



## Research article

# Rice residues can be converted to functional health nutrients through mushroom *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer production

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## Abstract

Growth of 9 mushroom *Pleurotus ostreatus* was investigated on six combinations of rice residues as substrate. The substrate composition and functional health nutrients of slowly dried mushrooms were also analyzed. Substrate formulae respectively composed of rice husk, rice bran (as supplement), and rice straw were designated as SI (625 g, 112.5 g, 350 g), SII (250 g, 112.5 g, 275 g) SIII (847.98 g, 134.80 g, 230.40 g), SIV (402.02 g, 134.80 g, 230.40 g), SV (847.98 g, 90.20 g, 230.40 g) and SVI (402.02 g, 90.20 g, 230.40 g). Effective yield response was observed in SI (58.82% husk, 10.59% bran and 30.59% straw) and SII (39.23% husk, 17.65% bran and 43.14% straw) at pH 7.01 and pH 7.12 ( $p < 0.05$ ), respectively, and a carbon-to-nitrogen ratio of 60.47 and 60.82, respectively. Biological efficiency and total dry yield, respectively, peaked in SII (67.31%, 66.92 g) and SI (52.29%, 54.94 g). Protein expression was in the range 42.12–65.48  $\mu\text{g/mL}$  and the total phenolic content expressed in milligram equivalents of gallic acid per gram dry weight of mushroom was high in all substrates: SI (186.33), SII (31.91), SIII (121.52), SIV (48.00), SV (44.15) and SVI (87.24); there were selenium traces in SI, SII and SIII. This study revealed that rice residues in adequate proportions are suitable as *Pleurotus ostreatus* substrates in terms of yield and functional health nutrients.

## Introduction

Rice production in sub-Saharan Africa (SSA) has significantly increased over the last decade as it has been cultivated in diverse agro-ecological zones coupled to the expansion in cultivation area (Diagne et al., 2013). Local production is widely promoted and continuously advocated in all countries of the region as per capita consumption remains at the peak compared to other regions of the world (Africa Rice Center, 2011). The rice sector is also regarded as a vital tool

in achieving the sustainable development goals of ensuring food security and increasing household incomes in SSA (Arouna et al., 2017). Paddy production yields in the region were 22.1 million t in 2014 (International Rice Research Institute, 2015). Rice is harvested as paddy, composed of approximately 72% edible endosperm, directly covered by 6% nutrient rich bran that is slowly digestible by hydrolases and 22% of outer protective husk layer made up of lignocellulose, hemicellulose, cellulose and silica (Monteiro et al., 2011; Soltani et al., 2015). Paddy can be processed to brown rice by

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hulling of the outer husk layer or to white milled rice by complete polishing of the bran layer. The increase in rice production therefore entails increased elimination of rice straw, rice bran and rice husk (Haefele et al., 2011). Rice farmers in SSA either burn rice straw in the fields, feed it to cattle or incorporate it in vegetable beds. Bran from rice mills is incorporated in animal feed and is available in large quantities as the consumption of brown rice is rare in the region (field observation). Rice husk, with its high lignocellulose content making it indigestible to rodents, is often abandoned behind mills, constituting a burden to both the miller and the environment (Ndindeng et al., 2015). Rice husk is a burden to value chain actors, due to its low bulk density, resorting to burning as the cheapest handling mode given that little or no profit is derived from it (Ndindeng et al., 2015). Rice bran, rice husk, and rice straw have limited value addition in SSA and where it is burnt, it leads to losses and health and environmental problems (Chou et al., 2009), and global warming that may negatively influence yields and quality in agricultural systems including rice (Zhao and Fitzgerald, 2013). Alternative exploitation of agricultural waste are being investigated worldwide including rice residues to foster human wellbeing while conserving the environment (Binod et al., 2010; Frimpong-Manso et al., 2011).

In Thailand, Delivand et al. (2011) exploited the conversion of rice straw to electricity through combustion while Binod et al. (2009) and Teghammar et al. (2012) exploited the fermentation and bioconversion of straw to bioethanol and biogas. Rice husk has been combusted for heat and electricity as well as composted to organic fertilizer (Lim et al., 2012a, 2012b). Phenolic compounds have been extracted from rice bran under subcritical water conditions (Pourali et al., 2010) while reports on bioactive compounds with positive health effects contained in rice bran have been documented (Prasad et al., 2011). In SSA, few reports are available on the profitable use of rice residues by actors in the rice value chain. Ndindeng et al. (2015) proposed the substitution of firewood with briquettes obtained from the hydraulic compression of rice milling by-products with the advantage of easy handling due to the increased bulk density.

Agricultural waste rich in complex polysaccharides like lignocellulose, hemicellulose and cellulose is used in the cultivation of edible mushroom (Belletini et al., 2016). Mushroom contains cellulases, laccases and other enzymes that digest complex polysaccharides to provide essential nutrients necessary for their proliferation (Belletini et al., 2016). Mushroom of the genus *Pleurotus* are reported to thrive on numerous agricultural wastes and this genus is considered a rich source of protein, minerals, antioxidants

and nutraceuticals (Danell and Eaker, 1992; Boonsong et al., 2016). *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer has been cultivated on cornstalks enriched with sorghum spent grain (Dzaka and Akpesey, 2017). Zhang et al. (2002) cultivated *Pleurotus ostreatus* on rice and wheat straw. Frimpong-Manso et al. (2011) investigated the influence of rice husk on the biological efficiency and nutrient content of *Pleurotus ostreatus*. Rice bran has been used as a supplement for mushroom cultivation (Adjapong et al., 2015; Sonnenberg et al., 2015). In SSA, few farmers exploit rice residues in mushroom production and reports on the sole use of rice residues as mushroom substrate are scarce. The current study aimed to investigate the yield response of *Pleurotus ostreatus* on alternate proportions of rice bran, rice husk and rice straw, and the nutritional content of some functional nutrients of the produced mushrooms.

## Material and Methods

### Rice residues

Rice bran and rice husk were collected from the rice mill at the Food Technology Laboratory of the Institute of Agricultural Research for Development (IRAD, Yaounde Cameroon), while rice straw was collected from the IRAD rice seed demonstration plot immediately after harvesting and threshing. Rice straw was chopped into small sizes of approximately 3 cm on a clean surface using a hand cutter. The residues were then separately dried in an oven at 40°C to constant weight before mixing into the desired proportions.

### Design of experiment

The alternate proportions of the rice residues used to formulate the mushroom substrate were set up in the Minitab 14 software for Windows (Minitab Inc.; PA, USA) using a central composite design in a quadratic model with the three factors being: rice bran (75–150g), rice husk (250–1,000g) and rice straw (150–350g). Fifteen different substrate formulations were investigated and six retained in the optimum design (Table 1) through preliminary testing that adjusted rice bran (the supplement) to limit contamination and over supplementation with nitrogen (Mantovani et al., 2007) which retards mycelial viability (Carlile et al., 2001). The treatment weights were allocated using a sensitive balance and thoroughly mixed before being adjusted to the initial moisture content of 68% using a 1% calcium carbonate (lime) solution. Four replicates of 2 kg per

**Table 1** Substrate combination generated from rice husk, rice straw and rice bran using central composite design and a quadratic model

Treatment	Substrate designation	Rice husk		Rice bran		Rice straw	
		Coded unit	Actual value (g)	Coded unit	Actual value (g)	Coded unit	Actual value (g)
1	SI	0	625.00	0	112.50	0	350.00
2	SII	0	250.00	1.68	112.50	0	275.00
3	SIII	-1	847.98	1	134.80	-1	230.40
4	SIV	-1	402.02	-1	134.80	1	230.40
5	SV	1	847.98	1	90.20	1	230.40
6	SVI	1	402.02	-1	90.20	1	230.40

treatment were weighed into polypropylene bags and pasteurized at 98°C for 8 hr in a mesh basket designed for steaming paddy during parboiling. The sterilized bags were opened under aseptic conditions to cool to room temperature before being inoculated with 8 g of the spawns. *Pleurotus ostreatus* spawns prepared on rice paddy, corncobs and agar were supplied by the Mycology Laboratory of IRAD, Bambui Research Center (Bamenda, Cameroon). The bags were tied with appropriate labels and placed on caged shelves in a dark room at 28°C and 80% relative humidity and watered twice a day to maintain adequate room humidity and moisture content of the substrates. Spawn colonization was monitored for 15 d, after which the door and windows of the room were opened for 2 hr to aerate the substrates.

#### *Substrate analysis*

Substrate composition and nutrient content were analyzed in the Soil, Plant, Fertilizer and Water Research Laboratory of IRAD, Yaounde (LASPEE, IRAD). After cooling of the sterilized substrates, 10 g of each treatment was collected, dried in the oven at 40°C and milled to pass through a 1.0 mm mesh as powder. The pH of homogenized solutions of 1 g powder prepared in 10 mL of distilled water and KCl (1:2.5 weight per volume basis) was determined using a pH meter. Total nitrogen was determined using the Kjeldahl method as detailed in Raja et al. (2014). The total carbon content was determined following the method described by Nelson and Sommers (1982).

#### *Production performance*

After colonization of the substrates by the mycelia which lasted 18 d, the number of fruiting bodies and maturity duration on each substrate for the first flush was noted followed by measurement of the total weight of fresh mushrooms harvested when the caps were about to flatten. Three flushes (first, second, third) were harvested within 6 wk at intervals of 8 d between flushes. The total fresh weight for the three flushes constituted the total yield per substrate and this value was used to calculate biological efficiency defined as the ratio of total fresh weight of harvested mushroom per dry weight of the substrate on which the mushroom was grown, expressed as a percentage. Harvested mushrooms were slowly dried at 30°C for 48 hr in an oven and the total dry yield per substrate determined.

#### *Nutrient analysis*

Total carbohydrates were extracted in 80% hot ethanol and quantified using the phenol sulfuric acid method with glucose as the standard. Absorbance was read at 490 nm in a UV-Visible spectrophotometer (Shimadzu, Japan). Total protein quantification was performed using Bradford's reagent and bovine serum albumin as the standard protein for the analysis following the extraction of 2.5 g of the ground mushroom powder in 1 M NaOH (Danell and Eaker, 1992) with incubation at 50°C in the water bath. Total ash

was determined by incineration of 5 g of dried mushroom in a muffle furnace at 600°C.

The mineral elements sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were extracted in concentrated hydrochloric acid solution (0.1 N) by mineralization of grey ash obtained by incinerating 2 g of mushroom at 450°C for 2 hr in the muffle furnace. The Na, K, Ca, and Mg contents were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) in a 725-ES device (Varian; CA, USA) while the phosphorus (P) content was estimated following color development with 1% ascorbic acid, 0.01% ammonium molybdate, and antimony sodium tartrate in concentrated sulfuric acid. Absorbance was read at 880 nm from which the phosphorus content was calculated. Selenium (Se) was determined using the ICP-AES device following the digestion of 3 g of the mushroom powder with nitrohydrochloric acid (aqua regia) prepared with double-distilled water.

The total phenolic content was quantified using Folin-Ciocalteu reagent following the extraction of 2.5 g fine powder in 100 mL of 95% methanol. Freshly prepared Folin-Ciocalteu reagent (2 mL, 1 N) was added to 0.5 mL of the extract in glass test tubes followed by the addition of Na<sub>2</sub>CO<sub>3</sub> (1.5 mL, 7.5%). The tubes were homogenized and incubated in the dark for 1 hr after which the absorbance was read at 760 nm. The external reference sample was gallic acid (10–200 mg/mL) and the phenolic content was expressed as milligram equivalents of gallic acid per gram dry weight of the mushroom (mg GAE /g dw).

#### *Statistical analysis*

Data collected in triplicate for all variables were entered into the Excel 2016 software (Office 365, Microsoft Corp.; WA, USA). Bar charts of the dry yield, proximate composition and total phenolic content were then generated for all the treatments. Analysis of variance with Duncan's multiple-range test was used to determine significant differences between the substrate compositions. Multiple comparison using the general linear model procedure was used to evaluate the effect of treatment on production performance and nutrient content. Mean separation between the treatments was adjusted using Bonferroni. Statistical analysis carried out with the SPSS 22.0 software (SPSS Inc.; IL, USA) at the 95% confidence interval.

## **Results and Discussion**

#### *Substrate composition and production performance*

The chemical composition of the different substrate treatments influenced both the biological efficiency and nutrient content of the produced mushrooms. It was observed (Table 2) that the combination of different proportions of rice residues led to significant variations in the substrate pH, carbon, nitrogen and carbon-to-nitrogen ratio, revealing differences in mycelial growth, nutrient and bioactive content of mushrooms across the substrates. The pH significantly varied across all the treatments ranging from 6.19 in SV to 8.86 in SIII. Kalmis et al. (2008) reported the pH levels for the optimum

**Table 2** Substrate pH, carbon (C), nitrogen (N), and carbon-to-nitrogen ratio (C:N) after sterilization

Treatment	pH	C (%)	N (mg/g)	C:N
SI	7.01 ± 0.03 <sup>b</sup>	60.36 ± 2.49 <sup>c</sup>	1.00 ± 0.09 <sup>a</sup>	60.47 ± 8.23 <sup>b</sup>
SII	7.12 ± 0.02 <sup>c</sup>	56.92 ± 0.78 <sup>b</sup>	0.93 ± 0.03 <sup>a</sup>	60.82 ± 1.73 <sup>b</sup>
SIII	8.86 ± 0.02 <sup>f</sup>	59.81 ± 1.42 <sup>c</sup>	1.23 ± 0.02 <sup>b</sup>	48.52 ± 2.13 <sup>a</sup>
SIV	7.80 ± 0.01 <sup>d</sup>	52.05 ± 1.11 <sup>a</sup>	1.14 ± 0.06 <sup>b</sup>	45.50 ± 3.34 <sup>a</sup>
SV	6.19 ± 0.06 <sup>a</sup>	56.02 ± 0.12 <sup>b</sup>	1.18 ± 0.03 <sup>b</sup>	47.36 ± 1.32 <sup>a</sup>
SVI	8.03 ± 0.02 <sup>e</sup>	55.11 ± 1.01 <sup>b</sup>	1.20 ± 0.02 <sup>b</sup>	45.69 ± 1.62 <sup>a</sup>
Total	7.50 ± 0.87	56.71 ± 3.12	1.11 ± 0.12	51.39 ± 7.55

See Table 1 for details of composition of substrates S1–SVI.

Values are mean±SD from three replicates.

Values in a column with different superscript letters are significantly different ( $p < 0.05$ ) at 5% level of significance using Duncan's multiple-range test.

mycelial growth for *Pleurotus ostreatus* were 6.5 and 7.0 on wheat straw and olive residues, respectively. For the rice residues in the current study, the highest total fresh yield was comparable in SI, SII and SIV with pH values of 7.01, 7.12, and 7.80, respectively. However, mushroom requires a wide range of pH for effective mycelial growth and formation of fruiting bodies (Bellettini et al., 2016). Total carbon ranged from 52.05% in SIV to 60.36% in SI with little significant variation. Total nitrogen ranged from 0.93% in SII to 1.23% in SIII leading to a similar significant variation in the carbon-to-nitrogen ratios of the substrates that ranged from 45.50 in SIV to 60.84 in SII. An overall mean value of 51.39 characterized the carbon-to-nitrogen ratio of all substrates. The average optimum carbon-to-nitrogen ratio for high *Pleurotus ostreatus* biological efficiency was reported to be 50 by Wang et al. (2015) and supported by Dzaka and Akpese (2017). However, the carbon and nitrogen contents producing this ratio were different, being 50:1 for the former and 46.55:0.93 for the latter. Contrary to these reports, in the current study, the C:N ranged from 45.50 to 60.82 giving the highest biological efficiency of 67.31% at C:N 60.82 and lowest biological efficiency of 18.41% at C:N 45.59 with corresponding nitrogen contents of 0.93% and 1.20%. *Pleurotus ostreatus* (being ligninolytic) requires a moderate nitrogen substrate to initiate ligninolytic activity (Knop et al., 2015) and to maintain effective mycelial growth and maximum formation of fruiting bodies (Yang et al., 2013). Other related production performance indicators, such as the highest number of shoots in the first flush and least duration to maturity, were observed in SI and SII with an average C:N ratio of 60. The difference in the C:N ratio and production performance observed across the substrates might have influenced the fermentation capacity of the fungus (Velioglu and Urek, 2015).

Production performance using SI to evaluate mean differences between the substrates is presented in Table 3. The number of shoots recorded for SI was highest and significantly different compared to SIII ( $p = 0.001$ ), SV ( $p = 0.005$ ), SVI ( $p = 0.005$ ) and SIV ( $p = 0.008$ ). Thus, in terms of mycelial viability, the treatment SI, composed of 58.82% rice husk, 10.59% rice bran and 30.59% rice straw produced the best results compared to the other treatments. The time required to reach maturity was 24 d in SI against 34 d in SIII and SV ( $p = 0.001$ ), 32 d in SVI ( $p = 0.005$ ), 30 d in SII ( $p = 0.03$ ) and 28 d in SIV. The total yield of fresh mushroom was 261.47 g from SI, 336.57 g from SII, 168.74 g from SIII and 228.75 g from SIV, where SI was not significantly different at 116.10 g ( $p = 0.01$ ) from SV and 92.05 g ( $p = 0.005$ ) from SVI. Therefore, the total fresh yield was highest

on SII whose treatment was composed of 39.23% rice husk, 17.65% rice bran and 43.14% rice straw. As expected, biological efficiency computed from the total fresh yield varied in the same manner as the total fresh yield with significant differences expressed for SV and SVI. In order to check actual differences in productivity between the flushes, the average dry weight per flush and total dry weight for the three flushes were compared. It can be observed from Fig. 1 that the first flush contributed most to the total dry yield compared to the second and third flushes, except in SV where the second flush was highest. The dry yield of the third flush visibly decreased in all substrates producing 12.38 g in SI through to 0.15 g in SV whose proportions were 72.56% rice husk, 7.72% rice bran and 19.72% rice husk. The total dry weight was highest in SII (66.92 g) compared to 17.77 g in SVI. These results were consistent with Wang et al. (2015) and Dzaka and Akpese (2017) who positively correlated a percentage increase in yield and biological efficiency to nitrogen supplementation.

#### Functional nutrients

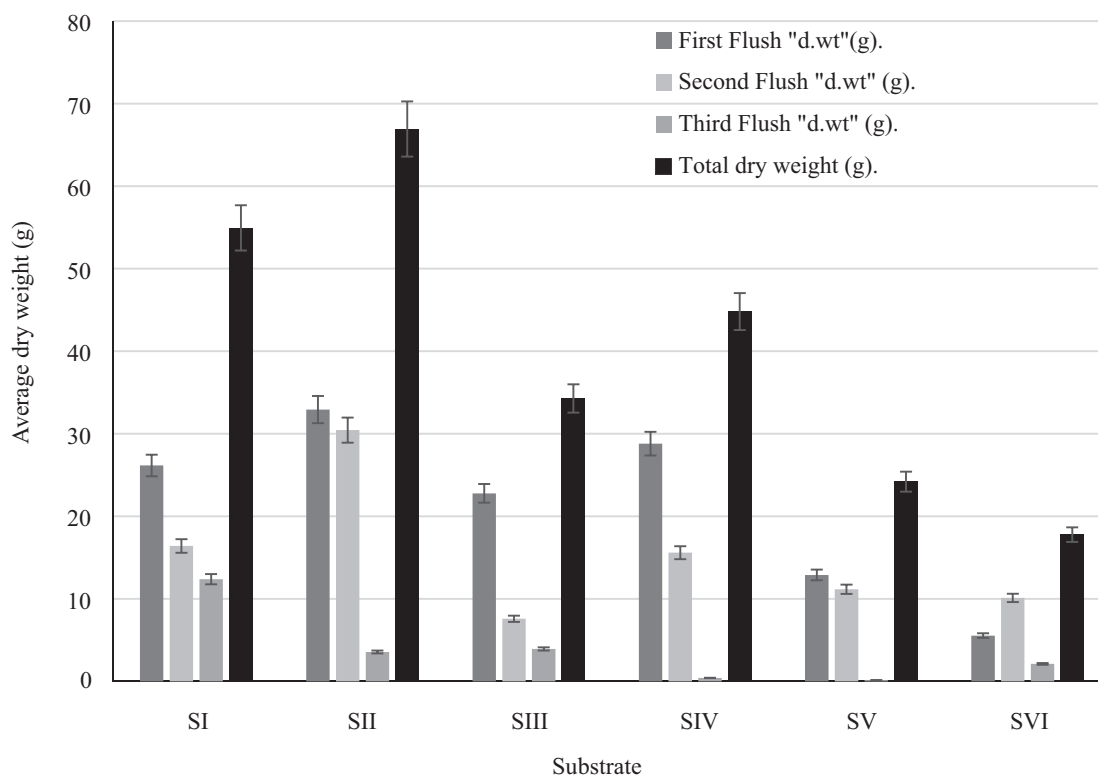
Mushrooms are regarded as a source of valuable nutrients and bioactive compounds with numerous functional properties (Ao and Deb, 2019). Carbohydrate and protein levels in all mushrooms were pronounced and comparable to earlier reports using similar methods in wild edible mushrooms (Manjunathan and Kaviyaran, 2011). The highest carbohydrate content was observed in SI (52.93 mg/mL) and the lowest in SV [28.71 mg/mL (Fig. 2)]. Total protein ranged from 42.12 µg/mL in SIII to 65.48 µg/mL in SVI. SII had 61.43 µg/mL while the total protein in SI was 58.61 µg/mL. Total ash with little variation ranged from 10.52% in SI to 13.25% in SIII averaging 11% across the substrates and was greater than the highest value of 8.19% obtained on woody substrates (Oyetayo and Ariyo, 2013). Differences across the substrates might be linked to differences in the carbon and nitrogen contents whose low ratio favors carbohydrate and polysaccharide expression while a high ratio favors amino acids, crude protein and 5'-nucleotide expressions during fungal development as observed in *Pleurotus eryngii* (Li et al., 2015). *Pleurotus ostreatus* grown on woody substrates was rich in total protein, providing eight of the 10 essential amino acids (Oyetayo and Ariyo, 2013). In the current study, the carbohydrate and ash contents varied slightly across the substrates, except for the protein content which was visibly lower in SVI.

**Table 3** Mean and mean differences of production performance indicators of *Pleurotus ostreatus* from the substrates

Dependent Variable	Substrate (J)	Mean	Mean difference (SI-J)	p-Value	95% Confidence interval	
					Lower bound	Upper bound
First flush (number of shoots)	SI	27.00	0.00	1.000	21.53	32.46
	SII	9.33	17.67*	0.005	4.72	30.62
	SIII	5.00	22.00*	0.001	9.05	34.95
	SIV	10.33	16.67*	0.008	3.72	29.62
	SV	6.33	20.67*	0.001	7.72	33.62
	SVI	5.66	21.33*	0.001	8.38	34.28
Time to maturity (d)	SI	24.33	0.00	1.000	21.63	27.03
	SII	30.66	-6.33*	0.033	27.96	33.36
	SIII	34.66	-10.33*	0.001	31.96	37.36
	SIV	28.33	-4.00	0.272	25.63	31.03
	SV	34.00	-9.67*	0.001	31.29	36.70
	SVI	32.66	-8.33*	0.005	29.96	35.36
Total yield (g)	SI	261.47	0.00	1.000	206.31	316.63
	SII	336.57	-75.10	0.349	281.41	391.73
	SIII	168.74	92.73	0.173	113.57	223.90
	SIV	228.75	32.71	0.936	173.59	283.92
	SV	116.10	145.30*	0.015	60.93	171.26
	SVI	92.07	169.43*	0.005	36.91	147.23
Biological efficiency (%)	SI	52.29	0.00	1	41.26	63.32
	SII	67.31	-15.02	0.349	56.28	78.34
	SIII	33.74	18.54	0.173	22.71	44.78
	SIV	45.75	6.54	0.936	34.72	56.78
	SV	23.22	29.07*	0.015	12.18	34.25
	SVI	18.41	33.88*	0.005	7.38	29.44

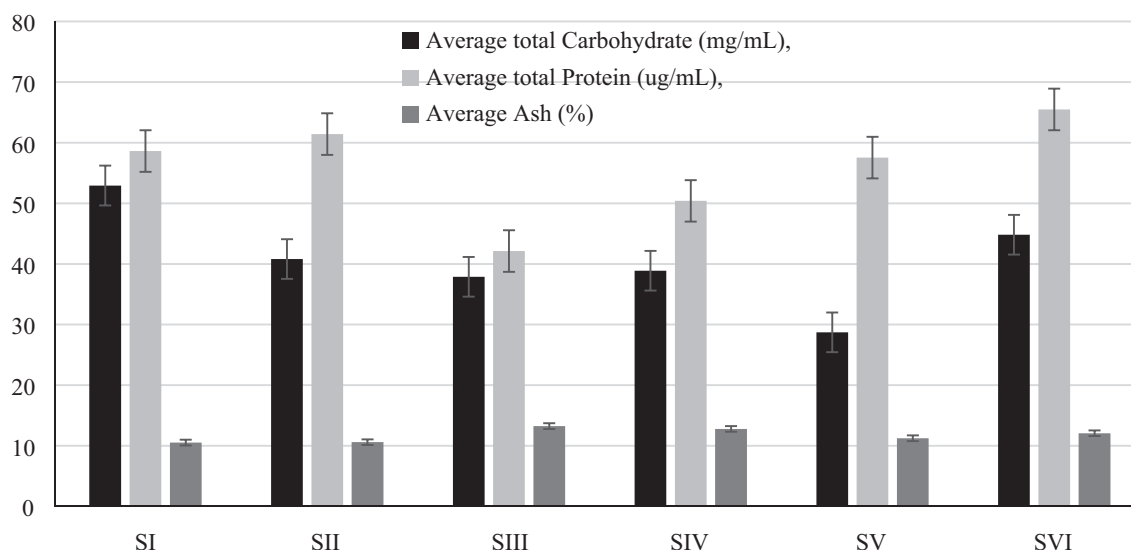
See Table 1 for details of composition of substrates S1–SVI.

\* mean difference is significant at 95% confidence interval when the substrate (SI) is compared with the other substrate (J) using Bonferroni multiple comparison adjustment.



**Fig. 1** Bar charts of dry mushroom yield harvested from three flushes cultivated on alternate rice residues, where error bars indicate  $\pm$  SD and see Table 1 for details of composition of substrates S1–SVI





**Fig. 2** Bar charts of total carbohydrate, total protein and ash content of dried mushroom cultivated on alternate rice residues, where error bars indicate  $\pm$  SD and see Table 1 for details of composition of substrates S1–SVI

Mean differences in some important health minerals of the dried mushrooms are presented in Table 4. Sodium slightly varied from 54.66 g/kg in SII to 56.30 g/kg in SVI through 56.10 g/kg in SI. A significant difference existed only between SI and SII ( $p = 0.03$ ). The potassium content of *Pleurotus ostreatus* from the rice residues was almost in the same range as sodium, with potassium greater than sodium in SII, SIV and SV. It ranged from 53.33 g/kg in SVI, 55.97 g/kg in SI to 72.51 g/kg in SII with significant variation ( $p < 0.001$ ). Similar results to those in these mentioned treatments have been observed in other mushroom species (Mallikarjuna et al., 2012) and are claimed to have blood pressure control ability in subjects suffering from hypertension (Houston, 2011). Calcium ranged from 2.26 g/kg in SVI to 3.16 g/kg in SI with significant differences expressed for SIII ( $p = 0.05$ ), SIV ( $p = 0.02$ ) and SVI ( $p = 0.01$ ) compared with SI. Magnesium ranged from 1.30 g/kg in SV to 2.54 g/kg in SIII. Lower levels were observed for magnesium compared to calcium, similar to the observations of Mallikarjuna et al. (2012) and Oyetayo and Ariyo (2013). However, Bhattacharjya et al. (2015) cultivated *Pleurotus ostreatus* on different sawdust substrates and reported higher values of magnesium compared to calcium. Calcium is recommended for bone formation and strength (Bonjour, 2011) while magnesium is primordial in the biochemical regulation of cell function (Wolf and Trapani, 2008). The phosphorus content differed significantly from 2.82 g/kg in SI to 9.03 g/kg in SIII with  $p < 0.001$  across all the substrates compared with SI. Its concentration was lower than that reported for other *Pleurotus spp.* (Mallikarjuna et al., 2012) and higher than that in *Pleurotus ostreatus* cultivated on different sawdust substrates by Bhattacharjya et al. (2015). When Frimpong-Manso et al. (2011) used rice bran and 2% rice husk without rice straw, higher values of calcium, phosphorus and carbohydrates were reported. Oyetayo and Ariyo (2013) also reported higher values of phosphorus. In addition to its role in bone health, phosphorus is a component of the genetic information of living cells. The micro element selenium

accumulated in only three of the treatments, namely SI (0.5 g/kg), SII (0.62 g/kg), and SIII (1.25 g/kg). The only significant difference was for SIII ( $p = 0.01$ ). The accumulation of selenium in plants is thought to be influenced by the soil content, redox status, pH and microbial activity (Medhi et al., 2013). Although mushrooms are generally regarded as potential selenium accumulators (Kalac, 2010), cultivated mushrooms including *Pleurotus ostreatus* are most often poor in selenium (Alam et al., 2008; Costa-Silva et al., 2011). Selenium enhancement technologies in mushroom have been practiced through substrate fortification using anhydrous sodium selenite [ $\text{Na}_2\text{SeO}_3$ ] (Werner and Beelma, 2002; Nunes et al., 2012). With selenium fortification of *Pleurotus ostreatus*, a high dosage of the substrate with selenite ( $[\text{Na}_2\text{SeO}_3] > 12.8 \text{ mg/kg}$ ) reduced mycelial proliferation (Nunes et al., 2012). Selenium is a limiting element for membranous protective selenoproteins such as glutathione peroxidase, deiodinase, selenoprotein-P and thioredoxin reductase where these complex enzyme systems in animals and humans protect cells against free radical damage or oxidative stress and also regarded as potential anti-inflammatory and anticarcinogenic agents (Mehdi et al., 2013).

In addition to selenium, high total phenolic content was observed in the substrates, specifically in SI, SIII and SVI (Fig 3) with a range from 31.91 mg GAE/g dw in SII to 186.33 mg GAE/g dw in SI with observable differences. The total phenolic contents in SIII, SVI, SIV and SV were 121.52 mg GAE/g dw, 87.24 mg GAE/g dw, 48.00 mg GAE/g dw and 44.15 mg GAE/g dw, respectively. The total phenolic content levels in the current study were comparable to earlier reports from commercial and wild mushrooms including *Pleurotus spp.* (Alvarrez-Parrella et al., 2007) but higher than the values (12.34–14.03 mg GAE/g dw) extracted in 50% volume per volume ethanol (Boonsong et al., 2016). Phenolic compounds are synthesized during solid state fermentation by fungi due to their secretory ability of hydrolytic and ligninolytic enzymes. Barbosa et al. (2008) converted ferulic acid (released by ligninolytic enzymes of the basidiomycete

*Phanerochaete chrysosporium* cultivated on green coconut husk) to vanillin. The total phenolic content in food is positively correlated with antioxidant capacity and hypoglycemic potential and thus is desirable in oxidative stress and diabetes suppression (Randhir and Shetty, 2007).

Huge quantities of rice residues are currently produced in SSA, requiring profitable exploitation. This study indicated that the oyster mushroom *Pleurotus ostreatus* can be profitably cultivated on rice residues when adequate proportions are combined of rice bran (as supplement), rice husk and rice straw. The best production substrates turned were: 1) SI (58.82% rice husk, 10.59% rice brown and 30.59% rice straw) with influential production parameters of pH 7.01, C:N 60.47 and nitrogen 1.00 mg/g; and 2) SII (39.23% rice husk, 17.65% rice bran and 43.14% rice straw) characterized by pH 7.12, C:N 60.82 and nitrogen 0.93 mg/g. Significant levels of functional health nutrients, such as protein and antioxidants, were generated across the substrates. Mushroom cultivation out-scaling is underway in Cameroon rice development hubs in response to the valuable exploitation of rice residues. The antioxidant capacity and the

glucose-lowering potential of mushrooms produced on high yielding rice substrates should be investigated.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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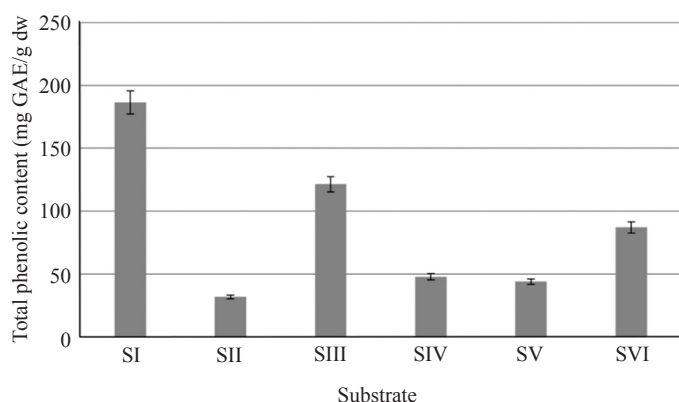
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**Table 4** Mean and mean differences of some important health minerals of *Pleurotu ostreatus* from the substrates

Dependent variable	Substrate (J)	Mean	Mean difference (SI-J)	p-Value	95% Confidence interval	
					Lower bound	Upper bound
Sodium (mg/100 g)	SI	56,10	0.00	1.000	55,49	56,71
	SII	54,66	1,44*	0,031	54,05	55,27
	SIII	55,86	0,24	0,989	55,25	56,47
	SIV	55,47	0,62	0,623	54,86	56,08
	SV	54,91	1,19	0,089	54,30	55,52
	SVI	56,30	-0,20	0,995	55,69	56,91
Potassium (mg/100 g)	SI	55,97	0.00	1.000	55,59	56,35
	SII	72,51	-16,54*	< 0.001	72,13	72,89
	SIII	52,74	3,23*	< 0.001	52,36	53,12
	SIV	65,10	-9,13*	< 0.001	64,72	65,48
	SV	68,09	-12,12*	< 0.001	67,71	68,47
	SVI	53,33	2,63*	< 0.001	52,95	53,71
Calcium (mg/100 g)	SI	3,16	0.00	1.000	2,86	3,47
	SII	2,93	0,23	0,835	2,62	3,23
	SIII	2,50	0,06*	0,049	2,19	2,80
	SIV	2,44	0,72*	0,029	2,13	2,74
	SV	2,77	0,38	0,409	2,47	3,08
	SVI	2,32	0,84*	0,011	2,01	2,62
Magnesium (mg/100 g)	SI	2,02	0.00	1.000	1,98	2,08
	SII	1,82	0,20	0,904	-0,47	0,88
	SIII	2,54	-0,52	0,182	-1,20	0,16
	SIV	1,54	0,48	0,232	-0,19	1,16
	SV	1,30	0,72*	0,035	0,04	1,40
	SVI	2,16	-0,13	0,982	-0,81	0,54
Phosphorus (mg/100 g)	SI	2,82	0.00	1.000	2,66	2,99
	SII	3,42	-0,59*	< 0,001	3,25	3,59
	SIII	9,03	-6,20*	< 0,001	8,86	9,19
	SIV	8,67	-5,85*	< 0,001	8,51	8,84
	SV	3,56	-0,74*	< 0,001	3,40	3,73
	SVI	6,85	-4,03*	< 0,001	6,69	7,02
Selenium (µg/g)	SI	0,50	0.00	1.000	0,21	0,78
	SII	0,62	-0,12	0,985	0,33	0,90
	SIII	1,27	-0,76*	0,014	0,98	1,55
	SIV	0,00	0,49	0,154	-0,27	0,29
	SV	0,00	0,50	0,145	-0,28	0,28
	SVI	0,00	0,50	0,145	-0,28	0,28

See Table 1 for details of composition of substrates S1–SVI.

\* mean difference is significant at 95% confidence interval when the substrate (SI) is compared with the other substrates (J) using Bonferroni multiple comparison adjustment.



**Fig. 3** Bar charts of total phenolic content of dried mushroom cultivated on alternate rice residues [expressed as milligram equivalence of Gallic acid per gram of dried mushroom (mg GAE/g dw)], where error bars indicate  $\pm$  SD and see Table 1 for details of composition of substrates S1–SVI

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