The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*)

Ha Thi Hoa¹, Chun-Li Wang^{2,*} and Chong-Ho Wang²

¹Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

²Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan

Abstract The study was conducted to compare the effects of different agro-wastes on the growth, yield, and nutritional composition of oyster mushrooms *Pleurotus ostreatus* (PO) and *Pleurotus cystidiosus* (PC). Seven substrate formulas including sawdust (SD), comcob (CC), sugarcane bagasse (SB) alone and in combination of 80 : 20, 50 : 50 ratio between SD and CC, SD and SB were investigated. The results indicated that different substrate formulas gave a significant difference in total colonization period, characteristics of fruiting bodies, yield, biological efficiency (BE), nutritional composition and mineral contents of two oyster mushrooms PO and PC. The results showed that increasing CC and SB reduced C/N ratio, and enhanced some mineral contents (Ca, P, and Mg) of substrate formulas. The increased amount of CC and SB of substrate formulas enhanced protein, ash, mineral contents (Ca, K, Mg, Mn, and Zn) of fruiting bodies of both mushrooms. Substrates with 100% CC and 100% SB were the most suitable substrate formulas for cultivation of oyster mushrooms PO and PC in which they gave the highest values of cap diameter, stipe thickness, mushroom weight, yield, BE, protein, fiber, ash, mineral content (Ca, K, and Mg) and short stipe length. However, substrate formula 100% CC gave the slowest time for the first harvest of both mushrooms PO and PC (46.02 days and 64.24 days, respectively). It is also found that the C/N ratio of substrate formulas has close correlation with total colonization period, mushroom weight, yield, BE and protein content of mushroom PO and PC.

Keywords Nutritional composition, Oyster mushroom, Substrates, Yield

Oyster mushroom (*Pleurotus* species) belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide following the *Agaricus bisporus* [1, 2]. However, Obodai *et al.* [3] reported that oyster mushroom is the third largest commercially produced mushroom in the world market. *Pleurotus* species are popular and widely

cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency (BE) [4]. Moreover, the interest of oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties [5]. *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures [1]. In comparison to other edible mushrooms, *Pleurotus* species need a short growth time and their fruiting bodies are not often attacked by diseases and pests [6, 7]. *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources. The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust [SD], waste paper, leaves, and sugarcane residue) can be used as mushroom substrates [8]. Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates [9, 10].

Pleurotus species are a rich source of protein, minerals (P, Ca, Fe, K, and Na) and vitamin (thiamine, riboflavin,

Mycobiology 2015 December, 43(4): 423-434
<http://dx.doi.org/10.5941/MYCO.2015.43.4.423>
pISSN 1229-8093 • eISSN 2092-9323
© The Korean Society of Mycology

*Corresponding author

E-mail: lelia@mail.npust.edu.tw

Received June 7, 2015

Revised August 18, 2015

Accepted September 29, 2015

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folic acid, and niacin) [11]. Apart from food value, their medicinal value for diabetics and in cancer therapy has been emphasized [12]. Numerous mushroom species contain a wide range of metabolites as antitumour, antigenotoxic, antioxidant, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial, and antiviral activities [13]. Several species of oyster mushrooms are very important in the field of medicine. *Pleurotus cystidiosus* (PC) is a strong antioxidant [14] while *Pleurotus ostreatus* (PO) also possesses antitumor activity [15].

Large volumes of unused lignocellulosic by-products are available in tropical and subtropical areas. These by-products are usually left to rot in the field or are disposed through burning [6]. Using locally available lignocellulosic substrates to cultivate oyster mushroom is one solution to transform these inedible wastes into accepted edible biomass of high market and nutrient values [6]. Presently, in Asia (including Taiwan), the main substrate used for the commercial cultivation of oyster mushroom is SD. Using large quantities of SD for mushroom cultivation causes reduction of wooded areas while information on the potential use of other locally available resources is lacking [16]. The potential shortages of SD and high potential of agro-waste residues are the reasons why we need to identify alternatives for sustainable cultivation of oyster mushrooms. The study was conducted to compare the effects of different agro-wastes on the growth, yield, and nutritional composition of oyster mushrooms PO and PC. The final aim is to find the best substrate formulas for effective cultivation of oyster mushrooms PO and PC.

MATERIALS AND METHODS

Mushroom material and spawn preparation. Two species of oyster mushroom PC (strain AG 2041) and PO (strain AG 2042) obtained from Plant Physiology and Value Added Microorganisms Laboratory (Department of Plant Industry, National Pingtung University of Science and Technology [NPUST], Taiwan) were grown on potato sucrose agar medium (PSA) at 28°C for regular subculture and maintained on PSA at 4°C for a maximum of 3 mon. Spawns were prepared in 850-mL polypropylene plastic bottles filled with 600 g acacia SD supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate (in terms of dry weight basis) and 60~65% water content, and then sterilized at 121°C for 5 hr. After cooling to room temperature, 10 mycelium discs (diameter 1 cm) of each oyster mushroom were inoculated into each bottle of sterilized spawn. The spawn was incubated at 28°C until the substrate fully colonized.

Substrate preparation and inoculation. Three lignocellulosic substrates including sugarcane bagasse (SB), corncob (CC), and SD (made from acacia wood) were obtained from Pingtung County, Taiwan. SB and CC were

dried and then ground into 0.5~1.5 cm length pellets and soaked separately in water over 4 hr. After draining excess water from those materials, they were used for replacing SD. In order to determine suitable substrates and suitable ratios for cultivation of two oyster mushrooms PO and PC, seven substrate formulas including SD, CC, SB alone and in combination of 80:20, 50:50 ratio between SD and CC; SD and SB (on dry weight basis) were investigated. Substrate 100% SD was used as control treatment. After mixing materials with the above proportion, they were supplemented with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.03% mono-potassium phosphate. Water content of the final mixture was adjusted to about 65%. Each lignocellulosic substrate formula after supplementing nutrient and distilled water was filled into 10 × 23-cm polyethylene plastic bags and sterilized in an autoclave at 121°C for 5 hr. The weight of every bag was approximately 1 kg. Twenty-four culture bags were used for each substrate formula. After substrates were cooled to room temperature, they were inoculated with the 2 g spawn per bag.

Incubation and harvest. The inoculated substrates were kept in an incubation room at 28°C and 60~70% relative humidity under dark condition. After the surface of substrates was entirely covered with mycelium, then the substrates were moved to a cropping room in which temperature was maintained at 24°C and kept at related humidity about 90% or above. For all substrate formulas, three flushes of mushroom PC and six flushes of mushroom PO were harvested from each of the culture bags when the in-rolled margins of the mushroom caps began to flatten. The time from inoculation to the first harvest and total harvesting time (from the first to the last harvest) were observed and recorded. At every flush, the harvested fruiting bodies were weighed and mushroom size was measured. The length and thickness of stipe, diameter of cap, and number of effective fruiting body per bunch were measured at the first, second and third flush and the means were also determined. At the end of the harvest period, the accumulated data were used to calculate total yield and the BE. BE is the ratio of fresh fruiting body weight (g) per dry weight of substrates (g), expressed as a percentage.

Substrate analysis. Substrate samples were dried by an oven at 40°C to a constant weight and ground to powder samples. Total carbon (C) content was determined according to the report of Nelson and Sommers [17], and total nitrogen (N) content was carried out on a 0.2 g sample by the Kjeldal method after 96% H₂SO₄ hot digestion [18]. Then the C/N ratio of each substrate was calculated. Electrolyte conductivity (EC) and pH were determined according to the methods of Cavins *et al.* [19] by using a pH meter (UltraBasic-UB10; Denver Instrument, New York, NY, USA) and EC meter (SC-2300 conductivity meter;

Suntex Instrument Co. Ltd., New Taipei City, Taiwan); 20 g of substrate was mixed with 200 mL water (ratio 1 : 10) to wet the sample to saturation, shaken for 15 min and left for 60 min and filtered before the measurements were made. The contents of mineral elements (P, K, Ca, Mg, Fe, Mn, Zn, and Cu) were analyzed by ICP atomic emission spectrophotometry using the Varian 725-ES device (Varian, Santa Clara, CA, USA) after element extraction in 0.1 N HCl acidic solution. These instruments were manufactured by HORIBA Jobin Yvon (Longjumeau, France).

Fruiting body analysis. Mushroom samples were dried by an oven at 40°C to a constant weight to calculate moisture content and then ground into power samples for other analysis. The samples were analyzed for nutritional composition (fat, carbohydrates, fiber, and ash) using the Association of Official Analytical Chemists procedures [20]. The protein content ($N \times 6.25$) of samples was estimated by the macro-Kjeldahl method [18]. The fat was determined by extracting a known weight of powdered sample with ethyl ether, using a Soxhlet apparatus. The ash content was measured by incineration at $600 \pm 15^\circ\text{C}$. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal/100 g) = $4 \times \text{Protein} + 4 \times \text{Carbohydrate} + 9 \times \text{Fat}$. The mineral contents (P, K, Ca, Mg, Fe, Mn, Zn, and Cu) were analyzed by ICP atomic emission spectrophotometry using

the Varian 725-ES device after element extraction in 0.1 N HCl acidic solution.

Experimental design and data analysis. The experiments were conducted in Plant Physiology and Value Added Microorganisms Laboratory, Department of Plant Industry, NPUST in Taiwan during autumn-winter season, 2014 (July to December). The experiment was arranged in a randomized complete block design with three replications and twenty-four culture bags per treatment. One-way analysis of variance (ANOVA) was conducted with Duncan's multiple range tests to compare the mean significant differences ($p < 0.05$) among treatments by using computer software SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Substrate analysis. Total C, total N, C/N ratio, pH, EC, and mineral content are very important factors for mycelium colonized and development of fruiting bodies. It is very important to determine the chemical and nutrient composition of the substrates, especially those used for commercial purposes [21]. There was a significant difference in chemical and nutritional composition properties among substrate formulas used in this study (Tables 1 and 2). Regarding total C, the value of this parameter was the highest in substrate formula 100% SB (55%), and the

Table 1. Chemical properties of experimental substrate formulas

Substrate formula	C (%)	N (%)	C/N ratio	pH (1 : 10)	EC (1 : 10, mS/cm)
100% SD (control)	44.12 cd	0.86 e	51.71 a	6.93 a	2.88 e
100% SB	55.00 a	1.20 a	45.83 c	6.70 e	4.20 a
50% SD + 50% SB	49.00 b	1.05 bc	46.67 bc	6.83 c	3.66 b
80% SD + 20% SB	46.25 c	0.95 cde	48.68 b	6.88 ab	3.26 c
100% CC	39.98 e	1.16 ab	34.57 e	6.75 d	3.58 b
50% SD + 50% CC	42.55 d	1.00 cd	42.55 d	6.84 bc	3.38 c
80% SD + 20% CC	43.00 d	0.88 de	49.05 b	6.91 a	3.05 d

Means with the same column followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

SD, sawdust; SB, sugarcane bagasse; CC, corncob.

Table 2. Mineral contents of experimental substrate formulas

Substrate formula	mg/100 g dry weight							
	Ca	Cu	Fe	K	Mg	Mn	P	Zn
100% SD (control)	376.38 d	0.11 d	55.72 bc	1,557.71 c	60.65 d	7.22 c	187.98 d	3.30 bc
100% SB	521.28 a	0.35 a	56.24 bc	2,673.79 a	94.27 a	8.02 ab	221.90 a	3.59 b
50% SD + 50% SB	446.98 b	0.26 b	57.38 bc	2,617.85 a	83.05 b	7.67 bc	220.36 a	3.47 b
80% SD + 20% SB	427.71 c	0.15 cd	53.47 c	1,758.33 c	77.44 bc	7.20 c	218.38 a	3.34 bc
100% CC	461.23 b	0.20 c	65.89 a	2,457.49 a	83.26 b	8.31 a	217.42 ab	4.38 a
50% SD + 50% CC	447.69 b	0.16 cd	61.70 ab	2,139.31 b	72.29 c	7.72 bc	208.25 c	3.56 b
80% SD + 20% CC	418.57 c	0.13 d	54.79 bc	1,760.50 c	69.63 c	7.23 c	211.89 bc	3.08 c

Means with the same columns followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

SD, sawdust; SB, sugarcane bagasse; CC, corncob.

lowest in 100% CC (39.98%). Substrate formula 100% SB showed the highest total N (1.20%) while 100% SD (control substrate), 80% SD + 20% CC, 80% SD + 20% SB showed the lowest total N (0.86%, 0.88%, and 0.95%, respectively). N content increased gradually with the decreasing amount of SD in substrate formula (Table 1). In the experiment, C/N ratio of substrate formulas significantly varied from 34.57 to 51.71 and the highest value was obtained in control substrate formula. The pH values of substrates ranged from 6.7 to 6.93, suitably for oyster mushroom cultivation. The highest pH values were obtained from 100% SD, 80% SD + 20% CC, 80% SD + 20% SB (6.93, 6.91, and 6.88, respectively). EC values significantly changed among substrate formulas and ranged from 2.88 to 4.20 (mS/cm). The highest EC value was recorded at substrate containing 100% SB (4.20 mS/cm). When increasing CC and SB in substrate formulas, C/N ratio and pH value of substrates decreased when compared to substrate containing 100% SD; however, EC of substrate formulas increased.

The main mineral content (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) of substrate formulas used in this study varied considerably (Table 2). Sales-Campos *et al.* [22] confirmed in their study that these elements are naturally present in all the raw materials used for preparation of the cultivation substrate. Substrate formulas containing CC or SB at the rates 50% and 100% were rich in mineral content compared to substrates containing 100% SD (except Fe, Cu Zn, and Mn). Among substrate formulas, 100% SB contained the maximum amount of Ca (521.28 mg/100 g). Ca content of substrate involving 100% CC was the second highest and not significantly different with Ca content of substrates

containing 50% SB and 50% CC. In general, Cu and Zn contents of all substrate formulas were low. Fe content of substrate formula ranged from 53.47 to 65.89 (mg/100 g) and the highest value was obtained at substrate 100% CC (65.89 mg/100 g). Substrate 100% SB and 100% CC also gave the highest values of K (2,673.79 and 2,457.49 mg/100 g), Mn (8.02 and 8.31 mg/100 g), and P (221.90 and 217.42 mg/100 g), respectively. An increasing trend of mineral content was observed when SD was gradually replaced by SB or CC in substrate formulas.

Effect of different substrate formulas on morphological parameters. Seven different types of substrates were investigated to determine the growth, yield, nutritional composition of two oyster mushrooms PO and PC. The results in Table 3 showed that, there were significant differences in morphological parameter of both oyster mushrooms PO and PC grown on seven substrate formulas. Colonization of mushroom PO was completed in between 30.03~40.06 days after incubation while total colonization period of oyster mushroom PC varied from 48.25 to 55.02 days. Both oyster mushrooms PO and PC took the longer time (35.08~40.06 and 53.60~55.02 days, respectively) to complete colonizing in substrates containing 100% CC and 50% CC compared to other substrate formulas.

The latest first harvest (46.02 days) of oyster mushroom PO was recorded from substrate containing 100% CC and significantly later than that of other substrate formulas while the longest time for the first harvest of oyster mushroom PC also obtained from substrate containing 100% CC (64.24 days). However, it was not significantly

Table 3. Effect of different substrate formulas on morphological parameters and characteristics of fruiting body of oyster mushrooms PO and PC

Substrate formula	Total colonization period (day)	First harvest (day)	Harvesting period (day)	Cap diameter (mm)	Stipe length (mm)	Stipe thickness (mm)	No. of effective fruiting bodies/bunch	Mushroom weight (g/bunch)
PO								
100% SD (control)	30.03 c	42.27 b	45.22 a	70.62 d	38.22 a	8.52 b	10.32 a	38.76 c
100% SB	32.24 bc	42.44 b	45.50 a	84.83 ab	35.74 b	10.22 ab	8.06 b	42.95 ab
50% SD + 50% SB	32.20 bc	43.06 b	45.24 a	83.56 bc	38.16 a	10.06 ab	8.25 b	41.75 b
80% SD + 20% SB	33.12 bc	42.38 b	45.62 a	83.05 bc	39.21 a	10.10 ab	8.55 b	39.24 c
100% CC	40.06 a	46.02 a	43.80 ab	86.74 a	35.28 b	11.06 a	7.93 b	45.10 a
50% SD + 50% CC	35.08 b	44.02 b	42.04 b	82.75 bc	35.20 b	10.86 a	8.07 b	43.14 ab
80% SD + 20% CC	33.26 bc	43.12 b	45.82 a	80.87 c	38.34 a	9.84 ab	8.20 b	38.87 c
PC								
100% SD (control)	48.25 b	60.00 c	45.18 b	95.68 d	57.84 a	35.08 c	2.32 a	60.53 c
100% SB	50.84 b	60.42 c	48.57 a	102.05 b	50.22 bc	38.22 bc	2.09 ab	65.19 ab
50% SD + 50% SB	50.42 b	63.86 a	47.64 a	98.56 c	50.21 bc	36.42 bc	1.85 c	62.48 bc
80% SD + 20% SB	50.83 b	63.12 ab	47.72 a	95.04 d	52.02 b	38.32 bc	2.05 bc	61.78 bc
100% CC	55.02 a	64.24 a	45.00 b	106.24 a	52.21 b	44.32 a	2.23 ab	67.05 a
50% SD + 50% CC	53.60 a	62.06 abc	42.40 c	101.18 bc	46.64 c	39.66 b	2.27 ab	63.91 abc
80% SD + 20% CC	49.87 b	61.03 bc	47.06 ab	100.04 bc	46.06 c	36.00 bc	2.12 ab	61.50 c

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

Fig. 1. Relationship between C/N ratio and total colonization period (A), mushroom weight (B), mushroom yield (C), BE (D), protein content (E) of oyster mushrooms PO and PC. BE, biological efficiency; PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*.

different from that of substrate formulas containing 50% CC, 50% SB, and 20% SB. Total harvesting period (from the first harvest to the last harvest) of oyster mushroom PO and PC ranged from 42.04 to 45.82 days and 42.40 to 48.57 days, respectively. Although the time for colonization period and the first harvest of oyster mushrooms PO, PC obtained from substrate containing 100% CC were the longest, total harvesting period of both oyster mushroom was the same or shorter than that of other substrates (except mushroom PC grown on substrate formula 50% SD + 50% CC). This was explained by the difference in total C, total N of substrate formulas hence the difference in C/N ratio. C/N ratio had more effects on the mycelium growth, the formation and development of fruiting body.

Fig. 1A showed that there was a negative correlation between the C/N ratio of substrates used and total colonization period. The result was similar to the finding of Alborés *et al.* [23] who revealed that there was a positive correlation between the C/N ratio of substrate and mycelium growth rate. However, substrate with lower C/N ratio supported for fruiting bodies better than substrate with high C/N ratio. Naraian *et al.* [24] reported that mycelium growth and primordial development of *Pleurotus florida* were dependent on the lignocellulosic materials, especially the C/N ratio. These findings were similar to the results reported by Yang [25] that higher C/N ratio favored the mycelium growth, and lower C/N ratio favored the fruiting body growth. Yang [25] also determined that oyster

mushroom PO grown on substrate 80% cotton seed hull with C/N = 34.87 needed longer time to complete colonization period than substrate 80% rice straw and 80% wheat straw with C/N = 49.19 and C/N = 64.63, respectively.

Mycelium growth in this study was far slower than the finding of Dahmardeh *et al.* [26] that colonization period of oyster mushroom took three weeks and fruiting bodies appeared after 2~3 days. Whereas Bughio [27] revealed that oyster mushroom PO (Jacq. Ex. Fr.) Kummer took 43.25~53.00 days for pinhead formation after spawn inoculating in case of using wheat straw and sorghum leaves. The results of present study were in agreement with the finding of Bugarski *et al.* [28] who found that the first fruiting body occurred on different days depending on substrates.

Effect of different substrate formulas on characteristics of fruiting body.

There was significant difference in cap diameter of both oyster mushrooms grown on different substrate formulas (Table 3, Fig. 2). In case of oyster

mushroom PO, cap diameter was the highest (86.74 mm) on substrate formula 100% CC, and the lowest (70.62 mm) diameter was recorded on control substrate 100% SD. In case of oyster mushroom PC, the highest value of cap diameter (106.24 mm) was obtained from substrate containing 100% CC, and the lowest cap diameter was observed at substrate formulas 80% SD + 20% SB and 100% SD (95.04 and 95.68 mm, respectively).

The length and thickness of stipe of oyster mushrooms PO and PC significantly differed on different substrates (Table 3, Fig. 2). In case of mushroom PO, stipe length ranged from 35.28 to 39.21 mm, while the thickness of stipe varied from 8.52 to 11.06 mm. Stipe length of mushroom PC ranged from 46.06 to 57.84 mm and stipe thickness ranged from 35.08 to 44.02 mm. The stipe length values of mushrooms PO and PC grown on control substrate formula were the same or significantly higher than those of other experimental substrates while the values of the thickness were the same or lower. On the other hand, the cap thickness of both oyster mushrooms PO and PC

Fig. 2. Fruiting bodies of oyster mushrooms PO (A) and PC (B) grown on 100% SD (a); 100% SB (b); 50% SB (c); 20% SB (d); 100% CC (e); 50% CC (f); and 20% CC (g). PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

grown on control substrate formula was lower than that of other substrate formulas (data not shown). In substrate formulas with 50% CC, 100% CC, and 100% SB, the marketable quality of mushrooms PO and PC was improved by shortening mushroom stipe length, and enlarging mushroom cap diameter. Substrates containing 100% CC and 50% CC also gave the good marketable quality of oyster mushroom due to the higher stipe thickness of mushroom.

Effective fruiting body is the edible part of mushroom. The mean number of effective fruiting bodies per bunch exhibited significant difference among different substrate formulas (Table 3, Fig. 2). The result showed that the maximum fruiting body number of mushroom PO (10.32 fruiting bodies/bunch) was recorded at substrate 100% SD, followed by other substrate formulas (7.93~8.55 fruiting bodies/bunch) while the maximum fruiting body number of mushroom PC were obtained from substrate formulas 100% SD, 100% SB, 100% CC, 50% CC + 50% SD, 80% SD + 20% CC (2.32, 2.09, 2.23, 2.27, and 2.12 fruiting bodies/bunch, respectively). The lowest number of effective fruiting bodies of oyster mushroom PC was observed at substrate containing 50% SB. Effective fruiting bodies per bunch not only depended on substrate types, but also depended on oyster mushroom species. Between two oyster mushrooms, mushroom PO had higher effective fruiting bodies than that of mushroom PC. Mondal *et al.* [29] indicated that effective fruiting bodies of oyster mushroom *P. florida* ranged from 8.5 to 37.25 fruiting bodies/bunch, and it depended on substrate types used for cultivation.

The mushroom weight (g/bunch) of oyster mushroom depended on cap diameter, the length and thickness of stipe, the number of effective fruiting bodies per bunch and the thickness of cap. Mushroom weight closely related

with yield and BE. Mushroom weight of two oyster mushrooms PO and PC grown on different substrate types was significantly different (Table 3, Fig. 2). The highest mushroom weight of mushroom PO (45.10 g/bunch) was obtained from substrate 100% CC, followed by 43.14, 42.95 g/bunch obtained from substrate 50% SD + 50% CC and 100% SB respectively. The lowest mushroom weight was recorded at control substrate and substrate containing 20% SB and 20% CC (38.76, 39.24, and 38.87 g/bunch, respectively). Mushroom weight of mushroom PC was significantly higher than that of mushroom PO and it ranged from 60.53 to 67.03 g/bunch. Substrate 100% CC also gave the highest mushroom weight of mushroom PC (67.05 g/bunch). However, it was not significantly different from that of substrate formulas containing 50% CC and 100% SB. The lowest mushroom weight of mushroom PC was observed at substrate 100% SD and 80% SD + 20% CC (60.53 and 61.50 g/bunch). The increase amount of CC or SB in substrate formulas enhanced mushroom weight of both oyster mushrooms PO and PC compared to control substrate. Correlation analyses (Fig. 1B) demonstrated that C/N ratio of substrate formulas used in this experiment closely correlated with mushroom weight of oyster mushroom PO and PC ($R^2 = 0.82$ and $R^2 = 0.95$, respectively).

Effect of different substrate formulas on yield and BE.

The yield is one of the main purposes of mushroom cultivators. Both oyster mushrooms PO and PC grown on different substrates showed the significant difference in term of mushroom yield (Table 4). Mushroom PO had six flushes while mushroom PC had only three flushes. The significantly highest mushroom yield of PO and PC was obtained from the first flush, followed by the second flush

Table 4. Effect of different substrate formulas on yield and BE of oyster mushrooms PO and PC

Substrate formula	1 st flush (g/bag)	2 nd flush (g/bag)	3 rd flush (g/bag)	4 th flush (g/bag)	5 th flush (g/bag)	6 th flush (g/bag)	Total yield (g/bag)	BE (%)
PO								
100% SD (control)	70.65 b	53.24 c	39.44 b	30.15 d	22.00 b	17.06 b	232.54 d	46.44 e
100% SB	76.30 ab	61.32 a	45.10 a	35.10 abc	23.08 b	16.80 b	257.70 b	65.65 a
50% SD + 50% SB	75.55 ab	60.06 ab	45.68 a	32.02 bcd	20.12 b	17.08 b	250.51 c	58.94 b
80% SD + 20% SB	75.38 ab	50.30 c	38.60 b	31.02 cd	25.07 b	15.06 b	235.43 d	52.32 c
100% CC	77.68 a	56.16 abc	45.76 a	37.9 a	31.05 a	22.05 a	270.60 a	66.08 a
50% SD + 50% CC	76.88 a	62.00 a	43.80 ab	36.02 ab	23.62 b	16.50 b	258.82 b	58.82 b
80% SD + 20% CC	71.00 b	54.00 bc	39.20 b	28.96 d	22.00 b	18.06 ab	233.22 d	48.59 d
PC								
100% SD (control)	77.76 b	62.82 b	41.01 d	-	-	-	181.59 d	36.27 e
100% SB	81.67 ab	68.22 a	45.67 ab	-	-	-	195.56 a	49.54 a
50% SD + 50% SB	79.22 ab	65.03 ab	43.20 bcd	-	-	-	187.45 cd	44.11 b
80% SD + 20% SB	78.09 b	64.10 b	43.15 bcd	-	-	-	185.34 cd	41.19 c
100% CC	84.72 a	67.98 a	48.44 a	-	-	-	201.14 a	50.14 a
50% SD + 50% CC	80.46 ab	66.02 ab	45.24 bc	-	-	-	191.72 bc	43.57 b
80% SD + 20% CC	78.30 ab	64.12 b	42.08 cd	-	-	-	184.5 cd	38.44 d

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

BE, biological efficiency; PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

and the trend gradually decreased at next flushes. The total yield of mushroom PO ranged from 232.54 to 270.60 g/bag. Substrate formulas 100% CC gave the highest total yield (270.60 g/bag) followed by substrate formulas containing 50% CC and 100% SB (258.82 and 257.70 g/bag, respectively). Substrates containing 100% CC, 50% CC, and 100% SB had the higher values of the yield of mushroom PO at almost all flushes and thus their total yield was higher than that of other substrate formulas. In case of mushroom PC, the significantly highest total yield was obtained from substrate formulas 100% CC and 100% SB (201.14 and 195.56 g/bag, respectively), followed by substrate containing 50% CC (191.72 g/bag). Control substrate formula (100% SD) gave the lowest mushroom yield (181.59 g/bag); however, it was not significantly different from that obtained from substrates containing 50% SB, 20% SB, and 20% CC.

In term of BE, mushroom PO had higher value than that of mushroom PC at all substrate formulas. In general, substrates gave the higher yield also gave the higher value of BE. Whereas, substrate 100% SD showed the lowest mushroom yield, as well as the lowest BE of both oyster mushrooms PO and PC. The highest BE of mushroom PO was obtained from substrate formulas 100% CC and 100% SB (66.08% and 65.65%, respectively). The second highest BE was observed from substrates containing 50% SB and 50% CC (58.94% and 58.82%, respectively). BE of mushroom PC ranged from 36.27% to 50.14%. Substrate formulas 100% CC, 100% SB, 50% CC + 50% SD, 50% SB + 50% SD were more suitable for both oyster mushrooms PO and PC in terms of mushroom yield and BE compared to other substrate formulas.

In general, BE of two mushrooms PO and PC in present research was far lower in comparison to other studies. Bhattacharjya *et al.* [30] observed that BE of PO grown on different SD substrates ranged from 187.0% to 213.2%. BE at 139.0% of PO was found in the combination substrates of rice straw and weed plant at 1 : 1 ratio [31]. Yang *et al.* [32] also reported that BE of mushroom PO ranged from 51.3% to 125.6% on rice and wheat straw basal substrate supplemented with cotton seed hull. However, the results of this study were similar to some previous studies. Liang *et al.* [33] found that BE (39.55–58.33%) of oyster mushroom *Pleurotus pulmonarius* was grown on the stalks of three grass plants in Taiwan. Using wood chips and wheat bran, BE values at 60.7% and 54.3% were achieved for PO and *P. pulmonarius* [34].

The differences in terms of yield and BE of both oyster mushrooms grown on different substrate types were due to the differences in physical and chemical composition of substrate formulas such as cellulose/lignin ratio and mineral contents, pH, EC of substrate, especially C/N ratio. The low nitrogen in SD was one of factors affecting on the overall yield and BE compared to other substrates. When SD was gradually replaced by SB or CC in the substrate formulas, nitrogen enhanced thus C/N ratio decreased. In the present experiment, the lower C/N ratio of substrate

supported better mushroom yield than the higher C/N ratio. The result was similar to the result of Mintesnot *et al.* [35] who indicated that organic residues having greater C/N ratio reduced the yield and quality of three oyster mushrooms (PO, *P. florida*, and *P. sajor-caju*). Correlation analyses (Fig. 1C and 1D) demonstrated that C/N ratio of substrate formulas used in this experiment closely correlated with total yield and BE of oyster mushroom PO ($R^2 = 0.89$ and $R^2 = 0.71$, respectively) and oyster mushroom PC ($R^2 = 0.86$ and $R^2 = 0.73$, respectively). The optimum C/N ratio found for biological yield and BE was 35.7 for PO and 40.6 for *P. florida* [36]. The values were within the range of the C/N ratio 32/1–150/1 reported by Chang and Miles [37] to be effective for primordial induction in *Pleurotus* species. Taurachand [38] reported that SB contained cellulose and sucrose which are easily degraded by oyster mushroom and provide energy for mushrooms. The result was in agreement with Philippoussis *et al.* [39] who stated that the yield of medicinal mushroom (*Lentinula edodes*) grown on CC was higher than that on wheat straw and oak wood SD. Wang *et al.* [40] showed that there was a positive correlation between BE and degradation of cellulose and hemicellulose whereas a negative relationship between BE and lignin degradation was observed. Philippoussis *et al.* [41] also reported that there is a strong negative correlation between mushroom yield (mushroom number and BE) and C/N ratio of substrate. Quimio [42] indicated that cellulose rich in organic substance was one of the best substrates for the cultivation of oyster mushrooms. Substrates with high lignin and phenolic content decreased the activity of cellulose, but less lignin would enhance enzyme activity and thus ensure higher mushroom yield and BE [43].

Effect of different substrate formulas on nutritional composition of fruiting body.

Fundamental food characteristic as ash, fiber, protein, fat, carbohydrate content and energy value of oyster mushroom grown on different substrate formulas were presented in Table 5. Effect of substrate formulas was not significant on moisture content (89.71–91.56%) of mushroom PO while different substrate formulas significantly affected on moisture content of mushroom PC (Table 5). The highest moisture content (92.45% and 91.13%) of oyster mushroom PC was obtained from substrates 100% SB and 100% SD, respectively. This result was due to the water holding capacity of substrate. The lowest moisture content (86.95%) of oyster mushroom PC was recorded at substrate 100% CC. This might be the poor nature of this substrate in water holding capacity as compared to the other substrates. The result was quite close to the value stated by Mintesnot *et al.* [35] who reported that 85.6–93.4% moisture content of *Pleurotus sajor-caju*. Similar moisture content (80.0–92.5%) was reported for *Pleurotus* species grown on different agro-wastes [44, 45]. Moisture content was also influenced by mushroom age, growing environments, mushroom strains, and postharvest environments [44].

Table 5. Effect of different substrate formulas on nutritional composition of oyster mushrooms PO and PC

Substrate	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Ash (%)	Energy (kcal/100 g)
PO							
100% SD (control)	91.06 a	19.52 d	1.32 c	22.00 c	51.26 a	5.90 b	295.00 a
100% SB	91.56 a	27.13 ab	2.00 b	29.25 a	34.94 cd	6.68 ab	266.28 cd
50% SD + 50% SB	89.71 a	24.17 bc	2.50 a	28.75 a	37.88 c	6.70 ab	270.70 b
80% SD + 20% SB	89.37 a	21.88 cd	2.78 a	24.02 bc	44.97 b	6.35 ab	292.42 a
100% CC	90.57 a	29.70 a	2.67 a	29.75 a	30.78 d	7.10 a	265.95 d
50% SD + 50% CC	90.37 a	25.65 b	1.80 b	28.25 ab	37.50 c	6.80 ab	268.80 bc
80% SD + 20% CC	89.89 a	20.89 d	2.08 b	23.04 c	47.62 ab	6.37 ab	292.76 a
PC							
100% SD (control)	91.13 a	15.68 d	2.05 c	20.05 c	55.92 a	6.30 b	304.85 a
100% SB	92.45 a	22.18 ab	2.30 bc	22.79 ab	45.25 cd	7.48 a	290.42 c
50% SD + 50% SB	87.69 b	18.66 c	3.28 a	24.5 ab	46.86 c	6.70 ab	291.60 c
80% SD + 20% SB	88.77 b	17.95 cd	3.33 a	25.05 a	47.27 bc	6.40 b	290.85 c
100% CC	86.95 b	24.54 a	3.00 ab	24.25 ab	40.64 d	7.57 a	287.72 c
50% SD + 50% CC	88.19 b	21.47 b	2.80 abc	23.58 ab	44.85 cd	7.30 a	290.48 c
80% SD + 20% CC	87.14 b	16.9 cd	2.33 bc	22.45 b	51.93 ab	6.39 b	296.29 b

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

The study indicated that the fruiting bodies of oyster mushrooms PO and PC grown on all substrate formulas are quite rich in protein, carbohydrate, fiber and low in fat content making them excellent foods that can be used in low caloric diets. Protein of mushrooms PO and PC ranged between 19.52~29.70% and 15.68~24.54%, respectively on dry weight basis. The highest protein of mushrooms PO and PC (29.70% and 24.54%, respectively) were recorded from fruiting bodies grown on 100% CC, that was not significantly different with substrate 100% SB. This value was slightly similar to the range of Kurtzman [44] in which the protein on dry matter basis in oyster mushroom could range between 20~40%. However, protein content of mushroom PC grown on substrate formulas containing 100% SD, 50% SB, 20% SB, and 20% CC was lower than the finding of Kurtzman [44]. Protein of oyster mushrooms PO and PC enhanced with increasing amount of CC and SB in substrate formulas used. The differences in protein content of mushrooms PO and PC grown on different substrates could be due to the varying nitrogen content of substrates. The high nitrogen content of SB and CC contributed to the higher protein content of fruiting bodies while SD provided less nitrogen and it could be attributed to the nitrogen utilization efficiency by the species.

The negative correlation between high protein content of mushrooms (PO, PC) and low C/N ratio of substrate (Fig. 1E) implies that nitrogen is essential for synthesis of protein in mushroom fruiting bodies. This result was in line with Ragunathan and Swaminathan [46], the protein contents of mushroom depended on biological, chemical differences and the C/N ratio of substrates. Wang *et al.* [40] reported that not only protein content in fruiting body but also nature of protein were affected by substrate used. The result was confirmed with the finding of Mshandete

and Cuff [47] that protein content of edible mushrooms was influenced by species/train specific and the growing substrate.

Fat and fiber content on dry weight basis of oyster mushroom PO varied from 1.32% to 2.78% and 22.00% to 29.75%, respectively while these values of mushroom PC ranged from 2.05% to 3.33% and 20.05% to 25.05%, respectively. The highest fat value of mushroom PO was obtained from substrate formula 100% CC and not significant with that of substrate containing 20% SB and 50% SB. Substrate formula 100% SD gave the lowest value of fat and fiber contents of both mushrooms PO and PC. Whereas the highest carbohydrates of both oyster mushrooms PO (51.26%) and PC (55.92%) were observed from control substrate. The fiber content obtained in this study is higher than that reported by Wang *et al.* [40] as 5.97~6.42%. The total carbohydrate content of oyster mushroom PO and PC (30.78~51.26% and 40.64~55.92%, respectively) was also found in this study. Patil *et al.* [10] reported that carbohydrate content of PO (jacq. Fr.) Kumm ranged from 50.50~55.33% grown on paddy straw, soybean straw and wheat straw. However, the findings of Sharma *et al.* [48] was 30.24~42.26% of PO grown on different substrates.

Ash content ranged from 5.90% to 7.10% of mushroom PO and 6.30% to 7.57% of mushroom PC. In comparison to control substrate formula, only substrate formula 100% CC gave a significant difference in ash content of mushroom PO. Other substrates gave the same result in ash content, whereas the highest value of ash content of mushroom PC was observed from substrate containing 100% CC, 50% CC, 100% SB, and 50% SB and it was significantly higher than that of mushroom PC grown on control substrate formula. The ash content in this experiment was in accordance with the value reported by Bonatti-Chaves *et al.* [49] when PO

and *P. sajor-caju* were grown on different lignocellulosic wastes.

Substrate not only affected on protein, carbohydrate, and fat but also influenced on total energy of oyster mushrooms PO and PC (Table 5). Total energy contribution of the samples ranged between 265.95~295 kcal/100 g dry weight for oyster mushroom PO and 287~304.85 kcal/100 g dry weight for oyster mushroom PC. In general, oyster mushrooms were low in calorie food because they provided low amount of fat. The result was quite similar to the findings of Khan *et al.* [50] who stated that energy of *P. florida* and PC were 250.1 kcal/100 g dry sample and 262.8 kcal/100 g dry sample.

Effect of different substrate formulas on mineral contents of fruiting body.

Minerals in diet are essential for metabolic reactions, healthy bone formation, transmission of nerve impulses, regulation of water, and salt balance [51]. The mineral content of oyster mushrooms PO and PC varied with different substrates (Table 6). In general, mushrooms PO and PC grown on seven substrate formulas had good quality in terms of mineral contents. Potassium (K) content was higher compared to other minerals in mushrooms PO and PC. When SD was replaced by SB and CC at different rates in substrate formulas, the mineral contents of mushrooms PO and PC was similar or in the trend improved. The highest Ca content in PO was recorded at substrate 100% SB (345.06 mg/100 g dry weight); however, it was not significant with that at substrate formulas containing 50% SB, 100% CC, and 50% CC. The maximum Ca content of mushroom PC was also found on substrate formula 100% CC (337.14 mg/100 g) and 100% SB (348.98 mg/100 g).

Substrate formulas containing 100% CC, 100% SB gave not only the highest Ca content but also the maximum values of Cu (2.55 and 2.22 mg/100 g), Fe (14.64 and 14.85 mg/100 g), K (2,624.16 and 2,573.79 mg/100g), Mg (225.17 and 337.07 mg/100 g), and P (717.49 and 732.37 mg/100 g) in mushroom PO. However, these values were not significantly different with the Ca, Cu, Fe, and Mg content of mushroom PO grown on 50% CC and 50% SB. The highest Zn and Mn content of mushroom PO also achieved on substrate 100% CC. In case of mushroom PC, the maximum Cu content (3.04 mg/100 g) was achieved on substrate formula 100% SB; however, it was not significantly different with that on substrates containing 100% SD and 20% CC. The highest value of Fe content was recorded at substrates containing 100% SD, 100% CC, 50% CC, and 20% CC. The lowest K value was observed from substrate formula 100% SD. It was significantly lower than that on other substrate formulas. Substrate formula 100% CC also showed the highest content values of Mg, Mn, and Zn while substrate formula 100% SB gave no significant difference in the Mn content of mushroom PC compared to control substrate formula (100% SD) and it was significantly lower than that obtained from substrate formula 100% CC. The P content of mushroom PC varied from 558.14~629.44 mg/100 g. Substrate formula containing 20% CC had the highest value of the P content and significantly higher than that of mushroom PC grown on other substrate formulas (except substrate 100% SD).

Difference in mineral content of mushroom not only depended on mushroom species but also depended on substrates used. That was due to mineral concentration of substrate formulas. On the other hand, EC of substrate is one of factors affected on mineral uptake of mushroom.

Table 6. Effect of different substrate formulas on mineral contents of oyster mushrooms PO and PC

Substrate formula	mg/100 g dry weight							
	Ca	Cu	Fe	K	Mg	Mn	P	Zn
PO								
100% SD (control)	336.02 b	2.08 b	14.83 ab	1,424.37 c	217.76 bc	2.06 c	620.35 d	8.69 b
100% SB	345.06 a	2.22 ab	14.85 ab	2,573.79 a	237.07 a	3.06 b	732.27 a	8.56 b
50% SD + 50% SB	338.90 ab	2.18 ab	14.16 ab	2,551.19 a	231.57 ab	2.93 b	727.19 a	8.58 b
80% SD + 20% SB	334.28 b	2.11 b	14.18 ab	1,858.33 bc	218.40 bc	2.06 c	720.67 a	8.51 b
100% CC	340.08 ab	2.55 a	14.64 ab	2,624.16 a	225.17 ab	3.69 a	717.49 ab	11.45 a
50% SD + 50% CC	338.87 ab	2.43 ab	14.97 a	1,972.64 b	221.60 abc	2.16 c	687.23 c	8.77 b
80% SD + 20% CC	336.75 b	2.17 ab	13.79 b	1,627.17 bc	208.80 c	1.59 d	699.24 bc	7.61 c
PC								
100% SD (control)	332.52 c	2.86 ab	16.14 a	1,986.57 b	213.71 b	3.90 bc	606.32 ab	9.82 b
100% SB	348.98 a	3.04 a	15.51 bc	2,264.51 ab	224.72 ab	4.46 b	575.30 bc	10.49 ab
50% SD + 50% SB	342.71 bc	2.63 b	15.28 c	2,158.42 ab	222.72 ab	4.27 b	577.39 bc	9.34 b
80% SD + 20% SB	336.05 bc	2.64 b	15.20 c	2,125.45 ab	220.96 ab	3.93 bc	580.73 bc	10.38 ab
100% CC	337.14 ab	2.22 c	15.65 abc	2,308.42 a	228.27 ab	5.19 a	583.43 bc	11.60 a
50% SD + 50% CC	339.36 b	1.98 c	15.89 ab	2,100.19 ab	214.99 b	4.39 b	558.14 c	10.55 ab
80% SD + 20% CC	341.01 bc	2.98 ab	16.09 a	2,250.31 ab	233.07 a	3.41 c	629.44 a	10.11 b

Means within the same column of each oyster mushroom followed by the same letters are not significantly different at $p \leq 0.05$ according to Duncan's multiple range test.

PO, *Pleurotus ostreatus*; PC, *Pleurotus cystidiosus*; SD, sawdust; SB, sugarcane bagasse; CC, corncob.

Substrate 100% SB had the highest P content (Table 2); however, P uptake of mushroom PC was low because of high EC of substrate (Table 1). The result in mineral contents of this research was similar to the findings of Ahmed *et al.* [45] for the Fe content; Ahmed *et al.* [45] and Patil *et al.* [10] for the K content; Patil *et al.* [10] for the P and Ca content.

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