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Physicochemical properties and storage stability of mushrooms (*Pleurotus ostreatus*) cultivated on single (sawdust) and mixed substrates (sawdust and oil palm fibre)

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ABSTRACT

Oyster mushroom nutrients have been rated side by side with proteins in meat and eggs. Oyster mushrooms are high in vitamins and essential amino acids, but their cultivation is poor. Oil palm fibre is being used in making material strength in mechanical engineering with little or no use in the cultivation of food products. This study determined the physicochemical properties and storage stability of mushrooms cultivated on single and mixed substrates (sawdust and oil palm fibre). Oil palm fibre mixed with sawdust at different ratios (95:5, 90:10 and 85:15) was used to grow oyster mushrooms in other to turn waste to wealth. The oyster mushrooms cultivated on both single and mixed substrates were subjected to proximate composition analysis (22.99, 4.54, 6.93, and 6.98 %, for crude protein, fat, fibre, and ash), mineral content (5.92 mg/100g for sodium and 25.76 mg/100g for potassium), amino acid profile (155.85 mg/g for a total essential amino acid), fatty acid profile (43.82% for linoleic acid), anti-nutritional factor, and storage stability for three months. The mushrooms cultivated on the mixed substrate (oil palm fibre + sawdust) have a higher proximate composition, mineral content, amino acid profile, and fatty acid profiles than mushrooms cultivated on a single substrate (sawdust). Although the peroxide value of the oyster mushroom samples from the mixed substrates increased with storage time, the peroxide value was lower than 10 meq/kg of fat throughout the storage period, which means the samples could still be considered stable during storage. Cultivation of oyster mushrooms on mixed substrates of oil palm fibre and sawdust should be encouraged for highly nutritious oyster mushroom production. Oil palm fibre can also serve as a raw material in mushroom cultivation.

Introduction

Mushroom growing is all in one biotechnological process for the implementation and use of organic waste rich in lignocellulose (Mandeel et al., 2005). Mushroom nutrients have been rated side by side with proteins in meat and eggs, vitamins, and essential amino acids (Sánchez, 2010). Oyster mushrooms contain both essential and non-essential amino acids (Deepalakshmi and Mirunalini, 2014) and the cultivation of oyster mushrooms has improved all over the world due to their nutritional composition (Akindahunsi and Oyetayo, 2006) and ability to withstand harsh environmental conditions.

Nutritional composition of oyster mushrooms (*Pleurotus ostreatus*) grown on softwood (*Daniella oliver*) and hardwood (*Anogeissus leiocapus*) was reported by Ogundele et al. (2017). They reported a higher yield of oyster mushrooms (*Pleurotus ostreatus*) from softwood and higher nutritional composition of oyster mushrooms from hardwood. Also, Ogundele et al. (2014) reported the effects of pure and mixed substrate on the nutritional composition of oyster mushrooms (*Pleurotus ostreatus*). Oyster mushrooms (*Pleurotus ostreatus*) harvested from mixed substrate were reported to have higher nutritional composition than those harvested from pure substrate.

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Solid waste management has emerged in concert with the best challenges facing several developing countries. Daily human activities such as burning sawdust from sawmills caused a lot of environmental pollution in several cities in developing nations worldwide (Olukanni et al., 2016). The factors affecting such a high rate of waste generation include lifestyle, financial gain growth, and increasing use of disposable materials (Olukanni and Oresanya, 2018). Sawmills have been one of the major industries where waste is generated, and such waste (sawdust) has been found useful as a substrate in the cultivation of mushrooms (Ogundele et al., 2017).

Another type of waste generated from the palm oil industry is oil palm fibre, which is commonly used in making fire briquette in developing countries (Embrandiri et al., 2015). Oil palm fibre has been found useful in the engineering field to make concrete (Musa et al., 2019) and fire boilers in developed countries (Hassan and Ghaffari, 2014). Oil palm fibre is rich in crude oil (2%) (Koba and Ishizaki, 1990) and it may serve as a good substrate for the production of mushrooms (*Pleurotus ostreatus)* with higher crude oil content. Okjuoya (1991) reported a high yield of Pleurotus tuber-regium cultivated of oil palm fibre alone, which suggest that oil palm fibre can be used to cultivate other types of oyster mushrooms.

The use of sawdust in the cultivation of mushrooms is being reported by different researchers (Hoa et al., 2015; Ogundele et al., 2014 and 2017). Sawdust, which is a waste material, is being found useful in cultivating the various types of mushrooms which are sold and help in supplementing animal proteins. Other waste products (sugarcane bagasse and corncob) are being combined with sawdust to cultivate oyster mushrooms (Hoa et al., 2015) and such mushrooms were reported to have higher nutritional composition and yield. Since oil palm fibre can be easily sourced in our country and contains better nutritional content than sawdust. The combination of the two as the substrate in cultivating oyster mushrooms will help to reduce waste in our country and also provide a substrate with higher nutritional composition for a higher yield (Hoa et al., 2015).

In mushroom cultivation, to the best of our knowledge, there is no report on the use of oil palm fibre in combination with sawdust as a substrate for the growth of mushrooms. It can be hypothesized that the use of oil palm fibre with sawdust from hardwood (*Anogeissus leiocapus*) will help in producing mushrooms which are rich in oil and crude fibre. Also, the use of oil palm fibre in combination with sawdust from hardwood (*Anogeissus leiocapus*) will help in converting waste to wealth. Hence, this study is aimed at determining the effects of the mixed substrate (oil palm fibre and hardwood sawdust) on the physicochemical properties and storage stability of oyster mushrooms cultivated on the mixed substrate, since sawdust and oil palm fibre are very common in the country (Nigeria) and they are both counted as waste material.

Materials and methods

Preparation of Spawn

Oyster mushroom spawn was prepared using the method described by Ogundele et al. (2017), the method was used to prepare oyster mushroom spawn. Briefly, sorghum grains, sterile plastic bottles, cotton plants, aluminium foil, water, and previous spawns were used in preparing the spawn. Light dirt and dust were removed by inspiration, and sorting was used to remove unwanted materials like stone, metallic elements, and broken food grains. Sorted Sorghum grains were soaked in clean water overnight after washing. The following day, it was re-washed, drained, and steamed for 30-45 min (70 °C). The steamed grains were drained and spread to cooled in a sterile covered compartment. Three-quarter of the sterile bottles were filled with sorghum and the bottle's oral cavity was wadded with cotton wool and wrapped with aluminium foil. Wadded bottles were sterilized in an autoclave (Vertical autoclave, Mycological equipment, UK) at 121 ºC for 15 minutes and allowed to cool, and inoculated with 1/10 of the previous spawn as inoculums. The inoculated bottles were incubated until the mycelium completely colonized the emergence medium.

Preparation of substrate

Pure sawdust from hardwood (*Anogeissus leiocapus*) was obtained from the sawmill within the town (Offa, Kwara State). Oil palm fibre was collected from local palm oil producer also in Offa Kwara State. Sawdust and oil palm fibre were mixed in different ratios (sawdust alone, 95:5 oil palm fibre, 90:10 oil palm fibre, and 85:15 oil palm fibre). These substrates were mixed with calcium carbonate (0.5%), rice bran (0.5%), (to improve the nutritional composition of the substrate), and water (to moisten the substrate). All the component constituents were mixed with a mixer (spiral mixer h30, NY) and water was added to make a 65% moisture level of the mixture. The mixture was left for 45 minutes for water absorption and moisture uniformity within the substrate.

The substrate (500 g) was then filled into polyethylene bags (Size 7" X 10") and their lip was hacked by inserting water absorbing cotton wool with the help of a plastic neck and a rubber stripe. The substrates were pasteurized using an autoclave (Vertical autoclave, Mycological equipment, UK) at 121 °C for 1 h, cooled to room temperature (25 °C) and inoculated with oyster mushrooms (*Pleurotus ostreatus*). The inoculated bags were incubated in the culture room at 25 °C, during which the inoculated room temperature was maintained (25 °C). Also, the humidity of the incubated room was maintained (70-80 %) by the sprinkling of water on the floor four times a day and measured using a hand-held hygrometer (MDM 25, UK).

Harvesting and preparation of oyster mushroom fruits

Fruits were harvested on the substrates three times (2 weeks interval). Each harvest was weighed, and the fresh weight was recorded. The second harvest and the third harvest of the oyster mushroom fruit were also weighed, and the fresh weight recoded. The three harvest samples were dried in a forced-air oven (Whirlpool, South Africa) at 40 °C. Dried samples were milled into powder (Waring blender, Canada) and stored at refrigeration temperature (4 °C) for further analysis.

Proximate composition of oyster mushroom flour

AOAC (2006) standard methods were used to determine moisture content (AOAC – 934.01), crude fat content (AOAC – 922.06), crude fibre (AOAC - 978.10), and ash (AOAC – 923.03). Protein (AOAC – 990.03) content (Nx6.25) was determined by the micro Kjeldahl method. Carbohydrate contents were calculated by way of difference.

Mineral analysis of oyster mushroom flour

Mineral contents (sodium, potassium, magnesium, copper, phosphorous, and zinc) of the oyster mushroom flour were determined using the AOAC (2006) standard method (AOAC, 2006 984.27). Ash dissolved in concentrated nitric acid, with 50 ml of deionized water was analysed using an atomic absorption spectrophotometer (GBC 904AA, EVISA, Germany). The flame photometer was used to determine the sodium and potassium content of the samples. Phosphorus was determined using a UV-spectrophotometer (BestEquip, USA). The formed complex (ammonium vanadate molybdate) was read at 450 nm.

Amino acid profile of oyster mushroom flour

The modified method of Sumbo and Victor (2014) was used to determine the amino acid content of the samples. The amino acid content of oyster mushroom flour was determined using the Technicon sequential multisample (TSM) analyser. Briefly, the defatted oyster mushroom flour was hydrolysed with HCl under vacuum in a sealed Pyrex tube for 24 h. The hydrolysed sample was cooled and filtered, after which it was dried at 40 °C. About 5 mL of acetate buffer (pH 2.0) and 5 to 10 µL was used to dilute the filtrate before injecting into the TSM amino acid analyser. The peak area was calculated as the concentration of each amino acid.

Fatty acid profile of oyster mushroom flour

The fatty acid profile of the oyster mushroom flour was determined by the method of Ekunseitan et al. (2017) with the help of gas-liquid chromatography (Omega-Wax Capillary Supelco, USA). The mixture of n-hexane, ethyl ether, and acetic acid (70: 28: 2) was used as a mobile phase in thin-layer chromatography with silica gel G60 (Merck, Darmstadt, Germany) to separate the fatty acid composition in the flours.

Storage stability of oyster mushroom flour

The storage stability of oyster mushroom flour was studied. About 100 g of flour was packed in opaque polyethylene bags (150 x 180 mm- 40mic) and placed on the shelf at ambient temperature (25 °C). During storage, changes in peroxide values were analysed at a fourteen-day storage interval (2 weeks) for 90 days of storage (3 months).

Peroxide value of oyster mushroom flour

Peroxide values of the oil extracted from oyster mushroom flour by petroleum ether were determined as described in the methods of AOAC. (2006-965.33). Five (5 g) sample of oil was weighed into a 250 ml Erlenmeyer flask and then 30 ml of acetic acid chloroform solution (3:2) solution was added. The flask was swirled until the sample was dissolved, and 0.5 ml of saturated potassium iodide (KI) solution was also added. The solution was allowed to stand with occasional swirling for 1 min and then 30 ml of distilled water was added. The

solution was titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until the colour changed to light yellow. 0.5 ml of 1% soluble starch indicator was added. The formed blue solution was titrated with more sodium thiosulfate until the blue colour disappeared.

Peroxide value was calculated as:

$$\text{Peroxide value (meq (PV/kg)) sample} = \frac{SxNx1000}{Weight\ of\ the\ sample}$$

where S = ml of $Na_2S_2O_3$ and N = 0.01 sodium thiosulfate.

Anti-nutrient determination

The total cyanide content of the oyster mushroom flour was determined by the standard method of AOAC (2000). About 5 g of the flour was soaked in the mixture of 3 ml of orthophosphoric acid and 50 ml of distilled water overnight to free the bound cyanide. The distillate was titrated against $AgNO_3$ (0.01 mol/l). The tannin content of the flour (oyster mushroom flour) was determined by the method of Akindahunsi & Oyetayo (2006). 200 mg of the flour was extracted with acetone (70%). 0.5 ml of folin and 2.5 ml of $NaCO_3$ (20%) were added to the extracted sample and read at 750 nm wavelength (spectrophotometer). The result was compared with the prepared serial diluted tannic acid standard solution. Phytate content was determined using the method described by Frühbeck et al. (1995). The wavelength of 500 nm was utilized to quantify the absorbance of the result of the response between phytate, ferric chloride, and sulphosalicylic acid. Phytate extracted from the oyster mushroom flour was purified with 0.66M HCl by anion exchange chromatography (Dowex 1 anion-exchange resin) before the reaction with the ferric chloride and the sulphosalicylic acid reagent. The phytate content was determined from a standard curve prepared with sodium phytate (Sigma-Aldrich) solution and expressed as mg/100g sample.

Statistical Analysis

All data obtained was subjected to analysis of variance (ANOVA) using the Statistical Package for Social Science (IBM Corp. Released, 2012). Significant means were separated using the Duncan's multiple comparison tests at 5% level of probability.

Results and discussions

Oyster mushroom yield

The average pinhead, fresh fruit height, fresh fruit weight and average dry weight of oyster mushrooms harvested on the substrates are shown in Table 1. Fresh fruit harvested on sawdust substrate has the least pinhead, fruit height, and fruit weight, while the parameters mentioned increased in the fresh fruits harvested on other substrates (sawdust + oil palm fibre at different ratio). The mixed substrate with the highest percentage of oil palm fibre has the highest values in all the measured parameters. The increase in the size of all the measured parameters in the mushroom fruits may be due to the nutrients that were present in the oil palm fibre (Chiejina and Osibe, 2015). Hoa et al. (2015) reported the effects of different substrates on the yield and nutritional qualities of two mushrooms (*Pleurotus ostreatus* and Pleurotus cystidiosus). They reported that the substrate with higher carbon and nitrogen ratio gives the best yield, and such yield has higher nutritional composition. Furthermore, Hoa et al. (2015) attributed the differences in protein content of the mushrooms cultivated on a different substrate to the ability to utilize nitrogen present in the substrate efficiently, in which some mushroom species may not be able.

Proximate composition

Table 2 shows the proximate composition of oyster mushrooms grown on a mixed substrate (sawdust and oil palm fibre). Mushrooms grown on sawdust alone gave the highest moisture content (7.59%) and slightly decreased to 7.49, 7.10, and 7.01 % as oil palm fibre content increased in the mixed substrates A, B, and C, respectively with no significant difference ($P > 0.05$) between the mushrooms grown on substrate A (SD + 5% oil palm fibre). The protein contents of the oyster mushrooms grown on the mixed substrate were higher than oyster mushrooms grown on sawdust alone. There was no significant difference among the protein contents of the mushrooms grown on the mixed substrate, but there was a significant difference ($P < 0.05$) between the protein content of the oyster mushrooms grown on sawdust alone and that of the mixed substrate. Although there was a slight increase in value among the oyster mushrooms grown on the mixed substrate.

The crude fat values of the oyster mushrooms grown on the mixed substrate were higher than oyster mushrooms grown on sawdust alone. The crude fat content of the oyster mushrooms grown on mixed substrate increased with the increase in the amount of oil palm fibre added to sawdust.

Table 1. Average weight of harvested fresh (three flushes) and dried Oyster mushrooms

Samples	Average pin Head (mm)	Average fresh fruit Height (mm)	Average fresh fruit weight (g)	Average dry weight (g)
A	12.22±0.3[a]	8.21±0.3[a]	135.64±0.3[a]	20.34±0.3[a]
B	15.40±0.5[b]	10.21±0.5[b]	165.36±0.5[b]	25.72±0.2[b]
C	16.21±0.1[c]	10.56±0.2[b]	183.77±0.1[c]	28.96±0.2[c]
D	16.91±0.1[c]	11.22±0.1[c]	208.65±0.1[d]	32.56±0.1[d]

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly (p>0.05) different according to the LSD test.* Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre).

Table 2. Proximate composition (%) of oyster mushrooms grown on mixed substrate (sawdust and oil palm fibre)

Samples	Moisture	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate
A	7.59±0.4[b]	26.85±0.3[a]	0.94±0.1[a]	5.77±0.3[a]	6.06±0.2[a]	52.79±0.6[d]
B	7.49±0.3[b]	29.24±0.6[b]	1.32±0.3[b]	6.73±0.2[b]	6.68±0.2[b]	48.54±0.5[c]
C	7.10±0.5[a]	29.68±0.4[b]	1.56±0.5[c]	6.84±0.3[b]	6.91±0.3[b]	47.91±0.4[b]
D	7.01±0.4[a]	29.99±0.6[b]	2.54±0.3[d]	6.93±0.4[b]	6.98±0.4[b]	46.55±0.3[a]

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly (p>0.05) different according to the LSD test.* Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre).

There was a significant difference ($P < 0.05$) in crude fat values of all the oyster mushrooms, with oyster mushrooms grown on a mixed substrate (SD + 15% OPF) having the highest value (4.54%). The crude fibre of all the oyster mushrooms grown on both sawdust and mixed substrate (SD+OPF) have different values with significant differences at $P < 0.05$. The crude fibre value of the oyster mushrooms grown on sawdust was lower than for oyster mushrooms grown on the mixed substrate. The ash content of oyster mushrooms grown on sawdust alone was lower than that of oyster mushrooms grown on a mixed substrate. The carbohydrate content of the samples was significantly different at $P < 0.05$. Oyster mushrooms grown on sawdust alone have the highest value of carbohydrates (52.79%), while the carbohydrates of the oyster mushrooms grown on mixed substrate decreased with an increase in the amount of OPF added.

The differences in moisture, crude protein, and crude fat content observed among the samples may be due to the difference in the substrates used to cultivate the oyster mushrooms. The addition of oil palm fibre to sawdust may be attributed to the increase in crude protein, crude fat, crude fibre, and ash contents of the oyster mushrooms harvested on the mixed substrate when compared with oyster mushrooms grown on sawdust alone. This is because oil palm fibre has been reported to contain 5.6% of nitrogen, 1.9% of lipids, and fibre (homocellulose 59.6%, and lignin 28.5% (Koba and Ishizaki, 1990) when compared to sawdust which contained 0.9% of nitrogen and 60.8% of carbon (Phonphuak and Chindaprasirt, 2014). The composition of the nutrients which are present in the substrate used in the cultivation of mushrooms has been reported to contribute to the yield and the nutritional composition (Hoa et al., 2015). The protein content (26.85%) of the oyster mushrooms grown on sawdust alone was similar to the protein content (26.67%) of the previous reports on the oyster mushrooms cultivated on sawdust of hardwood (Ogundele et al., 2017). The decrease in the carbohydrate content of the oyster mushrooms grown on the mixed substrate may be due to an increase in other nutritional components (Crude protein, crude fat, fibre, and ash).

Minerals

Table 3 shows the mineral composition of oyster mushrooms on sawdust and mixed substrate (Sawdust alone (SD), sawdust + oil palm fibre (SD+OPF)). All the minerals (sodium, potassium, magnesium, copper, phosphorus, and zinc) increased with an increase in the percentage of oil palm fibre added to sawdust. Potassium was the most abundant mineral present in all the mushrooms (24.56 mg/100 g grown on sawdust alone to 25.76 mg/100 g on SD + 15% OPF), followed by sodium (4.32 mg/100 g grown on sawdust alone to 5.76 mg/100 g on SD + 15% OPF). Copper was the least abundant mineral with the value ranging between 0.15 mg/100 g for oyster mushrooms grown on sawdust alone (SD) and 0.18 mg/100 g for oyster mushrooms grown on SD + 15% OPF. The increase in the minerals may be attributed to the addition of oil palm fibre to sawdust to grow the oyster mushrooms.

This result agrees with the report of Akindahunsi and Oyetayo (2006) who reported higher potassium content (3.3 mg/100 g) in *Pleurotus tuber-regium* obtained in the market. The potassium content in the study was higher (4.32 mg/100 g) than the report of Akindahunsi and Oyetayo (2006) and this may be due to a different type of mushrooms analysed, since *Pleurotus ostreatus* can utilize the nutrients in the substrate than *Pleurotus tuber-regium* which is cultivated by the use of its sclerotia (Okjuoya, 1991).

Table 3. Mineral content (mg/100g) of oyster mushrooms grown on mixed substrate (sawdust and oil palm fibre)

Minerals	A	B	C	D
Sodium	4.32±0.3[a]	5.01±0.3[b]	5.92±0.3[b]	5.76±0.3[b]
Potassium	24.56±0.5[a]	25.26±0.5[b]	25.56±0.5[b]	25.76±0.2[b]
Magnesium	1.56±0.1[a]	1.60±0.2[b]	1.67±0.1[b]	1.67±0.2[b]
Copper	0.15±0.1[a]	0.17±0.1[b]	0.18±0.1[b]	0.18±0.1[b]
Phosphorus	1.58±0.2[a]	1.78±0.2[b]	1.76±0.1[b]	1.76±0.1[b]
Zinc	0.31±0.1[a]	0.37±0.1[b]	0.35±0.1[b]	0.35±0.1[b]

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly (p>0.05) different according to the LSD test.* Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre).

Table 4. Amino acid profile (mg/g) of oyster mushrooms grown on mixed substrate (sawdust and oil palm fibre)

Amino Acids	A	B	C	D
Tryptophan	5.21±0.1[a]	6.31±0.4[b]	6.40±0.3[b]	6.51±0.2[b]
Threonine	21.40±0.4[a]	25.01±0.5[b]	25.21±0.5[b]	25.50±0.5[b]
Isoleucine	12.31±0.3[a]	19.20±0.4[b]	19.51±0.4[b]	19.41±0.4[b]
Valine	22.40±0.2[a]	27.81±0.3[b]	27.60±0.6[b]	27.50±0.5[b]
Lysine	16.41±0.1[a]	20.41±0.5[b]	20.61±0.2[b]	20.81±0.3[b]
Methionine	1.20±0.3[a]	2.10±0.1[b]	2.21±0.1[b]	2.40±0.1[b]
Phenylalanine	12.10±0.5[a]	16.81±0.3[b]	16.90±0.3[b]	17.10±0.2[b]
Histidine	9.81±0.1[a]	11.60±0.4[b]	11.51±0.4[b]	11.71±0.2[b]
Leucine	21.40±0.3[a]	24.31±0.5[b]	24.21±0.3[b]	24.51±0.3[b]
Total EAA	**122.24±0.4[a]**	**153.56±0.5[b]**	**154.36±0.6[c]**	**155.85±0.5[d]**
Aspartic acid	29.20±0.4[a]	36.81±0.3[b]	36.91±0.6[b]	36.90±0.3[b]
Glutamic acid	41.81±06[a]	52.61±0.5[b]	53.01±0.5[b]	52.91±0.4[b]
Glycine	3.61±0.1[a]	4.10±0.2[b]	4.31±0.1[b]	4.20±0.2[b]
Alanine	19.40±0.2[a]	23.21±0.4[b]	23.40±0.3[b]	23.61±0.3[b]
Cystine	1.61±0.1[a]	2.40±0.1[b]	2.51±0.2[b]	2.50±0.1[b]
Arginine	20.41±0.3[a]	26.71±0.3[b]	26.80±0.5[b]	26.51±0.5[b]
Serine	7.40±0.5[a]	10.10±0.4[b]	10.41±0.4[b]	10.31±0.2[b]
Proline	1.80±0.3[a]	2.90±0.2[b]	2.71±0.3[b]	2.80±0.1[b]
Tyrosine	1.21±0.1[a]	2.31±0.1[b]	2.30±0.1[b]	2.51±0.3[b]
Total Amino acids	**248.6±0.8[a]**	**314.71±0.7[b]**	**316.72[b]±0.8[b]**	**318.10[b]±0.9[b]**

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly (p>0.05) different according to the LSD test.* Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre). EAA= Essential Amino Acids

The mineral content results in this study were higher than the mineral content of oyster mushrooms grown on sawdust of hardwood (Ogundele et al., 2017). The mineral content of the oyster mushrooms grown on mixed substrate was expected to be higher than that of oyster mushrooms grown on sawdust alone because the ash content of oyster mushrooms on mixed substrates was higher. Mineral content of mushrooms is being associated with the biological nature and chemical composition of the substrates used for cultivation (Jin et al., 2018).

Amino acid Profile

The amino acid profile of the oyster mushrooms grown on sawdust alone (SD) and mixed substrate (SD + OPF) was shown in Table 4. Threonine, valine, and leucine were higher among the essential amino acids present in the oyster mushrooms grown on both substrates. Other essential amino acids are also present in adequate proportions. The essential amino acids in oyster mushrooms grown on the mixed substrate were higher than that of those grown on sawdust alone. There was no significant difference ($P > 0.05$) among the essential amino acid of *Pleurotus ostreatus* grown on different substrates. When compared with the essential amino acid value of oyster mushrooms grown on sawdust alone, the essential amino acid values from oyster mushrooms grown on the mixed substrate were higher. The total essential amino acids for all the oyster mushroom grown on the two substrates (SD alone & SD+OPF) showed that oyster mushrooms grown on mixed substrates are better than those grown on the single substrate.

Glutamic acid and aspartic acid were higher in value among the non-essential amino acids of oyster mushrooms grown on sawdust alone and sawdust mixed with oil palm fibre. The other non-essential amino acids like (arginine & alanine) also have higher values (20.41 & 19.40 mg/g). There was an increase in the value of non-essential amino acids determined from the oyster mushrooms grown on a mixed

substrate (SD + OPF). The value of non-essential amino acids from oyster mushrooms grown on a single substrate (SD) was lower than that of oyster mushrooms grown on the mixed substrate.

The increase in the value of essential amino acids from the mushrooms grown on the mixed substrate may be attributed to the mixed substrate used to cultivate the oyster mushrooms. Oil palm fibre which was mixed with sawdust contained 3.6% of protein which may contribute to the high value of essential amino acids determined in the oyster mushrooms grown on the substrate. Aspartic and glutamic non-essential amino acids are the precursor from which the backbones of amino acids are formed, this may be responsible for their high values in the mushrooms. Akindahunsi and Oyetayo, (2006) reported a high value of threonine, valine, and leucine among the essential amino acids and aspartic and glutamic acids among the non-essential amino acids of *Pleurotus tuber-regium*. Although, the values of amino acids reported by Akindahunsi and Oyetayo, (2006) were higher than the values of amino acids in this study. This may be due to different types of oyster mushrooms and the process of cultivation which was not known for *Pleurotus tuber-regium* analysed by Akindahunsi and Oyetayo, (2006). The increase in the amino acids of oyster mushrooms grown on the mixed substrate (SD+OPF) showed that oil palm fibre can be used to cultivate oyster mushrooms, although it was not used alone in this study.

Fatty acid profile

The fatty acid profile of the oyster mushrooms grown on SD alone and SD+OPF is shown in Table 5. The oyster mushrooms contained both saturated fatty acids (lauric (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) and unsaturated fatty acids (oleic acid (C18:1) and linoleic acid (C18:2). Palmitic acid has the highest value among the saturated fatty acids and lauric acid has the lowest. Linoleic acid was the highest between the unsaturated fatty acids while oleic acid was the lowest. The values of unsaturated fatty acids in the mushrooms was higher than the value of saturated fatty acids in this study. This result is supported by the findings of (Pedneault et al., 2007) who reported that oleic and linoleic acids were the most common unsaturated fatty acid lipids of oyster mushrooms.

Abeer et al. (2013) also reported that oleic and linoleic acids are common precursors during biosynthesis and metabolism of other fatty acids. The fatty acid profiles of the oyster mushrooms grown on a single substrate (SD) were lower in both saturated and unsaturated fatty acids, and the value of the fatty acids increased with an increase in the amount of oil palm fibre added to sawdust to grow the oyster mushrooms.

The increase in the amount of the fatty acid profile of the oyster mushrooms grown on the mixed substrate may be attributed to the addition of oil palm fibre to sawdust to cultivate or grow the oyster mushrooms. This is supported by the increase in crude fat (Table 1) value of the oyster mushrooms cultivated/grown on the mixed substrate (SD+OPF). The increase in the fatty acid profile may be due to the residue of crude palm oil which may be present in oil palm fibre mixed with sawdust to grow the oyster mushrooms. The oyster mushrooms were able to utilize the nutrients in the substrate to produce oyster mushrooms with high fatty acids.

The increased value of unsaturated fatty acids compared to saturated fatty acids observed in this study showed that the mixed substrate (SD+OPF) was better than sawdust alone to grow oyster mushrooms. Laoteng et al. (2011) reported that an increase in saturated fatty acids can be used as a selection criterion for the unfavourable conditions for the cultivation of oleaginous fungi. They further reported that the accumulation of lipids and the synthesis of saturated fatty acids in fungi are complex and closely associated with cell physiology and the environmental adaptation of cells.

Table 5. Fatty acid profile (%) of oyster mushroom (*Pleurotus ostreatus*) flour grown on mixed substrate (sawdust and oil palm fibre)

Samples	Fatty acid profile					
	Lauric acid (C12:0)	Myristic acid (C14:0)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
A	2.21±0.1^{a}	5.81±0.2^{a}	16.51±0.5^{a}	5.41±0.1^{a}	10.92±0.4^{a}	42.60±0.5^{a}
B	5.71±0.2^{b}	6.22±0.2^{b}	20.81±0.3^{b}	5.90±0.5^{a}	16.81±0.3^{b}	42.81±0.4^{a}
C	7.20±0.3^{c}	8.11±0.1^{c}	24.52±0.4^{c}	6.21±0.1^{b}	19.62±0.5^{c}	43.61±0.3^{b}
D	9.51±0.2^{d}	10.72±0.5^{d}	27.61±0.2^{d}	7.53±0.3^{c}	22.81±0.4^{d}	43.82±0.4^{b}

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly (p>0.05) different according to the LSD test.* Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre).

The increase in the nutritional composition of the oyster mushrooms grown on mixed substrate propels us to determine the anti-nutritional factors of the oyster mushrooms grown on both substrates. This was done to ensure that the oyster mushrooms grown on both substrates (SD alone and mixed substrate (SD+OPF) have a lower concentration of anti-nutrients.

Anti-nutritional factors

Table 6 shows the results of the anti-nutritional factors of oyster mushrooms grown on the substrates. The total cyanide content of the samples (oyster mushrooms) ranged from 0.10 mg/100 g in oyster mushrooms grown on sawdust alone to 0.26 mg/100 g in oyster mushrooms grown on a mixed substrate (SD+15% OPF). The total cyanide content increase with the increase in the percentage of oil palm fibre added to sawdust to grow the mushrooms. The tannin content in the oyster mushrooms followed the pattern of cyanide. The tannin content ranged between 0.21 mg/100 g in oyster mushrooms grown of the single substrate (sawdust) and 0.35 mg/100 g in oyster mushrooms grown on the mixed substrate. The phytate content of the oyster mushrooms grown on sawdust was 0.13 mg/100 g, while the phytate content of oyster mushrooms grown on mixed substrate ranged from 0.24 mg/100 g (SD+5% OPF) to 0.39 mg/100 g (SD+15% OPF). It can be said that the addition of oil palm fibre to sawdust to grow the oyster mushrooms contributed to the increase in the anti-nutritional factors determined in this study, but the value of the anti-nutritional factors was below the values that can inhibit nutrient present in the oyster mushrooms.

Cyanide is present in blood at a lower concentration (12 µmol), increase in the amount of cyanide in blood can lead to the inhibition of the respiratory chain which can cause respiratory problems (Leavesley et al., 2008) and this made the World Health Organisation (WHO) set a safe limit of 10 ppm total cyanide in food (FAO/WHO, 1995). Although, different countries set a different safe limit of total cyanide in food (Burns et al., 2012). The value of cyanide in this study was higher than the value of cyanide reported by Akindahunsi and Oyetayo, (2006) for *Pleurotus tuber-regium*. This may be due to a different cultivar of mushrooms and a different growth medium (Hoa et al., 2015). The presence of oil palm fibre in the substrate may also be responsible for the increase in tannin and phytate contents of the oyster mushrooms harvested on the mixed substrate when compared to oyster mushroom harvested on a single substrate. The tannin content in this study is similar to the tannin content reported for *Pleurotus tuber-regium* by Akindahunsi and Oyetayo (2006). The phytate value of the oyster mushrooms in this study was lower than the value reported by Akindahunsi and Oyetayo (2006) and this may be due to the cultivar of the analysed mushrooms (*Pleurotus tuber-regium*). The value of the phytate may not hinder the uptake of the minerals present in the oyster mushrooms because phytic acid forms a complex with mineral ions rendering them bio-unavailable for the body (Lopez et al., 2002).

Peroxide value during storage

Fig. 1 shows the results of the peroxide value of stored oyster mushrooms grown on single and mixed substrate kept at room temperature for 3 months. The peroxide value of the extracted oil from the oyster mushrooms increased with the increase in storage time.

Oyster mushrooms grown on single substrate had the least peroxide value and the oyster mushrooms cultivated on a mixed substrate with 15% oil palm fibre (OPF) had the highest peroxide value. The peroxide value was higher in all the samples (oyster mushroom) cultivated on a mixed substrate when compared with the single substrate throughout the storage period. This may be attributed to the increase in crude fat in the samples which undergo oxidation. Oxidation of free fatty acid in crude fat leads to the production of peroxide (Choe and Min, 2006). Production of lipid hydroperoxide has been reported to be low at room temperature and this is because hydroperoxides are relatively stable at room temperature (Choe and Min, 2006), and this may be responsible for the values obtained in all the samples which were lower than 10 meq/kg fat throughout the storage period.

Table 6. Anti-nutritional content (mg/100g) of oyster mushrooms grown on mixed substrate (sawdust and oil palm fibre)

Samples	Total cyanide	Tannin (% TA)	Phytate
A	$0.10^{a}\pm0.1^{a}$	$0.21^{a}\pm0.1^{a}$	$0.13^{a}\pm0.1^{a}$
B	$0.20^{b}\pm0.2^{b}$	$0.26^{a}\pm0.1^{a}$	$0.24^{b}\pm0.1^{b}$
C	$0.24^{b}\pm0.2^{b}$	$0.32^{b}\pm0.2^{b}$	$0.32^{c}\pm0.2^{c}$
D	$0.26^{b}\pm0.1^{b}$	$0.35^{b}\pm0.3^{b}$	$0.39^{c}\pm0.1^{c}$

Mean + SD (n=3). *Means followed by the same letter within the same column are not significantly ($p>0.05$) different according to the LSD test.*
Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre).

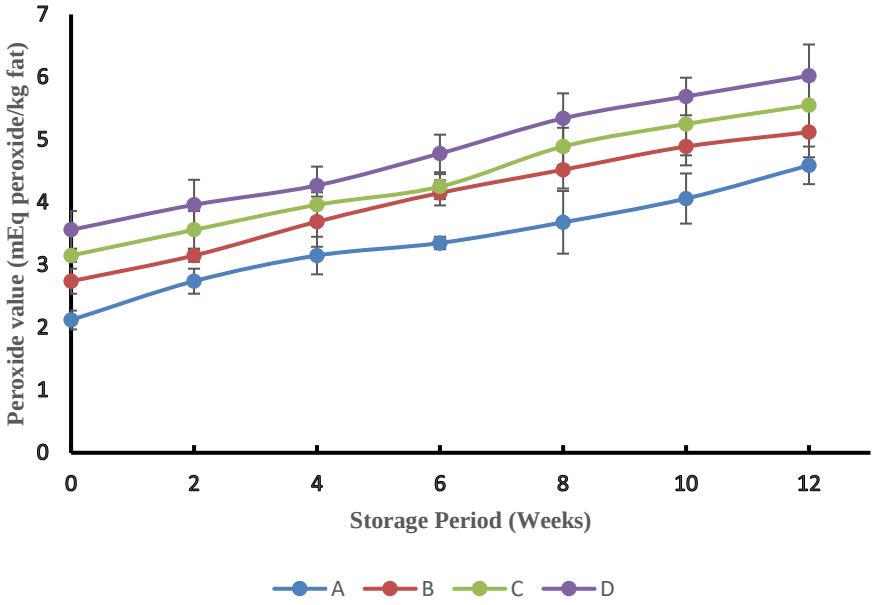

Fig. 1. Peroxide value of stored oyster mushroom (*Pleurotus ostreatus*) flour grown on mixed substrate (sawdust and oil palm fibre). Keys: A is oyster mushrooms cultivated on single substrate (sawdust alone); B is oyster mushrooms cultivated on mixed substrate (sawdust + 5% oil palm fibre); C is oyster mushrooms cultivated on mixed substrate (sawdust + 10% oil palm fibre); D is oyster mushrooms cultivated on mixed substrate (sawdust + 15% oil palm fibre)

Conclusion

The use of oil palm fibre with sawdust helps to improve the crude fat, crude fibre, and ash content of the oyster mushroom. The addition of 15% of oil palm fibre to sawdust can be a good substrate for the cultivation of oyster mushrooms which are stable at room temperature during storage. The oyster mushrooms cultivated on both substrates have good nutritional qualities, but the oyster mushrooms grown on the mixed substrate is better in terms of protein, amino acid profile, and fatty acid profile when compared with oyster mushrooms grown on sawdust alone, but higher in anti-nutritional compounds.

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