doi:10.14720/aas.2024.120.4.18542 Original research article / izvirni znanstveni članek

Influence of different substrates on fruiting bodies yield and antioxidant properties of oyster mushroom

Anita UDOVIČIĆ [1], Mirza VALJEVAC [1], Melina HEĆO [1], Dženeta FAZLIĆ [2], Lutvija KARIĆ [1], Ćerima ZAHIROVIĆ SINANOVIĆ [1], Senad MURTIĆ [3, 4]

Received April 08, 2024, accepted November 18, 2024
Delo je prispelo 08. april 20XX, sprejeto 18. november 2024

Influence of different substrates on fruiting bodies yield and antioxidant properties of oyster mushroom

Abstract: The present study was designed to evaluate the influence of different substrates on the yield and antioxidant properties of oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) cultivated in grow bags. The substrates used in this study were: (1) maize straw, (2) beech sawdust supplemented with wheat bran at a rate of 20 %, (3) mixture of maize straw and spent coffee grounds in a ratio of 70 : 30, and (4) mixture of maize straw and spent coffee grounds in a ratio of 50 : 50. Total phenolics, total flavonoids and total antioxidant activity of oyster mushroom were determined by the Folin-Ciocalteu method, Aluminum chloride method and Ferric Reducing Antioxidant Power assay respectively. The highest fruiting bodies yield of oyster mushroom was obtained from substrate 4 while the least was from substrate 1. Total phenolic contents ranged from 3.80 mg in oyster mushroom grown on substrate 4 to 4.85 mg of gallic acid equivalents g^{-1} dry mass in oyster mushroom from substrate 2. Total flavonoid contents were very low in all analysed mushroom extracts. There was no significant difference between total antioxidant activities of oyster mushroom grown on different substrates.

Key words: coffee grounds, *Pleurotus ostreatus*, sawdust, straw, total phenolic and flavonoid contents

Vpliv različnih gojišč na pridelek trosnjakov in antioksidacijske lastnosti bukovega ostrigarja

Izvleček: Namen raziskave je bil ovrednotiti vpliv različnih gojišč na pridelek trosnjakov in antioksidacijske lastnosti bukovega ostrigarja (*Pleurotus ostreatus* (Jacq.) P. Kumm.) gojenega na substratu v vrečah. V raziskavi so bila uporabljena naslednja gojišča: (1) koruznica, (2) bukova žagovina dopolnjena z 20 % pšeničnih otrobov, (3) mešanica koruznice in usedline kave v razmerju 70 : 30, (4) mešanica koruznice in usedline kave v razmerju 50 : 50. Vsebnost celokupnih fenolov, celokupnih flavonoidiov in antioksidacijska aktivnost bukovega ostrigarja so bile določene s Folin-Ciocalteujevo metodo, metodo aluminijevega klorida in preiskusom antioksidacijske moči z redukcijo železa. Največji pridelek trosnjakov je bil pri uporabi gojišča 4, najmanjši pri gojišču 1. Vsebnost celokupnih fenolov je bila v območju od 3,80 mg, kadar je ostrigar rastel v gojišču 4 do 4,85 mg, izraženo kot ekvivalent galne kisline na gram suhe mase ostrigarja, ko je ta rasel v gojišču 2. Celokupna vsebnost flavonoidov je bila pri vseh analiziranih vzorcih zelo majhna, pravtako ni bilo značilne razlike v antioksidacijski aktivnosti ostigarja rastočega na različnih gojiščih.

Ključne besede: usedlina kave, *Pleurotus ostreatus*, žagovina, slama, celokupna vsebnost fenolov in flavonoidov

1 University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Vegetable Crops, Sarajevo, Bosnia and Herzegovina
2 University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Food Technology, Sarajevo, Bosnia and Herzegovina
3 University of Sarajevo, Faculty of Agriculture and Food Sciences, Department of Plant Physiology, Sarajevo, Bosnia and Herzegovina
4 corresponding author: murticsenad@hotmail.com

1 INTRODUCTION

Oyster mushroom cultivation attracts attention of scientists, traders and consumers due to low production costs and high biological efficiency. The interest of oyster mushrooms is also increasing due to its taste, high nutritional value and health benefits (Guragain et al., 2024). Therefore, it is not surprising that oyster mushrooms, scientifically known as *Pleurotus* species are one of the most cultivated edible mushrooms in the world (Obodai et al., 2003; Shnyreva et al., 2017).

In comparison to other cultivated edible mushrooms, oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) requires little growth time, approximately 60 days from media cultivation inoculated with fungi until the fruiting body in bag is ready to be harvested. It is also well known that fruiting bodies of oyster mushroom have good resistance to pests and bacterial and fungal diseases (Bhatti et al., 2024).

Pleurotus ostreatus can grow on a wide range of substrates, including grass clippings, sawdust, straw, coffee grounds, coco-coir, cottonseed hulls and even office paper (Hoa et al., 2015). *Pleurotus ostreatus*, as well as other mushrooms, utilize their mycelium to penetrate into organic substrates in which they grow. As they grow within the substrate, they exude enzymes that convert complex organic molecules into simpler substances which can then be absorbed and utilized (Girmay et al., 2016). In this light, it is obvious that the yield of *Pleurotus ostreatus* largely depends on the structure and chemical composition of the growing media, and therefore, the choice of substrate is of vital importance for the growth of both mycelium and fruiting body of *Pleurotus ostreatus*. Besides, the mineral composition, nutritional value as well as the antioxidant properties of mushrooms also depends on the type of substrate (Oyetayo and Ariyo, 2013).

The aim of this study was to evaluate the influence of different substrates on the yield and antioxidant properties of *Pleurotus ostreatus* cultivated in grow bags. We hypothesized that changes in the structure and chemical composition of the growing media would affect the yield and antioxidant properties of *Pleurotus ostreatus*.

2 MATERIALS AND METHODS

2.1 LOCATION OF STUDY SITE

The experiment was conducted from November 2023 to March 2024 at the experimental station of the Faculty of Agriculture and Food Science in Sarajevo (43°49'34.41" N and 18°19'18.47" E).

2.2 SUBSTRATE COLLECTION

Maize straw was collected from a farmer's field from around the Sarajevo town and transported to the experimental station. After transportation, the maize straw was sun-dried for 2 to 3 days and then stored in a dry place. Beech sawdust was purchased from the wood market, and coffee grounds from the coffee bar located in Sarajevo. Coffee grounds consisted of the waste created by brewing coffee during its final preparation stages. Freshly brewed coffee grounds were used for substrate preparation.

2.3 SUBSTRATE PREPARATION

The maize straw-based substrate was prepared as follows: dry maize straw was chopped into small pieces approximately 2–5 cm long. Exactly 10 kg was weighed and put into a barrel made with high-density polyethylene. The barrel was filled with 20 l of hot water (80 °C). It was soaked for 24 h, and during this time, the barrel containing the maize straw was covered in order to extend the heat treatment. The purpose of this treatment was to destroy all pathogenic organisms that might be present in substrate and that could compete with the cultivated mushrooms. Following hot treatment, maize straw was removed, and strained using raffia baskets. It was allowed to settle for about 1 h to drain excess water.

The maize straw was also utilized for making substrates 3 and 4. These substrates were created by mixing maize straw with spent coffee grounds in a ratio of 70 : 30 and 50 : 50.

Substrate containing beech sawdust and wheat bran was prepared as follows: 10 kg of beech sawdust was weighed and placed in a styrofoam container, followed by 2 kg of wheat bran. The substrate components were mixed thoroughly before adding 25 l of water. Thereafter, the substrate components were mixed again until there was no more water at the bottom of the container.

Final moisture contents of each substrate were determined with an Infrared Moisture Determination Balance (Kett Electric Laboratory, Model FD-770) and ranged from 69 % in substrate 2: beech sawdust supplemented with wheat bran to 73 % in substrate 1: maize straw.

1.5 kg of each homogenized substrates (wet mass) was then placed in polypropylene bags (20.32 × 30.48 cm) and pasteurized at 65 °C for 8 h.

2.4 SUBSTRATE INOCULATION AND INCUBATION

Following pasteurization, filled bags were cooled at room temperature, and then were inoculated with Pleurotus ostreatus spawn at the rate of 10 % (150 g per bag on a w/w wet-weight basis). The spawn was evenly distributed throughout the substrate and then mixed by hand into it. Pleurotus ostreatus spawn was generously donated by Urban Farm Mikić (Orašje, Bosnia and Herzegovina), and was prepared using the method of spawn preparation outlined by Stamets and Chilton (1983). Grain spawns with the mycelium of Pleurotus ostreatus (strain number P10001 acquired from Mushrooms & Equipment Shop, Münster Germany, type of grain: sorghum) were used for inoculation.

After inoculation, the bags were incubated in a climate chamber at 22 °C in darkness at 80 % relative humidity for 20 days, until the mycelium was grown through the whole substrate. The bags were then exposed to 16 °C for an additional three days to encourage fructification after five to six holes were drilled along the length of each bag. Thereafter, the growth conditions in climate chamber were modified to 18 ± 2 °C, 85 % relative humidity and incandescent light (400 lux) for 12 h on/off cycle.

This study was carried out with four substrate treatments and three replications, so that a total of 12 grow bags (bags with inoculated substrate) were used. Matured *Pleurotus ostreatus* fruiting bodies (white in colour, with up curved pileus) were harvested to determine biological efficiency and yield. A total of three flushes was harvested and data were collected in mass (grams) for each flush. The biological efficiency (BE) of *Pleurotus ostreatus* was calculated using the following formula:

$$\text{BE (\%)} = \text{fresh mass of havested ,mushroom (g)/ substrated dry mass (g)} \times 100 \quad (1)$$

2.5 COLLECTION AND PREPARATION OF *PLEUROTUS OSTREATUS* FRUITING BODIES FOR ANALYSIS

Samples of complete fruiting bodies (100 g) from each substrate were dried in an oven at 40 °C to a constant mass. Then dried fruiting bodies were ground into a fine powder using an electric blender and stored in paper bags in dark cool place until analysis.

Preparation and extraction of mushroom samples for determination total phenolics and flavonoids and total antioxidant capacity was performed as follows: 1 g of mushroom powder was placed in an Erlenmeyer flask (100 ml), and then 25 ml of 30 % ethanol was added. Flasks were left to stand for 1 h with frequent shaking and then kept in the dark for 16 h. After that, the mixture was filtered through coarse filter paper into a 25 ml volumetric flask and diluted to the mark with extract solution (30 % ethanol). The extracts thus obtained were used to evaluate total phenolic and flavonoid contents and total antioxidant activities.

2.6 TOTAL PHENOLIC CONTENT ESTIMATION

The total phenolic content of mushroom extracts was determined by spectrophotometric method based on Folin-Ciocalteu reagent (Ough and Amerine, 1998). In brief, 0.1 ml of extract, 6 ml of distilled water, and 0.5 ml of Folin-Ciocalteu reagent (previously diluted with 1:2 distilled water in a ratio 1:2) were transferred into a 10 ml flask. After 5 min, 1.5 ml of saturated solution of Na_2CO_3 was added and mixed thoroughly. The flask was then filled to the mark with 30% ethanol solution and left at room temperature for 1 h, after which the absorbance was read at 750 nm. The gallic acid standard curve ranging from 0 to 500 mg l^{-1} was used to determine the total phenolic content of each sample, and then the obtained values were recalculated to dry mass of mushroom fruiting bodies (mg eq. GAE g^{-1} DM).

2.7 TOTAL FLAVONOID CONTENT ESTIMATION

The total flavonoid content of mushroom extracts was determined spectrophotometrically using the Aluminium chloride colorimetric assay (Zhishen et al., 1999). In brief, 1 ml of extract, 4 ml of distilled water, 0.3 ml of 5 % $NaNO_2$ and 0.3 ml of 10 % $AlCl_3$ were transferred into a 10 ml flask and mixed thoroughly. After 5 min, 2 ml of 1 mol l^{-1} NaOH was added and the flask was diluted to the mark with distilled water. The flask was then left at room temperature for 15 min, after which the absorbance was read at 510 nm. The catechin standard curve ranging from 0 to 100 mg l^{-1} was used to determine the total flavonoid content of each sample, and then the obtained values were recalculated to dry mass of mushroom fruiting bodies (mg eq. C g^{-1} DM).

2.8 TOTAL ANTIOXIDANT ACTIVITY ESTIMATION

The total antioxidant activity of mushroom extracts was determined spectrophotometrically using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). This method is based on the ability of the

antioxidants to reduce the brown-coloured Fe^{3+} complex to a blue-coloured Fe^{2+} complex at low pH. In brief, 100 µl of extract and 2000 µl of FRAP reagent (0.3 mol l^{-1} acetate buffer (pH = 3.6), 10 mmol l^{-1} TPTZ (2, 4, 6-tripyridyl-s-triazine) and 20 mmol l^{-1} $FeCl_3$ x 6 H_2O in a ratio 10:1:1) were transferred into a 10 ml flask and mixed thoroughly. After 15 min, the absorbance was read at 510 nm. The $FeSO_4 \times 7H_2O$ standard curve ranging from 0 to 2000 µmol l^{-1} was used to determine the total antioxidant activity of each sample and then the obtained values were recalculated to dry mass of mushroom fruiting bodies (µmol Fe^{2+} g^{-1} DM).

2.9 STATISTICAL ANALYSIS

All data collected was subjected to Analysis of Variance using a Microsoft Excel software program. When Fisher's F values were significant, the analysis was continued by comparing the means using the least significant difference (LSD) test at the threshold of $p < 0.05$.

3 RESULTS AND DISCUSSION

As shown in Table 1, the maximum fruiting bodies yield of *Pleurotus ostreatus* was obtained in substrate 4: maize straw mixed with spent coffee grounds in a ratio of 50:50 (414 g per bag), followed by substrate 3: maize straw mixed with spent coffee grounds in a ratio of 70:30 and substrate 2: beech sawdust mixed with wheat bran in a ratio of 80:20. The lowest yield was obtained in substrate 1: maize straw (368.6 g per bag).

In this study, *Pleurotus ostreatus* cultivated on substrates obtained by mixing maize straw with spent coffee grounds showed higher yield compared to other substrates, indicating that the spent coffee grounds can be used as an effective supplementary material for *Pleurotus ostreatus* growing media. Numerous studies have also shown a positive correlation between *Pleurotus ostreatus* yield parameters and substrates containing spent coffee grounds (Chai et al., 2021; Gemechu, 2023; Tambaru et al., 2023). Elsisura and Figueroa (2022) reported that spent coffee grounds contain large amounts of organic compounds (lignin (23.90 g 100 g^{-1} dry product), non-starch polysaccharides (60.40 g 100 g^{-1} dry product) as well as nutrients such as potassium and phosphorus, which are essential for the mushroom growth. In addition, spent coffee grounds have a high water-holding capacity (Turek et al., 2019), and that is also of vital importance for mushroom growth. So, it is not surprising that mushroom producers are increasingly using spent coffee grounds as a supplementary substrate for cultivating oyster mushrooms and other edible mushrooms.

However, on the other hand, growing mushrooms on substrates containing spent coffee grounds can be a limiting factor for mushroom growth. Namely, substance with a high water-holding capacity such as spent coffee grounds can decrease mycelium spreads through the substrate, increasing its incubation times (Bellettini et al., 2013). For the above reasons, spent coffee grounds can be used as a substrate for growing mushrooms but only if mixed with other organic substrates such as maize or wheat straw.

In this study, substrate 2: beech sawdust mixed with wheat bran in a ratio of 80:20 exhibited a higher fruiting bodies yield compared to maize straw (substrate 1), indicating that maize straw or other crop residues without nutritional additives such as wheat bran or coffee grounds are not the most suitable growing media for *Pleurotus ostreatus*. These findings are in line with those from previous studies (Nyochembeng et al., 2008; Carrasco et al., 2018).

In this study, substrates that gave higher mushroom fruiting bodies yield are also given a higher biological efficiency (BE) value. The highest percentage of BE in this

Table 1: Yield and biological efficiency of Pleurotus ostreatus grown on different substrates

Substrate*	Fruiting bodies yield per bag				
	I flush (g)	II flush (g)	III flush (g)	Total yield (g)	Biological efficiency (%)
Substrate 1	221.6 ± 28.7	120.0 ± 23.1	27.0 ± 11.3	368.6 ± 21.1c**	73.7 ± 6.9c
Substrate 2	282.2 ± 27.1	108.5 ± 24.3	-	390.7 ± 25.2b	78.1 ± 5.0b
Substrate3	247.4 ± 24.1	123.2 ± 14.1	30.5 ± 14.5	401.1 ± 15.4a	80.2 ± 3.6ab
Substrate 4	250.7 ± 25.2	150.5 ± 26.3	12.8 ± 4.3	414.0 ± 24.6a	82.8 ± 4.9a
$LSD_{0.05}$				20.4	0.4

* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50

**Averages denoted by the same letter indicate no significant difference ($p \leq 0.05$)

study was obtained from substrate 4: maize straw mixed with spent coffee grounds in a ratio of 50:50, followed by substrate 3: maize straw mixed with spent coffee grounds in a ratio of 70:30. These results are consistent with other studies in which coffee grounds have been identified as an important additive for improving substrate chemical composition and thus oyster mushroom yield and biological efficiency (Alsanad et al, 2021; Dissasa, 2022). The possible justification put forward is that coffee grounds provide nutrients, especially nitrogen and potassium, needed for mushrooms to grow. In addition, coffee grounds have a fast decomposition rate (Ballesteros et al., 2014).

Total phenolic and flavonoid contents and total antioxidant activity (FRAP) values of oyster mushrooms cultivated on different substrates are presented in Table 2.

The highest total phenolic contents were found in *Pleurotus ostreatus* cultivated on substrate 2: beech sawdust mixed with wheat bran in a ratio of 80:20, followed by substrate 1: maize straw and substrate 3: maize straw mixed with spent coffee grounds in a ratio of 70:30. The lowest total phenolic contents were obtained from *Pleurotus ostreatus* cultivated on substrate 4: maize straw mixed with spent coffee grounds in a ratio of 50:50. The results of this study also showed that *Pleurotus ostreatus* is an excellent source of phenolic compounds that have been shown to have strong anti-inflammatory, antiproliferative, and antioxidant properties.

Many scientists agree that the accumulation and synthesis of phenolic compounds in mushroom fruiting bodies strongly depend on growth conditions, including primarily substrate structure and chemical composition (Paz et al. 2012; Diamantopoulou et al., 2023). In this light, it is very important to choose a substrate that could increase the phenolic content in mushroom fruiting bodies without negatively affecting the yield. However, all the types of substrates used in this study did not have the desired effect of increasing total phenolic content in mushroom fruiting bodies without decreasing the oyster mushroom yield. For example, *Pleurotus ostreatus* cultivated on maize straw had a higher total phenolic content as compared to mushrooms grown on some other substrates, but at the same time the *Pleurotus ostreatus* yield on maize straw was the lowest. These findings strongly suggest that substrate composition has a significant impact on both mushroom growth and metabolism.

In this study, total flavonoid contents were very low in all analysed fruiting bodies and ranged from 0.11 in substrate 3 to 0.17 mg of catechin equivalents (CAE) g^{-1} dry mass in substrate 2. Numerous studies have also shown that flavonoids are present in mushrooms in very small quantities (Gan et al., 2013; Izham et al., 2022). In this light, Gil-Ramirez et al. (2012) point out that flavonoids cannot be synthesized by mushrooms. In view of this, the presence of flavonoids in *Pleurotus ostreatus* in this study could be due to the ability of *Pleurotus ostreatus* to absorb them from their substrate. However, the results of this study cannot confirm this hypothesis but neither the hypothesis that the flavonoids are metabolites synthesized by the mushroom body.

In this study, there was no significant difference in total antioxidant activities between *Pleurotus ostreatus* grown on different substrates. These findings are somewhat surprising given the fact that there was a significant difference in total phenolics and flavonoids among *Pleurotus ostreatus* grown on different substrates. In view of the above, it is obvious that phenolics are not the only large group of compounds that strongly contribute to the antioxidant capacity of mushrooms. Giavasis (2014) reported that polysaccharides in mushrooms also possess strong antioxidant activity and that among different types of mushroom polysaccharides with antioxidant

Table 2: Antioxidant properties of *Pleurotus ostreatus* grown on different substrates

Substrate*	Total phenolics (mm) (mg g^{-1} dry mass)	Total flavonoids (mg g^{-1} dry mass)	FRAP value (μmol Fe^{2+} g^{-1} dry mass)
Substrate 1	$4.65 \pm 0.73^{ab**}$	0.15 ± 0.02^{ab}	8.23 ± 1.92
Substrate 2	4.85 ± 0.42^{a}	0.17 ± 0.02^{a}	6.96 ± 2.28
Substrate 3	4.08 ± 0.58^{bc}	0.11 ± 0.03^{c}	6.50 ± 0.76
Substrate 4	3.80 ± 0.61^{c}	0.13 ± 0.03^{bc}	9.43 ± 3.32
$LSD_{0.05}$	0.63	0.03	-

* Substrate 1: maize straw, Substrate 2: beech sawdust + wheat bran in a ratio of 80:20, Substrate 3: maize straw + spent coffee grounds in a ratio of 70:30, Substrate 4: maize straw + spent coffee grounds in a ratio of 50:50

**Averages denoted by the same letter indicate no significant difference ($p \leq 0.05$)

activity, β-D-glucans are considered to be the most important. However, further research is required to confirm this hypothesis.

4 CONCLUSIONS

Generally, the present study confirmed that *Pleurotus ostreatus* can grow on different substrates. In this study, substrates obtained by mixing maize straw and spent coffee grounds produced a significantly higher yield and biological efficiency of *Pleurotus ostreatus* compared to the other substrates. Therefore, spent coffee grounds can be recommended as a suitable supplementary substrate to increase *Pleurotus ostreatus* yield. The study results also revealed that changes in the structure and chemical composition of the substrates used in this experiment did not significantly affect the total antioxidant activity of the oyster mushroom.

The authors declare that they have no conflict of interest related to the manuscript.

The data that support the findings of this study are available on request from the corresponding author (SM).

5 REFERENCES

Alsanad, A.M., Sassine, Y.N., El Sebaaly, Z., Fayssal, S.A. (2021). Spent coffee grounds influence on Pleurotus ostreatus production, composition, fatty acid profile, and lignocellulose biodegradation capacity. *CyTA - Journal of Food, 19(1),* 11–20. https://doi.org/10.1080/19476337.2020.1845243

Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I. (2014). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and Bioprocess Technology, 7,* 3493-3503. https://doi.org/10.1007/s11947-014-1349-z

Bellettini, M.B., Fiorda, F.A., Maiaves, H.A., Teixeira, G.L., Avila, S., Hornung, P.S., Junior, A.M., Ribani, R.H. (2019). Factors affecting mushroom Pleurotus spp. *Saudi Journal of Biological Sciences, 26*(4)*,* 633-646. https://doi.org/10.1016/j.sjbs.2016.12.005

Benzie, I.F., Strain, J.J. 1996: Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry, 239,* 70-76. https://doi.org/10.1006/abio.1996.0292

Bhatti, M.I., Jiskani, M.M., Wagan, K.H., Pathan, M.F., Magsi, M.R. (2007). Growth, development and yield of oyster mushroom, *Pleurotus ostreatus* (Jacq. Ex. Fr.) Kummer as affected by different spawn rates. *Pakistan Journal of Botany 39*(7), 2685-2692.

Carrasco, J., Zied, D.C., Pardo, J.E., Preston, G.M., Pardo-Giménez, A. (2018). Supplementation in mushroom crops and its impact on yield and quality. *AMB Express*, *8*(1), 146. https://doi.org/10.1186/s13568-018-0678-0

Chai, W.Y., Krishnan, U.G., Sabaratnam, V., Tan, J.B.L. (2021). Assessment of coffee waste in formulation of substrate for oyster mushrooms *Pleurotus pulmonarius* and *Pleurotus floridanus. Future Foods, 4,* 100075. https://doi.org/10.1016/j.fufo.2021.100075

Diamantopoulou, P., Fourtaka, K., Melanouri, E.M., Dedousi, M., Diamantis, I., Gardeli, C., Papanikolaou, S. (2023). Examining the impact of substrate composition on the biochemical properties and antioxidant activity of *Pleurotus* and *Agaricus* mushrooms. *Fermentation, 9*(7), 689. https://doi.org/10.3390/fermentation9070689

Dissasa, G. (2022). Cultivation of different oyster mushroom (*Pleurotus* species) on coffee waste and determination of their relative biological efficiency and pectinase enzyme production, Ethiopia. *International Journal of Microbiology, 2022*(8)*,* 1-10. https://doi.org/10.1155/2022/5219939

Elsisura, I.B., Figueroa, M.A.G. (2022). Growth and yield performance of oyster mushroom cultivated in combined cassava peels, coconut residue and coffee waste substrates. *American Journal of Environment and Climate, 1*(1)*,* 1–11. https://doi.org/10.54536/ajec.v1i1.206

Gan, C.H., Nurul Amira, B., Asmah, R. (2013). Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). *International Food Research Journal, 20*(3)*,* 1095-1102.

Gemechu, G.W. (2023). Growth, yield and yield related parameters of oyster mushroom (*Pleurotus ostreatus*) as affected by proportion of coffee husk and wheat bran. *International Journal of Agriculture, 8*(3)*,* 11-14. https://doi.org/10.47604/ija.2155

Giavasis I. (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology, 2014,* 162-173. https://doi.org/10.1016/j.copbio.2014.01.010

Gil-Ramírez, A., Pavo-Caballero, C., Baeza, E., Baenas, N., Garcia-Viguera, C., Marína, F.R., Soler-Rivas, C. (2016). Mushrooms do not contain flavonoids. *Journal of Functional Foods, 25,* 1-13. https://doi.org/10.1016/j.jff.2016.05.005

Girmay, Z., Gorems, W., Birhanu, G., Zewdie, S. (2016). Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express, 6*(1)*,* 87. https://doi.org/10.1186/s13568-016-0265-1

Guragain, D.P., Shrestha, B., Bajracharya, I. (2024). A low-cost centralized IoT ecosystem for enhancing oyster mushroom cultivation. *Journal of Agriculture and Food Research*, *15*, 100952. https://doi.org/10.1016/j.jafr.2023.100952

Hoa, H.T., Wang, C.L., Wang, C.H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology, 43*(4)*,* 423-434. https://doi.org/10.5941/MYCO.2015.43.4.423

Izham, I., Avin, F., Raseetha, S. (2022). Systematic review: Heat treatments on phenolic content, antioxidant activity, and sensory quality of Malaysian mushroom: Oyster (*Pleurotus* spp.) and Black Jelly (*Auricularia* spp.). *Fron-*

tiers in Sustainable Food Systems, 6, 882939. https://doi.org/10.3389/fsufs.2022.882939

Lopez, J.C.C., Thepanondh, S., Sachdev, H., Avelar, A.M.P., Leon, M.C.D.C. (2021). Sustainability and economic feasibility through the production of oyster mushroom (*Pleurotus ostreatus* (Jacq.) P. Kumm.) derived from the waste of coffee-industry: A case study in the western area of San Salvador, El Salvador. *Polish Journal of Environmental Studies, 30,* 5617-5628. https://doi.org/10.15244/pjoes/135700

Nyochembeng, L.P., Beyl, C.A., Pacumbaba, R.P. (2008). Optimizing edible fungal growth and biodegradation of inedible crop residues using various cropping methods. *Bioresource Technology, 99*(13)*,* 5645-5649. https://doi.org/10.1016/j.biortech.2007.10.061

Obodai, M, Cleland-Okine, J, Vowotor, K.A. (2003). Comparative study on the growth and yield of Pleurotus ostreatus mushroom on different lignocellulosic by-products. *Journal of Industrial Microbiology and Biotechnology, 30*(3), 146-149. https://doi.org/10.1007/s10295-002-0021-1

Ough, C.S, Amerine, M.A. (1988). Phenolic compounds. In: Wiley (Ed), *Methods for analysis of must and wines*. USA: John Wiley and Sons.

Oyetayo, V.O., Ariyo, O.O. (2013). Micro and macronutrient properties of *Pleurotus ostreatus* (Jacq:Fries) cultivated on different wood substrates. *Jordan Journal of Biological Sciences, 6,* 223-226. https://doi.org/10.12816/0001537

Paz, M.F., Breyer, C.A., Longhi, R.F., Oviedo, M.S.V.P. (2012). Determining the basic composition and total phenolic compounds of Pleurotus sajor-caju cultivated in three different substrates by solid state bioprocess. *Journal of Biotechnology and Biodiversity, 3*(2)*,* 11-14. https://doi.org/10.20873/jbb.uft.cemaf.v3n2.paz

Shnyreva, A.A., Kozhevnikova, E.Y., Barkov, A.V., Shnyreva, A.V. (2017). Solid-state cultivation of edible oyster mushrooms, *Pleurotus* spp. under laboratory conditions. *Advances in Microbiology, 7*, 125-136. https://doi.org/10.4236/aim.2017.72010

Stamets, P., Chilton, J.S. (1983). *The Mushroom Cultivator. A Practical Guide to Growing Mushrooms at Home*. Agaricon Press, Olympia, Washington DC, 415 p.

Tambaru, E., Ura, R., Tuwo, M. (2023). The effect of coffee grounds and sawdust *Tectona grandis* L. f. as planting media for cultivation oyster mushroom *Pleurotus* sp. *IOP Conf. Series: Earth and Environmental Science, 1230,* 012071. https://doi.org/10.1088/1755-1315/1230/1/012071

Turek, M.E., Freitas, K.S., Armindo, R.A. (2019). Spent coffee grounds as organic amendment modify hydraulic properties in a sandy loam Brazilian soil. *Agricultural Water Management, 202,* 313-321. https://doi.org/10.1016/j.agwat.2019.06.006

Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64,* 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2