



Effect of different harvest period on the yield, proximate, mycochemical Composition of *Pleurotus pulmonarius*

Ndugbu M N, Onuegbu N C, Omeire G C, Nwokeke B C

Department of Food Science and Technology, Federal University of Technology, Owerri, Nigeria

Abstract

Mushrooms are cultivated for their nutritional attributes and potential application in industries especially in the food and pharmaceutical industry. *Pleurotus pulmonarius* is an edible mushroom with a high nutritive value and flavour. The effect of different harvest period (day 2, 3 & 4) on the yield, proximate, aroma and mycochemical composition of *Pleurotus pulmonarius* was determined. The mushroom was cultivated on three agro-waste materials in three replicates, harvested, and prepared for analysis. A total number of 42, 63 and 65 fruit bodies were harvested from the three groups (days 2, 3 and 4) respectively. Cap diameter (cm) of fruit bodies were in the range of 2.54 to 4.43 and increased with development. Fruit body weight followed a similar trend at 2.61g for fruit bodies from day 2, 4.62g for those of day 3 and 9.17g for day 4. Mean number of fruit bodies (NFB) from the three growth stages were not significantly different at $p \geq 0.05$, while significant differences ($p \leq 0.05$) were observed in the mean Cap Diameter and weight. The result of proximate analysis showed that the nutrient content of the samples varied. Total dietary fibre (TDF) of fruit bodies decreased with time from day 2(13.5%), day 3(12.54%), and day 4(11.29%). Carbohydrate also showed similar decrease with time 46.36 to 50.77%. The protein contents increases with developmental stages 21.05, 23.28 and 25.60% respectively. Ash and moisture content of fresh sample also increased with time from 8.46, 9.20 to 10.75% and 87.74, 88.66 and 87.83% for ash and moisture respectively. Fat content of fruit bodies were not significantly different ($p \geq 0.05$) across the various time treatment levels. The result of the mycochemical analysis showed that alkaloid, flavonoid, phenol, saponin and tannin content increases significantly ($p \leq 0.05$) with increase in developmental stages. Aroma intensity was highest with 6.38 for day 3, 5.03 for day 4 and 3.96 for day 2. The results obtained from this study revealed that mushrooms (*Pleurotus pulmonarius*) harvested at different developmental stages showed significant difference ($p \leq 0.05$) in their physical properties, nutrient and mycochemical contents.

Keywords: Mushroom, proximate, mycochemical, aroma intensity, yield, fruit bodies, agro- waste, macrofungi

Introduction

Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi (fungi which bear fruiting structures that are large enough to be seen with the naked eyes). They can either be epigeous or hypogeous, harvested wild or cultivated (Chang and Miles, 2004) [7]. The chief mushroom varieties cultivated are *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus ostreatus* and *Pleurotus pulmonarius*. *Pleurotus pulmonarius* has occupied the second position among globally cultivated edible mushrooms after *Agaricus bisporus* (Kamalebo and Boa, 2020) [12]. It is one of the mushrooms which are easy to cultivate on a variety of substrates, it also tolerates variations in temperature, making it suitable for commercial exploitation (Banik and Nandi, 2004) [4]. But here in Nigeria, mushroom cultivation has received low attention and hence, limiting its consumption as part of diet. Cereal crops straws are the most common agricultural waste used in the cultivation of mushrooms. This category of waste is of limited use and may constitute a form of environmental hazard if not disposed (Okwulehe and Okwujiako, 2008). Mushrooms have been universally recognized now as food and are grown on commercial scale in many parts of the world with high demand (Okwulehe and Odunze, 2004). The increased demand for mushrooms could be contingent upon the phenomenal rise in the unit costs of the conventional sources of animal protein such as beef, pork, chicken and fish (Riaz *et al.*, 2022) [32]. Therefore, mushrooms can be referred to as a close substitute to animal protein. Mushrooms have been considered as rich food

because they contain protein, sugars, glycogen, lipids, vitamins, amino acids and dietary fibre. They also contain important mineral nutrients which are required for normal functioning of the body (Jonathan *et al.*, 2006) [11]. Mushrooms are also high in antioxidant activity due to the phytochemicals present. The antioxidant compounds in oyster mushroom varieties can be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress (Okafor *e al.*, 2017) [23]. However, there are problems associated with wild mushroom consumption such as safety, availability and seasonality. *P. pulmonarius* is among the cultivatable species of mushroom in Nigeria, and yet it has been under-utilize for food and for flavour production in the food industry.

Mushrooms are very good sources of nutraceuticals, contributing to the general wellbeing of humans (Lim *et al.*; 2004) [15]. They are rich in unsaturated fatty acids, vitamins, phytochemical constituents and minerals, but low in calories (Chang and Miles, 2004) [7]. They are known as the meat of the vegetable world (Venturella, 2006). Mushrooms are generally rich in flavour compounds. The taste and aroma of commercially collected truffle mushroom is so intense that they are used as a flavouring instead of a separate dish (Wang and Marcone, 2011) [41]. However, just like any living organism, the mushroom properties are affected by several factors which include environmental conditions, growing site, type of substrates, mushroom type, developmental stages and part of the mushroom samples analyzed (Venkateshwarlu *et al.*; 1999) [39].

There have been few studies on nutritional values and mycochemical content of cultivated *Pleurotus pulmonarius*, also there is little information available on the effect of different harvest period on these nutrients and mycochemicals. In this regards, the appropriate time to consume mushrooms is as important as the mushrooms itself, since its properties are said to change with time.

Therefore, this study focuses on the effect of different developmental stages on yield, proximate composition, mycochemical content and aroma of *P.pulmonarius* cultivated on three agro waste straws. The result obtained from this research work will therefore reveal the effect of different harvest period on the yield, proximate composition, aroma intensity and mycochemical content of *P.pulmonarius*. It will also provide an alternative use of agro waste for *P.pulmonarius* cultivation. It will guide mushroom producers and consumers on the appropriate time of harvest to meet some nutritional needs.

Materials and methods

Procurement of samples and substrate

The spawn of *Pleurotus pulmunarius* was purchased from the mycology section, biotechnology department of the Federal Institute of Industrial Research, Oshodi (FIRO). Sorghum grains was purchased at Ekeukwu market Owerri. The substrates that were used in this research work were *Andropogon gayanus* (gamba grass), *Pennisetum purpureum* (elephant grass) and corn straws. The substrates were collected from farms of Michael Okpara University of Agriculture Umudike, Abia state.

Spawn multiplication

Spawn of *Pleurotus pulmonarius* was prepared using sorghum grains. Sorghum grains were washed in tap water and soaked overnight. Grains were then boiled in water in the ratio of 1:1 for 20minutes and mixed with 4%(w/w)CaCO₃ and 2%(w/w)CaSO₄ as described by Muhammad *et al*;(2007),to optimize pH and prevent clumping of grains respectively. Completely drained sorghum grains were then packed in 35cl glass bottles tightly plugged with cotton wool and sterilized in an autoclave at 121°C for 30minutes. After sterilization, the bottles were allowed to cool before they were inoculated with actively growing mycelia of *Pleurotus pulmonarius* by grain to grain transfer. Inoculation was done in the dark and left for 15days at 27°C until the grains were fully colonized by mycelia.

Preparation of the substrate

The straws were sun dried and chopped into tiny pieces approximately 1.5cm using machetes and used at the ratio of 1:1:1 (Adenipetun and Fasidi, 2003).

One kilogram (1000g) of substrates was measured in replicates into 400ml transparent poly bags. The substrates were soaked in water for three days to enhance fermentation. Excess water was drained off, the substrates were steam-pasteurized at a temperature of 85°C for 1hour using a big metal drum. It was allowed to cool while still in the drum.

Cultivation

The substrates were inoculated with the spawn of the fungus *Pleurotus pulmunarius*. Five grams of spawn was aseptically inoculated with each of the treatment. The spawn

was sprinkled on the substrates in layers and covered. The inoculated substrates were lightly watered every two days to maintain a high relative humidity of between 75-80%. A precision hygrometer (model 730) was used to monitor the temperature. The cultivation was done in triplicate and kept in sterile wooden racks in the mushroom house at 30±2°C (Adenipetun and Fasidi 2003).

The fruit bodies were harvested at different developmental stages (2, 3 and 4 days). They were oven dried at 40°C for two hours and pulverized. They were packed in an air- tight container for further use.

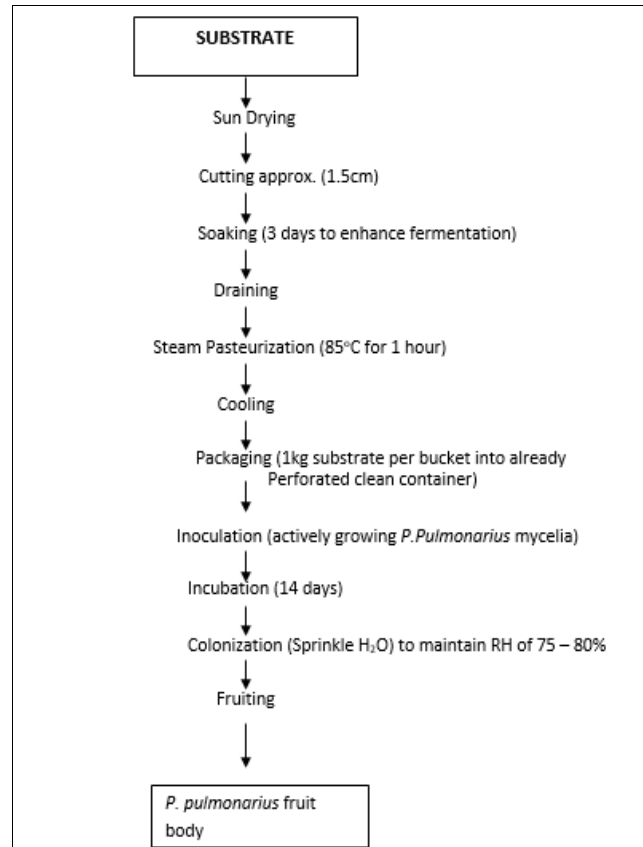


Fig 1: Flow chart for substrate preparation/cultivation

Quality evaluation of mushroom samples

The yield of *P.pulmonarius* was determined by recording the number of the fruit bodies after each of the developmental stages. Also the fresh weight of the fruit bodies was obtained by weighing using an electronic balance, while the cap diameter was obtained using a transparent plastic ruler.

Determination of aroma intensity of *Pleurotus pulmonarius*

Aroma intensity of the dried fruit bodies were determined at different stages of growth (2, 3 and 4 days old) using the sense of smell. This was done using the 9 point hedonic scale with twenty panellists, after which the scores was analyzed statistically.

The 9-point hedonic scale was measured as follows:

- 9-- Extremely intense
- 8-- Very much intense
- 7-- Moderately intense
- 6-- Slightly intense
- 5-- Neither intense nor mild
- 4-- Slightly mild

- 3-- Moderately mild
- 2-- Very much mild
- 1-- Extremely mild

Source: Lim and Fujimaru, (2010) [16].

Proximate Composition of *Pleurotus pulmonarius*

The proximate composition (protein, fat, ash, dietary fibre, and moisture content) of *Pleurotus pulmonarius* was determined according to the method described by AOAC, (2005) while carbohydrate was determined by difference.

Mycosynthesis of *Pleurotus pulmonarius*

Mycosynthesis composition (alkaloid, saponin, phenol, flavonoid and tannin content) of *Pleurotus pulmonarius* was determined using the method of Trease and Evans, (2004) [37].

Results and discussion

Morphological characteristics of *P. pulmonarius* fruit bodies

Results on Table 1 shows the effect of stages of development on some morphological characteristics of *Pleurotus pulmonarius*. A mean number of 42, 63 and 65 fruit bodies were harvested for days 2, 3 and 4 respectively. This result showed that as harvesting time increased from day 2 – 4, number of fruit bodies increased. Also the mean cap diameter (CD) and mean weight increased significantly from 2.54 to 4.43cm and 2.61 to 9.17g respectively as a result of growth.

It has been reported that genetics and substrate alone, are not the only factors responsible for the size of mushroom fruit bodies, rather age also has been found to be of essence (Chang, 2013) [6]. Maturity in mushroom fruit bodies are usually expressed on their morphological parts such as pileus (cap), stipe and physiological features such as spore

development and discharge (Stamets, 2000 [35]; Chang and Miles, 2004 [7]; Chang, 2013) [6]. The mean cap diameter as well as weight recorded at full maturity stage (day 4) of the studied oyster mushroom is in accordance with the results obtained by Okwulehie and Okwujiako, (2008) [28], Okoi and Iboh, (2015) [25]; Okwulehie *et al.*, (2018) [26].

Table 1: Effect of different developmental stages on some morphological characteristics of *Pleurotus pulmonarius* fruit bodies

| Time (Days) | NFB | CD (cm) | Wt (g) |
|-------------|-----------------------|-------------------------|-------------------------|
| 2 | 42 ^a ±2.51 | 2.54 ^b ±0.28 | 2.61 ^c ±0.50 |
| 3 | 63 ^a ±4.53 | 3.05 ^b ±0.59 | 4.62 ^b ±1.50 |
| 4 | 65 ^a ±4.35 | 4.43 ^a ±2.03 | 9.17 ^a ±2.01 |

NFB=Number of Fruiting Bodies, CD= Cap Diameter, Wt=Weight, Means followed by the same alphabet within column are not significantly different (p ≥ 0.05) by the LSD at means ± SD (n=3).

Proximate composition of *Pleurotus pulmonarius* fruit bodies

The results on the effect of age on the proximate composition of *P. pulmonarius* fruit bodies showed a steady increase on protein contents. The values were 21.05, 23.28±1.46 and 25.60% in fruit bodies harvested at day 2, 3 and 4 respectively. Lillian *et al.*, (2007) [14] reported a similar increase in their research on fruit body of *Lactarius* sp mushrooms. With the high values of protein contained in the studied mushroom, its consumption can help to prevent protein energy malnutrition in rural areas.

Ash content of fruit bodies was highest at day 4 (10.75%), and lowest at day 2 (8.46%) while TDF of fruit bodies decreased with time at day 2(13.5±0.78%), day 3 (12.54±0.47%) and day 4 (11.29±0.91%).

Table 2: Effect of time on the proximate composition of *P. pulmonarius* fruit bodies

| Time (days) | Protein (%dmb) | Ash (%dmb) | TDF (%dmb) | Fat (dmb) | MC (% wet basis) | CHO (%dmb) |
|-------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
| 2 | 21.05 ^c ±1.58 | 8.46 ^b ± 0.91 | 13.5 ^a ±0.78 | 2.88 ^a ±0.32 | 87.74 ^b ±0.23 | 50.77 ^a ±2.85 |
| 3 | 23.28 ^b ±1.46 | 9.20 ^b ±1.02 | 12.54 ^b ±0.47 | 2.66 ^a ±0.52 | 88.66 ^a ±0.28 | 47.95 ^b ±2.20 |
| 4 | 25.60 ^a ±1.52 | 10.75 ^a ±1.26 | 11.29 ^c ±0.91 | 2.64 ^a ±0.52 | 87.83 ^b ±0.02 | 46.36 ^b ±2.57 |

Means followed by the same alphabet within column are not significantly different by the LSD at (p≤0.05), means±SD (n=3). TDF= total dietary fibre, MC=moisture content, CHO=carbohydrate

Fat contents (2.64-2.88%) of the mushroom harvested at all stages of maturity were not significantly different (p≥0.05). The values agree with the values reported by Onyeizu *et al.*, (2017) who cultivated *P. Pulmonarius* on different wood log substrates. Okhuoya and Okogbo (1991) [24], Okwulehie and Odunze, (2004) [27] maintained that mushrooms generally contain low fat and oil, and hence are often recommended as good supplement for people with heart challenges.

Carbohydrate content decreased as the maturity period increases from 50.77 to 46.36%. Chang and Miles (2004) [7] reported that the high carbohydrate contents of mushrooms was due to the high ligno-cellulosic compositions in the substrate where they grow, which the mushrooms were able to break using extra cellular enzymes. From Table 2, it was observed that the moisture content of the mushroom samples ranges from 87.33 to 88.17%. The highest amount of moisture was recorded for day 3. This high moisture content is an indication that fresh mushrooms cannot keep for long time. This is because high water activity enhances

microbial growth. The high amounts of dietary fibre and protein in *P. Pulmonarius* fruit bodies as generally observed in this study has been attributed to the nature of substrate (Nwoko *et al.*, 2016) [21]. This further substantiates the claims by Obodai *et al.*, (2003) [22], Adejoye and Fasidi, (2009) [2] and Okoi and Iboh, (2015) [25] which in separate experiments noted that the nutritional composition of mushrooms could reflect the chemical composition of the substrate used, as mushrooms are able to carry out extra-cellular digestion of the decomposed substrate during growth and development. The consumption of dietary fibre rich diets has health-protective effects and disease-reversal benefits (Kaushik, 2016) [13]. Also, the high protein content at day 4 suggests it can be used as an ingredient in high protein food products.

Values obtained in all the studied parameters (protein, Ash, TDF, fat, MC and CHO) are in accordance with those reported by various researchers such as Okwulehie *et al.*, (2008) [28], Sharad, (2013) [34], Patil *et al.*, (2008) [31], Syed *et*

al., (2009) [36], and Okoi and Iboh, (2015) [25]. The carbohydrate content was lower than the values reported by Nwoko *et al.*, (2018) [20] for mature *Lentinus squarrosulus* fruit bodies harvested from log substrates. The reason for such result could however, be species or substrate dependent.

There are other factors that determine the choice of time to harvest mushroom fruit bodies. They include species (Chang, 2013) [6], toxicity (Das, 2005) [8] and commercial factors (Chang, and Miles, 2004 [7]; Stamets, 2000) [35]. For instance, *Lentinussquarrosulus* fruit bodies cultivated on *Dacryodesedulis* logs are soft with low carbohydrate content when young, but rich with high protein content at full maturity (Nwoko *et al.*, 2018) [20]

Table 3: Mycochemicals contents and aroma intensity of *P. pulmonarius* fruit bodies

| Time (days) | Alkaloid (%) | Flavonoid (%) | Phenol (%) | Saponin (%) | Tannin (%) | A.I |
|-------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 2 | 0.54 ^c ±0.02 | 0.05 ^c ±0.01 | 0.10 ^c ±0.01 | 0.32 ^c ±0.05 | 0.02 ^b ±0.00 | 3.96 ^b ±1.81 |
| 3 | 0.56 ^b ±0.02 | 0.06 ^b ±0.01 | 0.11 ^b ±0.01 | 0.35 ^b ±0.06 | 0.03 ^a ±0.00 | 6.38 ^a ±1.71 |
| 4 | 0.57 ^a ±0.02 | 0.07 ^a ±0.01 | 0.12 ^a ±0.01 | 0.38 ^a ±0.06 | 0.03 ^a ±0.00 | 5.03 ^a ±2.11 |

Means with same superscript within the column are not significantly different ($p \leq 0.05$), A.I.= aroma intensity.

The values recorded in this study for alkaloids, flavonoid, phenol and saponin were higher than those reported by Okwulehi and Nosike, (2015) in experiments involving *Pleurotus pulmonarius* cultivated on *Mangifera indica* wood logs which were 0.077, 0.046, 0.019 and 0.310% in dry mass basis. Variation in the quantities of these physiologically important compounds in the fruit bodies, were due to different substrates used or varietal differences. This affirms the report by Chang and Miles, (2004) [7] which stated that the nutritional and mycochemical composition of mushrooms to a large extent depends on the substrate where the mushroom was grown.

The mycochemicals determined in this research has so many health benefits, which has already been established by research. Studies have shown that flavonoids are excellent radical scavengers of the hydroxyl radical (Muhammad *et al.*, 2018) [19]; due to their ability to inhibit the hydroxyl radical and donate hydrogen atom. Flavonoids contain compounds with anti-inflammatory activity. Flavonoids inhibition of the immune and inflammation responses can be associated with their inhibition of the enzymes responsible for the activities (Tripoli *et al.*, 2007) [38]. Flavonoids also act as anti-carcinogens and anti-bacterial agents. Therefore, regular consumption of this mushroom especially at full maturity will protect the body against these free radicals.

Saponins are involved in the prevention of parasitic fungal diseases (Edeoga and Erieta, 2001) [9] while tannins have been used to facilitate healing of wounds, as anti-tumor agents and perform a wide range of anti-infective actions (Tripoli *et al.*, 2007) [38]. Alkaloids have powerful effect in animal physiology and are important in pharmaceutical industries, for drug manufacturing. It is also stated that alkaloids are stimulants and acts by prolonging the action of several hormones.

Harvesting mushroom at full maturity stage is more ideal, especially for mushroom farmers (Chang, and Miles, 2004 [7]; Stamets, 2000 [35]; Nwoko, *et al.*, 2016). Apart from making more profit, due to increase in biomass and weight, the biological benefits of high accumulation of phytochemicals in well mature mushroom fruit bodies

Mycochemicals concentration and flavour intensity of the fruit bodies

Results obtained from this study revealed that fruit bodies harvested at days 2, 3 and 4 had increasing levels of alkaloid contents of 0.54, 0.56 and 0.57% respectively, flavonoid content of 0.05, 0.06 and 0.07 respectively, phenol content of 0.10, 0.11 and 0.12% respectively, saponin content of 0.32, 0.35 and 0.38 respectively, while tannin content was 0.02, 0.03 and 0.03% respectively (Table 3). The results of this study agrees with findings of many researchers (Loedolff and Shuan, 2014) [17] who revealed that as plants and mushrooms grow older, there is always a corresponding increase in the accumulation of most phytochemicals and other active component in their various parts.

cannot be over-emphasized, thus suggesting that *P. pulmonarius* fruit bodies should be allowed to reach up to day 4 before harvesting.

In addition to nutritional value, mushrooms have some unique colour, flavour and aroma characteristics which attract their consumers (Sabir *et al.*, 2003) [33]. Ozturk *et al.* (2015) [30]. reported that the flavour experienced from eating mushrooms, or any other food, comes from a combination of taste, texture, temperature, spiciness, and aromatic qualities. In this investigation, it was observed that *P. pulmonarius* fruit bodies harvested at the 3rd day had the highest score (6.38±1.71) for aroma intensity (A.I), while the lowest (3.96±1.81) was recorded in fruit bodies harvested at the 2nd day. Flavours are generally volatile compounds (Ghweta *et al.*, 2014) [10] and could be responsible for low A.I as recorded in older fruit bodies compared to those of 3rd day. One of the most important consumer consideration in food acceptability is the flavour of the foods. Caglarırmak, (2007) and Ghweta *et al.*, (2014) [10] noted that the major consumer acceptance of Oyster mushroom is due to its characteristic flavour which is mainly contributed by 1-octen-3-ol.

Conclusion

The substrates used in this study has the potential to produce healthy fruitbodies and therefore should be incorporated in commercial mushroom production in Nigeria. Results indicated that as harvesting time increased from day 2 – 4, number of fruit bodies, mean cap diameter and mean weight increased significantly. Protein and ash contents of the oyster mushroom increased as the fruit bodies mature from day 2-4, while total dietary fibre and carbohydrate decreased as the fruit bodies became older. Developmental stages did not significantly affect the concentration of fat in the studied oyster mushroom. The concentration of studied mycochemicals such as alkaloids, flavonoids, phenols, saponins and tannins in the *P. pulmonarius* fruit bodies increased as the mushroom matures (2-4days). The results of this study suggest that as the fruit bodies grow older, their mycochemicals accumulation increases. Also where protein

and ash are important consideration, fully matured (day 4) *P. pulmonarius* fruit bodies should be consumed. But, if higher total dietary fibre, carbohydrate and flavour intensity are preferred, then consumption of younger (3days) fruit bodies of the oyster mushroom should be prioritised.

References

1. ACAO. Official Method of Analysis. 18th Edition. Association of Officiating Analytical Chemists, Washington DC, 2005. Method 935.14 and 992.24
2. Adejoye OD, Fasidi IO. Biodegradation of agro-wastes by some Nigerian white rot fungi. *Bioresources*, 2009;4(2):816–824.
3. Adenipetum CO, Fasidi IO. Effects of Animal Manures on the Growth and Fruit Body Production of *Pleurotussajorcaju* on cassava peels. *Advance Food Sci*, 2003;25(2):125–129.
4. Banik S, Nnadi R. Effects of supplementation of rice straw with biogas residual slurry manure on the yield, protein and mineral content of oyster mushroom. *Ind. Crop Prod*, 2004;20:311–319.
5. Calglarirmak N. The nutrients of exotic mushroom (*Lentinula edodes*) and (*Pleurotus sp.*) and an estimated approach to the volatile compounds. *Food Chem*, 2007;105:1188–1194.
6. Chang ST. Training manual on mushroom cultivation technology. Asian and Pasific Centre for Agricultural Engineering: PR Chi, 2013, 24–122.
7. Chang ST, Chang S, Miles PG. *Mushroom Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact*. CRC press, 2004, 69–73.
8. Das N. Heavy Metal Biosorption by Mushrooms. *Natural Radiance*, 2005;4(6):454–459.
9. Edeoga HO, Eriata DO. Alkaloids, tanins and saponin content of some medicinal plants *Journal Med. Aromatic Plant Sci.*, 2001;23:344–349.
10. Ghweta SG, Shilpa AR, Rahul CR, Jai SG, Dayanand CK, Akshaya KS. Important nutritional constituents, flavour components, antioxidant and antibacterial properties of *Pleurotus sajor-caju*. *Journal food science and technology*, 2014;51(8):1483–1491.
11. Jonathan G, Adetolu A, Ikpebievie O, Dombob W. Nutritive value of common wild edible mushrooms from southern Nigeria. *Global Journal of Biotech & Biochem*, 2006;1(1):16–21.
12. Kamalebo MH, boaDe Kesel A. Wild edible ectomycorrhizal fungi: an underutilized food a. resource from the rainforests of Tshopo province (Democratic Republic of the Congo). *J Ethnobiology Ethnomedicine*, 2020;16:8. <https://doi.org/10.1186/s13002-020-0357-5>.
13. Kaushik G. Edible mushrooms as source of dietary fibre and its health effects. *Journal of physical sciences*, 2016;21:129–137.
14. Lillian B, Paula B, Leticia ME, Isabel CFR. Effect of Fruiting Body Maturity Stage on Chemical Composition and Antimicrobial Activity of *Lactarius sp.* Mushrooms. *J Agric food chem*, 2007;55(21):8766–71.
15. Lim BO, Yamada K, Cho B, Jeon T, Hang SJ, Kang SA, Park DK. *Biosci. Biotechnol. Biochem*, 2004;68:11:2391–2394.
16. Lim J, Fujimaru T. Evaluation of the labelled hedonic scale under different experimental conditions. *Food Quality and Preference*, 2010;2:230–251
17. Loedolff B, Shuan P. Functional foods: miniature plants that pack a big punch! *Frontiers for young minds*, 2014, 6(52).
18. Mohamed AA, Mohamed S, Shabbier A, Asif HM. Protein and fat contents of various *Pleartus spp* raised on different waste materials. *Pak.J. Agr.Sci*, 2007;44(3):140–143.
19. Muhammad AS, Mohammed IU, Ameh M, Bello I, Haliru BS, Bagudo HA, et al. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis* L) and banana (*Musa sapientum* L) in Sokoto Metropolis. *Journal of Applied Biotechnology and Bioengineering*, 2018;5(3):172–182.
20. Nwoko MC, Ehumadu CR, Ukpai KU. Proximate and Bioactive Compounds Evaluation of Young and Mature Fruit Bodies of *Lentinus S quarrosulus* (Mont.) Sing. Collected from two Log Substrates in Umudike, Abia State Nigeria. *World Journal of Biology and Medicinal Science*, 2018;5(3):64–69.
21. Nwoko MC, Okwulehie IC, Achufusi JN. Nutritional composition of *Pleurotus ostreatus* (Jacq.) P. kumm. fruit bodies cultivated on deciduous tree logs in Umudike, Abia State, Nigeria. *International Journal of Research and Technology*, 2016;5:102–108.
22. Obodai M, Cleland J, Vowotor KA. Growth and yield of *Pleurotus ostreatus* on different lignocellulosic by-products. *Journal of Ind. Microbial. Biotechnol*, 2003;30:146–149.
23. Okafor UI, Omemu AM, et al. Nutritional composition and antinutritional properties of maize ogi cofermented with pigeon pea. *Journal of food science and nutrition*, 2017;23(3):65–70.
24. Okhuoya JA, Okogbo FO. Culyivation of *Pleurotus tuber-regium* (Fr.) Sing. On various farm waste product *Acad. Sci*, 1991;71:1–3.
25. Okoi AI, Iboh CI. The effects of different substrates on sporophore yield, mineral and nutrient composition of *Pleurotus tuber-regium* friesinger in Calabar, Nigeria *International Journal of Agricultural Science Research*, 2015;4(6):126–131.
26. Okwulehie IC, Nwoko MC, Achufusi JN, Onyeizu UR, Ezera VN. Yield and Some Macro-Morphological Characters of *Pleurotus pulmonarius* (Fries) Quel. Fruit Bodies Cultivated on HCl-Optimized Oil Palm Bunch Substrate. *J Environ Anal Toxicol*, 2018;7:538. doi: 10.4172/2161-0525.1000538
27. Okwulehie IC, Odunze IE. Evaluation of the nutritional value of some tropical edible mushroom. *J. sustainable Agri. Environmnt*, 2004;6:157–162.
28. Okwulehie IC, Okwujako IA, Edeoga HO. Proximate, macroelement and vitamin composition of the fruit bodies of *Pleurotus ostreatus* (var florida) Eger, grown on different substrate and substrate supplementation. *Global Sci. Books*, 2008;2(2):184–188.
29. Okwulehie IC, Nosike EN. Phytochemicals and Vitamin Composition of *Pleurotus pulmonarius* cultivated on barks of some indigenous fruit trees supplemented with agro wastes. *Asian Journal of Plant Science and Research*, 2015;5(2):1–7.

30. Ozturk M, Tel-ayan, A, Muhammad, P, Terzioglu, Duru ME. Mushrooms: a source of exciting bioactive compounds. *Stud. Nat. Prod. Chem.*, 2015, 45.
31. Patil SS, Kadam RM, Shinde SL, Deshmukh SA. Effect of different substrate on productivity and proximate composition of *P. florida* *Intl. J. plant sci.*,2008;3(1):151-153.
32. Riaz S, Ahmad A, Farooq R, Ahmed M, Shaheryar M, Hussain M. Edible Mushrooms, a Sustainable Source of Nutrition, Biochemically Active Compounds and Its Effect on Human Health. In N. Shiomi, & P. A. Savitskaya (Eds.), *Functional Food*, 2022.
33. Sabir SM, Hagat H, Husain I, Gardesi SR. Proximate Analysis of mushroom Azad Kashmir Pak. *Journal Plant. Pathol*,2003;2(2):97-101.
34. Sharad CM. Productivity and proximate content of *Pleurotus sajor-caju*. *Bioscience Discovery*,2013;4(4):169-172.
35. Stamets P. *Growing gourmet and medicinal mushrooms*. Ten Speed Press, Berkeley, 2000, 150.
36. Syed AA, Kadam JA, Mane VP, Patil SS, Baig MMV. Biological Efficiency And Nutritional Contents of *P. florida* (Mont.) Singer, cultivated in different agro wastes. *Nature and Sci*,2009;7(1):44-48.
37. Trease GE, Evans WC. *pharmacology* (4thed) USA; WB Saunders, 2004, 243-283.
38. Tripoli E, La Guardia M, Giammanco S. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry*,2007;104(2):466-479.
39. Venkateshwarlu G, Chandravadana MV, Tewari RP. Volatile flavor components of some edible mushrooms (*Basidiomy-cetes*). *Flavor Fragr. J.*,1999;14:191-194. IUCN, 2006. Red List of Threatened Species. Version 2009. @<http://www.iucnredlist.org/apps/redlist/details/ful/61597/0>
40. Venturalla F. Chemical composition and nutritional value of pleurotus taxa growing on umbeliferous plants (apiaceae) *J. agric food chem*,2006;53:5997-6002.
41. Wang S, Marcone MF. The Biochemistry and Biological Properties of the World Most Expensive Underground Edible Mushroom: Truffle. *Food Research International*,2011;44:2567-2581.