrate formulas for carpophore size (Table 2). Eighty-five percent PPF supplemented with 13% WB (treatment 4) produced carpophores that were significantly larger than those produced on the other substrate formulas evaluated in this study. Substrates non-supplemented with WB (treatments 1 and 11) tended to produce smaller carpophores except for 98% MS (treatment 6) which yielded significantly larger carpophores compared to treatments 1 and 11 (Table 2).

Carpophore quality contributing parameters viz; cap diameter and stipe length as influenced by substrate formulas are shown in Table 2. Carpophore cap diameter ranged from 4.36 ± 0.6 to 7.11 ± 1.5 cm. Cap diameter was significantly ($p = 0.05$) wider on substrates containing 90% GS supplemented with 8% WB (treatment 13) compared to the other substrate formulas. Cap diameter of carpophores produced on PPF based formulas (treatments 1-5) differed insignificantly. Substrates containing 98% GS non-supplemented with WB (treatment 11) tended to produce significantly ($p = 0.05$) shorter carpophores compared to 85% MS supplemented with 13% WB (treatment 9) (Table 2).

**Days to production.** From the results shown in Table 2, the shortest time to first harvest was achieved with substrate formulas containing PPF supplemented with 18% WB (treatment 5). Eighty percent MS supplemented with 18% WB (treatment 10) resulted in the longest time to production which is 56 ± 10.61 days. In general, days to production increased as the rate of WB in the substrate formulas increased, except for PPF based formulas (treatments 1~5). It was possible to reduce the time to production in substrates containing 80~90% PPF by an average of 17.7 days compared to 80~90% MS by adding WB in amounts equivalent to 8~18% to the substrate formulas (Table 2). In addition, carpophores emerging from PPF supplemented with 8~18% WB (treatments 3~5) tended to have more synchronous maturation for the first harvest. For example, carpophores from treatments 3, 4, and 5 actually matured and were harvested within 2 days compared to 8 days of harvest between treatments 8, 9, and 10 (Table 2).

**Proximate composition and EV of carpophores.** Results of proximate composition and EV of carpophores harvested from the several substrate formulas are shown in Table 3. Considerable differences were found in the crude protein, fibre, fat, total ash, carbohydrates and EV of carpophores cultivated on the different substrates. From the results shown in Table 3, it was evident that substrates supplementation with WB improved the protein content in carpophores except PPF based formulas. Crude protein was > 2% higher in carpophores produced on 85% MS supplemented with 13% WB (treatment 9) compared to

**Table 3.** Proximate compositions (%) and energy values (kcal/100 g) for *Lentinus squarrosulus* carpophores harvested from different organic substrate formulae

| No. | Substrate formulae (% dry wt)[a] | Substrate type | Crude protein (%) | Crude fibre (%) | Total ash (%) | Crude fat (%) | Total carbohydrates (%) | Energy value (kcal/100 g) |
|---|---|---|---|---|---|---|---|---|
| 1 | 98 : 0 : 1 : 1 | PPF | 19.21[b] | 5.09[b] | 7.34[b] | 0.68[b] | 54.95[b] | 247.28[b] |
| 2 | 95 : 3 : 1 : 1 | PPF + WB | 18.31 | 3.73 | 8.13 | 0.72 | 55.25 | 246.27 |
| 3 | 90 : 8 : 1 : 1 | PPF + WB | 13.41 | 2.48 | 10.37 | 0.90 | 54.98 | 234.00 |
| 4 | 85 : 13 : 1 : 1 | PPF + WB | 16.05 | 2.70 | 9.29 | 0.89 | 55.07 | 241.14 |
| 5 | 80 : 18 : 1 : 1 | PPF + WB | 17.26 | 3.14 | 8.92 | 0.76 | 54.05 | 239.68 |
| 6 | 98 : 0 : 1 : 1 | MS | 9.30 | 5.17 | 6.71 | 0.91 | 64.58 | 256.72 |
| 7 | 95 : 3 : 1 : 1 | MS + WB | 9.77 | 4.56 | 6.95 | 0.84 | 63.91 | 255.04 |
| 8 | 90 : 8 : 1 : 1 | MS + WB | 10.46 | 3.80 | 7.96 | 0.55 | 65.16 | 258.76 |
| 9 | 85 : 13 : 1 : 1 | MS + WB | 19.05 | 4.16 | 7.60 | 0.78 | 53.66 | 243.18 |
| 10 | 80 : 18 : 1 : 1 | MS + WB | 15.11 | 3.61 | 7.40 | 0.63 | 57.76 | 245.87 |
| 11 | 98 : 0 : 1 : 1 | GS | 13.87 | 5.40 | 7.97 | 0.38 | 60.10 | 248.67 |
| 12 | 95 : 3 : 1 : 1 | GS + WB | 14.17 | 5.12 | 8.05 | 0.41 | 59.77 | 248.56 |
| 13 | 90 : 8 : 1 : 1 | GS + WB | 17.10 | 4.74 | 8.52 | 0.52 | 56.39 | 245.39 |
| 14 | 85 : 13 : 1 : 1 | GS + WB | NT | NT | NT | NT | NT | NT |
| 15 | 80 : 18 : 1 : 1 | GS + WB | NT | NT | NT | NT | NT | NT |

PPF, palm press fibre; MS, mahogany sawdust; GS, *Gmelina* sawdust; NT, not tested.

[a]Percentage dry weight ratio of constituents in the substrate formulas; organic substrate : wheat bran (WB) : $CaCO_3$ : sucrose.

[b]Values are an average of two replicate analyses.

98% MS non-supplemented with WB (treatment 6). However, crude protein content was not always linear in relation to increasing levels of WB. For example, crude protein in carpophores produced on 80% MS supplemented with 18% WB (treatment 10) (15.11%) was lower than those produced on 85% MS supplemented with 13% WB (treatment 9) (19.05%). Crude protein was considerably higher in carpophores cultivated on PPF based formulas (treatments 1~5), with the maximum value of 19.21% observed on 98% PPF non-supplemented with WB (treatment 1) compared to the other substrates. Crude fibre in carpophores cultivated on the different substrate formulas varied between 2.48% and 5.40%. The highest crude fibre was dictated in carpophores produced on 98% GS non-supplemented with WB (treatment 11). Total ash was considerably higher for carpophores cultivated on 90% PPF supplemented with 8% WB (treatment 3) compared to the other substrate formulas. Crude fat was lower in carpophores produced on GS based formulas with the lowest value (0.38%) observed on treatment 11 (Table 3). Total carbohydrates varied between 53.66% and 65.16% for carpophores cultivated on the different substrates. Carpophores produced on MS based formulas (treatments 6~10) were richer in total carbohydrates compared to the other substrates. Total carbohydrates was maximum (65.16%) in carpophores produced on 90% MS supplemented with 8% WB (treatment 8).

EV of carpophores calculated based on the crude protein, fat and total carbohydrate contents are shown in Table 3. EV was found to be higher for carpophores produced on MS based formulas compared to the other substrates. Maximum EV (258.76 kcal/100 g) was calculated for carpophores cultivated on treatment 8. Carpophores from treatment 3 had the lowest EV (234 kcal/100 g).

## DISCUSSION

Currently, information is limited on a standard formula that could be utilized for efficient production of *L. squarrosulus* under appropriate production technologies for the developing countries. In the present investigation, supplementary carbon and nitrogen were important factors for the production of *L. squarrosulus* on organic basal substrates. The preliminary test was conducted in Petri plates to determine the importance of sucrose to the growth of fungus mycelia on sawdust and PPF. Based on the observation from the test (data not shown), the addition of sucrose in the range of 1~2% significantly increased mycelia growth rate compared to non-amended substrates. Thus, substrates amended with fixed proportion of 1% sucrose were used for subsequent studies.

The magnitudes of carpophore yield responses to the different substrate formulas were significant as shown in Table 2. Highest yield and BE were obtained on a 13% WB supplement on PPF. This result is likely due to the fibrous nature of PPF which aided adequate aeration for the development of carpophores. Furthermore, substrate combinations such as PPF and WB comprising a mixture of large and fine particles are considered ideal for fruiting body formation. Abd Razak *et al.* [11] reported that a complex mosaic of sawdust, oil palm frond and spent grain substrates resulted to significant higher BE and total yield of *Auricularia polytricha* compared to sawdust alone. They asserted that besides chemical composition factors in substrates; substrate structure could play significant roles in improving the productivity of substrate formulations. This could also explain the relatively higher yield and BE obtained on 8% level of WB supplement for MS compared to the same level of supplement on GS. In the present study, substrate mixture of MS and WB had a larger particle size compared to the fine particles of GS and WB combinations. In fact, about 8% of MS was retained on US standard sieve size 10 (< 2 mm) while about 99% of GS passed through the sieve prior to mixing with the supplements. Although this is not strong to become conclusive as sawdust particle size depends on the thickness of saw blade and not wholly an inherent characteristic of the wood. However, it underscores the importance of sawdust particle size on the yield of mushrooms. Earlier reports by Royse and Sanchez-Vazquez [17] showed that substrates containing wood chips of < 0.85 mm particle size are not optimum for the production of shiitake.

Our result showed that 8% WB supplement may be optimal for carpophore yield of *L. squarrosulus* on sawdust based formulas. In addition, WB levels greater than 8% and 13% on sawdust and PPF-based formulas respectively, resulted in decreased yield. Thus, it appears that substrate type may be a major factor influencing yield responses to increasing levels of supplements.

Our observation that GS supplemented with 13~18% WB did not support carpophore formation despite extensive mycelia colonization and appearance of primordia on the substrates is not well understood. Increased level of nitrogen in the substrate formulas may not adequately explain this phenomenon. This is because PPF which had about 4.5-fold nitrogen compared to GS (Table 1) produced carpophores at both 13% and 18% WB supplementations. Therefore, it is probable that the very fine particles resulting from the mixture of GS and higher amounts of WB may explain in part the above phenomenon. Ohga [18] showed that as sawdust particle size decreased, the radial mycelia extension rate increased while mycelia biomass decreased for *Lentinus edodes*. It was suggested that oxygen ($O_2$) depletion was the cause of reduced mycelia biomass development in substrates containing smaller particle size [18]. Similarly, Royse and Sanchez-Vazquez [17] inferred that gas-exchange restriction in substrates prepared with smaller particle sizes may be responsible for sub-optimum yields observed for *L. edodes* grown on wood chips with particle size < 0.85 mm. Yang *et al.* [19] had also demonstrated with *Ganoderma lucidum* that mixed ratios of stillage grain, WB and ground rice did not form primordia and fruiting body successfully despite rapid mycelia colonization

of the mixed substrate. They concluded that too much stillage grain or WB might result in the diminution of the compost voidage.

Mushroom size is essential for its market evaluation and carpophores with wide pilei could be of interest in the promotion of mushroom marketing. In the present study, although carpophore size and quality were not always linearly related to supplement levels, addition of WB to substrates tended to increase size and quality of carpophores compared to substrate formulas non-amended with WB. Thus, amendment of cultivation substrates with WB could enhance the size and quality of harvested carpophores. The result of this study on quality of harvested carpophores is similar to Moonmoon *et al.* [20]. Yang *et al.* [21] demonstrated that supplementation of rice straw or wheat straw with WB and cotton seed hull could shorten mushroom stipe length and enlarge mushroom cap diameter.

The addition of WB supplement resulted in an increase in days to production of carpophores on sawdust based formulas. However, a decrease in days to production was observed for PPF based formulas amended with WB. This could be attributed to the probable low C : N ratio resulting from the combination of PPF and WB. Özçelik and Pekşen [22] showed that the C : N ratio of hazel nut based substrate formulas was positively correlated with earliness (first harvest) in *L. edodes*. Although the C : N ratios of the substrate formulas were not evaluated in the present study, it is likely that the mixture of PPF and WB with high nitrogen contents (Table 1) may result in a decrease in the C : N ratios of the formulas. Thus, growers could efficiently produce *L. squarrosulus* carpophores by utilizing substrate mixtures of PPF and 8~13% WB. In addition, consistent yields may also be guaranteed due to the observed synchronous maturation of carpophores for the first break on PPF supplemented with 8~13% WB. This is significant for developing countries where scattered harvest periods are un-desirable due to inadequate mushroom processing facilities; hence substrate formulas that could result in uniform maturation of carpophores in a particular harvest period are desirable for bulk processing. However, in situations where sawdust is preferred owing to its availability, increased productivity could be obtained at the expense of faster crop cycle with 8% WB supplementation. Okhuoya *et al.* [7] reported an increase in the time to first primordial emergence of *L. squarrosulus* cultivated on sawdust substrates supplemented with WB compared to non-supplemented controls. On the other hand, Shen and Royse [23] demonstrated with *Grifola frondosa* that higher levels of WB in basal ingredient of mixed oak sawdust shortened the crop cycle, but produced poorer quality mushrooms and lowered BEs. Due to the apparent varying results on the effect of WB additions on time to first harvest of mushrooms, it does appear that the type of substrate formulation and mushroom determine the influence of WB additions on days to production as shown by our findings. Özçelik and Pekşen [22] also demonstrated that the addition of 10% WB (75% hazelnut husk : 15% wheat straw : 10% WB) as a substitute for some portions of wheat straw in the substrate formula (75% hazelnut : 25% wheat straw) reduced earliness (time to first harvest) from 86.87 days to 77 days. However, in the same study, the addition of 10% WB (75% hazelnut husk : 15% beech wood-chip : 10% WB) as a substitute for some portions of beech wood-chip in the substrate formula (75% hazelnut husk : 25% beech wood-chip) did not significantly change earliness as *L. edodes* was first harvested at 82.67 and 82.83 days, respectively for both substrate formulas.

The proximate composition of carpophores cultivated on the different substrate formulas revealed that protein content was improved for carpophores harvested from sawdust based formulas supplemented with WB compared to those non-supplemented with WB. However, protein content tended to decrease for carpophores harvested from PPF based formulas supplemented with WB compared to those non-supplemented with WB. This result confirms the conclusions previously made by other investigators that not only the amount but also the nature of the nitrogen source present in the substrate influences the protein content of fruit bodies [24, 25]. Wang *et al.* [25] reported that supplementation of spent grain with WB had a greater potential to improve the accumulation of protein in *Pleurotus ostreatus* than did rice bran or corn bran. In the present study, protein values obtained for *L. squarrosulus* carpophores are within the range 10~20.5% reported for the commercially cultivated *L. edodes* by other authors [26, 27]. Addition of WB to substrate formulas decreased the crude fibre and in some cases increased the crude fat of harvested carpophores compared to substrates non-supplemented with WB. This is in line with an earlier research on supplementation of rice straw substrate with cotton seed showing decreased total dietary fibre and increased total lipid for harvested *Pleurotus florida* compared to rice straw substrate non-amended with cotton seed [15]. The crude fibre and fat contents obtained in our study were similar to or lower than values reported by other investigators for *L. squarrosulus* [6, 28]. Total carbohydrate contents obtained in our study were similar to or higher than values reported by Yang *et al.* [27] for some commercial mushroom species. Total EVs of carpophores obtained in the present study were lower than values reported for *L. squarrosulus* cultivated on mixed sawdust of *Triplochiton scleroxylon* and *Chlorophora excelsa* [29]. Thus, from our results, it is evident the protein and carbohydrate contents of *L. squarrosulus* could be comparable to other commercially cultivated mushroom species. In addition, the relatively low net EV obtained in this study justifies mushrooms as low calorie foods [15].

In conclusion, PPF supplemented with 13% WB, sucrose and $CaCO_3$ of 1% does appear to be a suitable substrate formula for efficient production of *L. squarrosulus*. However, due to the fibrous nature of PPF, mixtures of the substrate with WB may not be wholly homogenous as portions of

the supplement tended to settle at the bottom of the mixing box and hence not evenly distributed within the substrate. Thus, the possibility of using physical processing such as milling or grinding while maintaining large particle size may further improve the yield potential of PPF. In addition, studies focused on assessing the effects of other types of nutrient supplements and their combinations with several organic basal substrates may reveal a more productive substrate formula than we found in this study.

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