University of Debrecen
Faculty of Agricultural, Food Sciences and Environmental Management
Institute of Food Science
Head of Institute: Prof. Dr. Béla Kovács

CULTIVATION OF *PLEUROTUS OSTREATUS* MYCELIA ON APPLE POMACE MEDIA

Nur Fatihah binti Kamarudin
Bachelor (Science) of Food Engineering

Supervisor:
Gréta Törős Seresné
Departmental engineer
Ph.D. student

Co-supervisor:
Dr. Ferenc Peles
Associate Professor

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INTRODUCTION

This study presents a sustainable novel approach which was proposed for biotechnology and agricultural fields, apple pomace waste-based solid medium cultivation of *Pleurotus ostreatus* mycelia. This novel approach provides a solution to part of the global challenge facing agricultural by-products for more environmentally friendly and economical processes. This topic is both theoretically and practically significant because, if a fungus able to convert apple pomace — the remaining number of apples after processing — into mycelium & basidiocarp can be identified and optimized for this process, it could change the whole apple industry by using what would have been discarded as value-added raising medium for edible mushrooms. The primary objective of this introduction is to explore how previous theoretical research has been translated into producing apple pomace formulations for *Pleurotus ostreatus* mycelia growth kids grounded in their mode of action and to rationalize the potential impacts on the agri-food sector, including agriculture sustainability and circular economy aspects. There is a growing interest in the application of agro by products for sustainable agriculture in recent years. With the ever-increasing global population, demand for food and agricultural products is on constant increase resulting in more agricultural activities and hence generation of a large amount of agricultural waste. Apple pomace is produced in the apple processing industry as a waste rich in organic matter and could present environmental problems if not properly disposed. Nevertheless, with the aid of novel biotechnological strategies, apple pomace may serve as an excellent resource to expand *P. ostreatus* mycelia and reduce environmental contamination for the circular economy. The key point of this topic is that the solid medium based on apple pomace, by which *P. ostreatus* mycelia were cultivated, could be a theoretical process enabling harnessing nutritional contents embodied in apple pomace and its structure components to sustain growth and evolution of edible mushrooms. By this approach, we can not only provide a sustainable solution for the management of agricultural waste but also an opportunity for studying intra specific interaction with substrate dynamics. The understanding of the induced modifications in the molecular structure and composition of apple pomace and their effect on mycelial growth and nutritional quality of *P. ostreatus* might have theoretical implications for bioprocess optimization and implementation in biotechnology industry. The practice of solid-state fermentation for the cultivation of *P. ostreatus* mycelia on apple pomace-based media has several practical implications related to sustainable agricultural practices, waste treatment and production of value-added products. The apple pomace may be seen to fit into the paradigm of the circular economy, converting waste materials into resources thus releasing environmental impact. Moreover, the capability of producing high-quality edible mushrooms and bioactive compounds could enhance the practical importance of this subject in food production, nutrition, and biopharmaceutical applications as well. Collectively, the theoretical import and industrial relevance of substrate-specific growth of *Pleurotus ostreatus* mycelia on solid media derived from apple pomace reveal opportunities to improve knowledge on resource utilization formation in biotechnologies, promote agricultural sustainability and enhance quality value-added product development. The present article should serve as an overview and introduction to the theory, practice, and prospects for this creative initiative and highlight its importance to biotechnology, agriculture, environmental sustainability.

1. RAISING A TOPIC

Over the last few years, use of agricultural by-products to enhance sustainable agriculture has gathered in-depth attention. Out of which, apple pomace has received significant attention because it could potentially be utilized for a variety of applications due to formation during the process (BHUSHAN et al., 2008). Apple pomace by-products can be used for an efficient and eco-friendly preparation of high-quality mulberry sub paste, which could lead to the development of both sustainable and cost-effective processes for generating revenue from apple waste. On top of that, it is the opportunity to create such product with valuable properties usable in multiple branches (COSTA et al., 2022). By investigating the hidden potential of apple pomace as an ideal material for biotechnological purposes, researchers can make significant strides in accomplishing a truly circular economy. The conception of the circular economy is that it allows us to convert waste materials into useful resources, reducing dependence on non-renewable sources and decreasing the negative effects on the environment resulting from industrial activity. Besides, the use of apple pomace as a substrate for bioprocesses not only provides new business opportunities in the apple field but also drives the growth of biotechnology and food sectors (AMPESE et al., 2022). Therefore, the application of an apple pomace by-product is proposed as a new and multidimensional horizon for regional development offering potential advantages in terms of ecologic-functional synergy (ecosystem services benefits) which will also promote economic gains. Approaching a unique inquiry, this study investigates the neglected avenues of apple pomace utilization in upgrading oyster mushroom production especially on *Pleurotus ostreatus*.

The development and improvement of novel solid media that use different amounts of apple pomace are the main areas of attention. Our study aims to clarify the complex interaction between apple pomace as a substrate and its significant influence on *P. ostreatus* mycelia colonization in an effort to produce novel insights. Pushing the boundaries of what is currently understood, we aim to reveal the nutritional composition of apple pomace and how it affects mycelial growth. Our first (1) goal is to determine how important nutrients are and how they function in relation to mycelial colonization. In addition, we will assess important variables including colonization rate, mycelial density, biomass generated, and incubation conditions to provide us a thorough grasp of *P. ostreatus* growth conditions. Comparing our novel solid media to commercially available alternatives is an important step in making sure that our method is competitive and distinct in promoting the best possible mushroom growth. Exploring the critical function of the carbon-to-nitrogen (C/N) ratio, our study seeks to clarify its impact on mycelial growth. This entails a thorough investigation of techniques to adjust the C/N ratio, creating ideal circumstances for mycelial development as well as fruiting body formation. Evaluating possible risks, including heavy metal contaminations, connected with apple pomace is an important secondary (2) objective in our commitment to safety and integrity. Our research thoroughly examines potentially dangerous substances, including infections, pesticide residues, mycotoxins, and foreign materials. We then develop mitigation techniques for these risks in order to ensure the overall safety of mushroom farming. By completing these complex tasks, our research hopes to redefine the role of apple pomace in safe and sustainable mycelial development and add innovative knowledge to the field of mushroom farming.

2. LITERATURE REVIEW

2.1. Definition of *Pleurotus ostreatus* and Its Importance

Pleurotus ostreatus, the oyster mushroom, is a common edible species of the Pleurotus genus. It is largely popular for its use in the field of culinary and medicine, with a higher demand for *P. ostreatus* due to flavour, taste, high content of nutrition, and therapeutic characters (KÜES - LIU, 2000), whereas scientific classification of *P. ostreatus* mushrooms were belongs to (WAL et al., 2023),

Taxonomic Description:

- Kingdom: Fungi
- Phylum: Basidiomycota
- Class: Agaricomycetes
- Order: Agaricales
- Family: Pleurotaceae
- Genus: Pleurotus
- Species: *P. ostreatus*
- Binomial Name: *Pleurotus ostreatus*

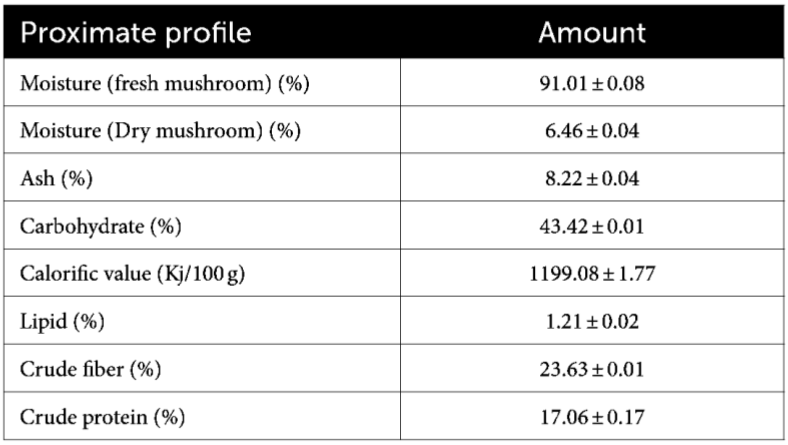
Mycological Description: *Pleurotus ostreatus'* fruiting body stands out for its unusual development pattern and appearance. While "*ostreatus*" and the English common name "Oyster" refer to the shape of the cap, which resembles the bivalve of the same name, the Latin word "Pleurotus" refers to the sideways growth of the stem with respect to the cap. *P. ostreatus* usually has an oyster-shaped fruiting body, which consists of a short or non-existent stalk and a broad, fan- or oyster-shaped cap. The cap's surface is typically velvety and smooth, with colour variations ranging from white to light brown (WAL et al., 2023).

Pleurotus ostreatus functions as a saprotroph, obtaining nutrition by consuming biological waste for example agricultural residues; wheat straw, rice straw, corn cobs, and sugarcane bagasse, forestry by-products; sawdust, wood chips, and wood shavings, paper waste; cardboard, newspaper, and office paper, coffee grounds, cotton waste and various types of plant residues and crop residues (LI et al., 2020). Saprotrophic fungi play a crucial role in ecosystem functionality, soil well-being, and nutrient cycling. Through the breakdown of organic substrates, saprotrophs release nutrients such as carbon, nitrogen, and phosphorus back into the soil, where they can be absorbed by plants and other organisms (AUSTIN et al., 2004; MCGONIGLE, 1995; WORRALL, 1989; WOLFE et al., 2012). The breakdown of organic matter by saprotrophs guarantees that different species can acquire nutrients. Particular parts of organic matter, including as cellulose, lignin, hemicellulose, chitin, and pectin, are targeted by the enzymes released by saprotrophs (HON, 1994). These enzymes break down complex biopolymers into simpler ones that the saprotroph may absorb for nourishment. This complicated mechanism is critical for maintaining nutrient balance in ecosystems and promoting overall ecosystem health. Because of its ability to thrive on various substrates, including agricultural waste, this fungus has evolved effective enzyme systems to break down complex organic molecules (ŠNAJDR et al., 2011). *P. ostreatus* ability to breakdown diverse substrates and return nutrients to the ecosystem is critical for maintaining the balance of nutrient cycles in nature.

2.1.1. Nutritional Values

Pleurotus ostreatus is an excellent supplement to a healthy diet due to its multiple benefits. The nutritional composition and values of *P. ostreatus* are as follows:

Table 1: Proximate content of *Pleurotus ostreatus*

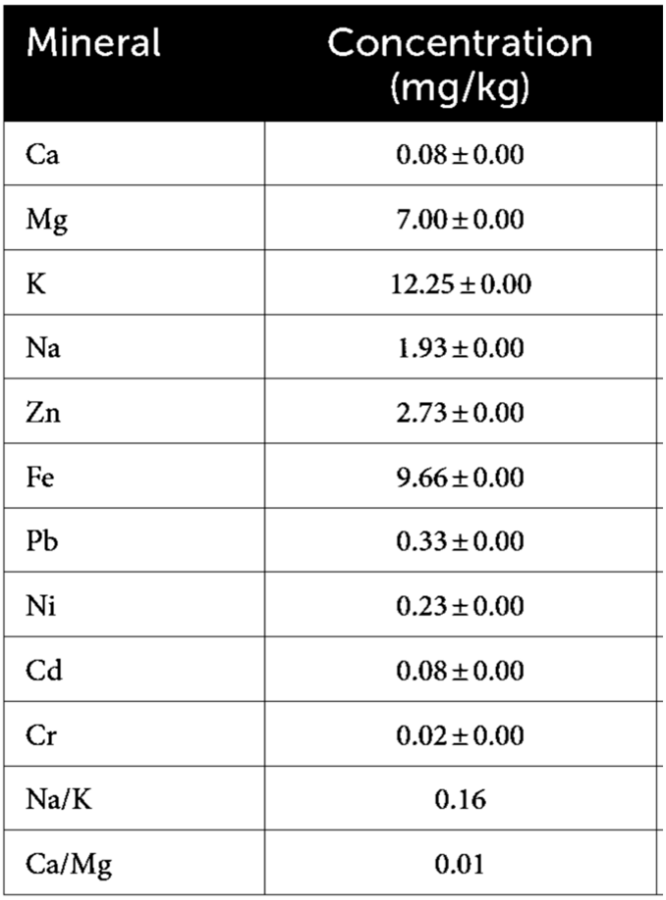


Proximate profile	Amount
Moisture (fresh mushroom) (%)	91.01 ± 0.08
Moisture (Dry mushroom) (%)	6.46 ± 0.04
Ash (%)	8.22 ± 0.04
Carbohydrate (%)	43.42 ± 0.01
Calorific value (Kj/100 g)	1199.08 ± 1.77
Lipid (%)	1.21 ± 0.02
Crude fiber (%)	23.63 ± 0.01
Crude protein (%)	17.06 ± 0.17

Sources: EFFIONG et al. (2024)

The proximate analysis of *P. ostreatus* revealed the percentage contents of carbohydrate, moisture, ash, fat, crude fiber, and crude protein. The carbohydrate content was the highest (43.42 ± 0.01%), followed by crude fiber (23.63 ± 0.01%), crude protein (17.06 ± 0.17), ash (8.22 ± 0.04%), and moisture (6.46 ± 0.04%) as the least (Table 1).

Table 2: Mineral Content of *Pleurotus ostreatus*

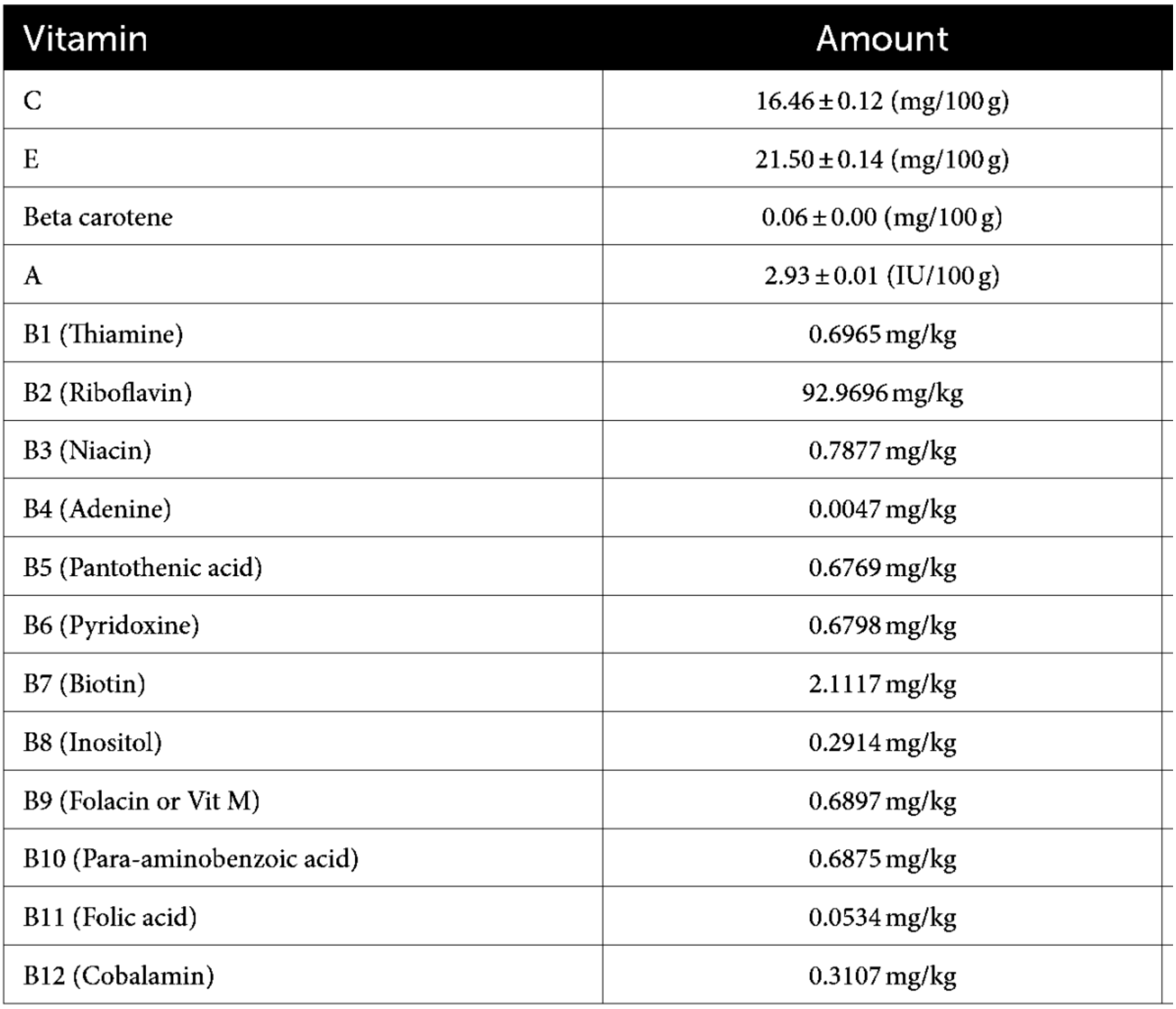


Mineral	Concentration (mg/kg)
Ca	0.08 ± 0.00
Mg	7.00 ± 0.00
K	12.25 ± 0.00
Na	1.93 ± 0.00
Zn	2.73 ± 0.00
Fe	9.66 ± 0.00
Pb	0.33 ± 0.00
Ni	0.23 ± 0.00
Cd	0.08 ± 0.00
Cr	0.02 ± 0.00
Na/K	0.16
Ca/Mg	0.01

Sources: EFFIONG et al. (2024)

Minerals are the ash that remains after the full combustion of dry mushrooms (BOADU et al., 2023). *P. ostreatus* contained high levels of K, Mg, Fe, Zn, and Na, but low levels of Ca. The heavy metal content (Pb, Ni, Cd, and Cr) was extremely low (Table 2). This shows that *P. ostreatus,* while being a saprophytic organism, is safe to consume and does not contribute to heavy metal poisoning. The calcium amount was likewise below the recommended daily intake (RDI) of 1,000 mg. This means that *P. ostreatus* is not a high-calcium food, but it is advantageous because it does not interfere with zinc and iron absorption from the diet. Zinc is necessary for health, with a recommended daily intake of 15 mg. The zinc concentration of *P. ostreatus* was 2.73 mg/kg. *P. ostreatus's* moderate zinc level indicates that when taken, it can help lower the severity of acute diarrhoea in young children and infants while not interfering with iron and copper metabolism.

Table 3: Vitamin content of *Pleurotus ostreatus*

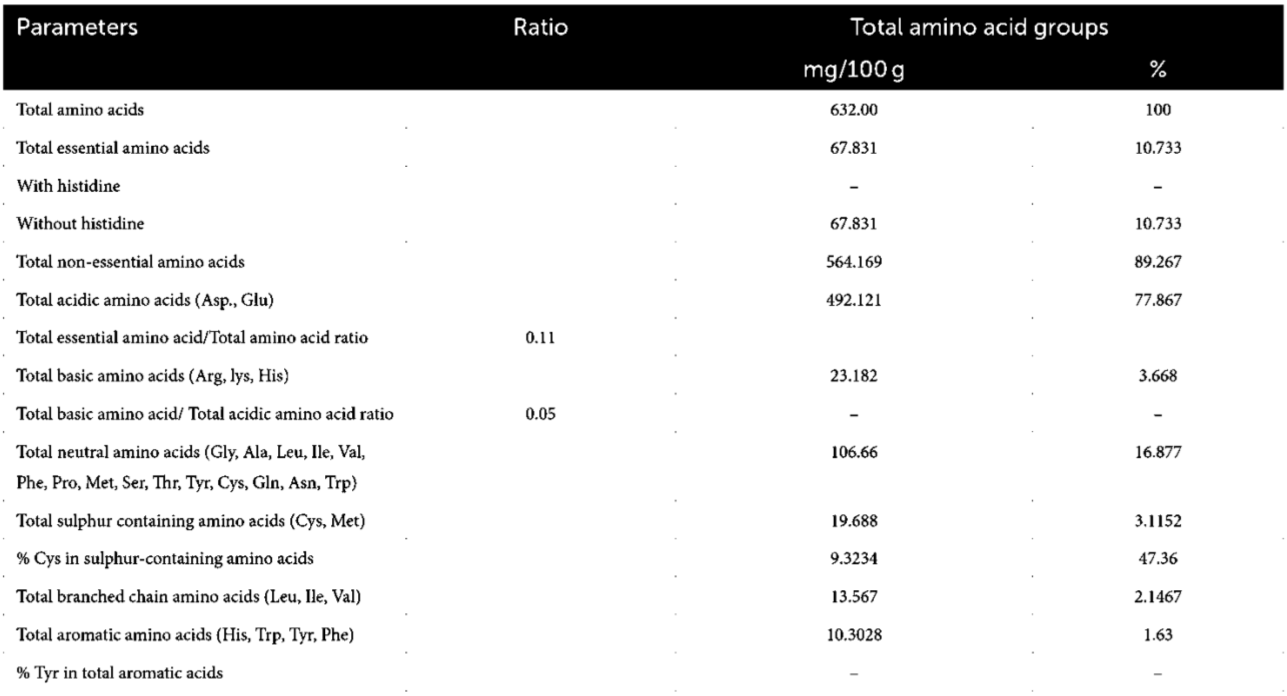


Vitamin	Amount
C	16.46 ± 0.12 (mg/100 g)
E	21.50 ± 0.14 (mg/100 g)
Beta carotene	0.06 ± 0.00 (mg/100 g)
A	2.93 ± 0.01 (IU/100 g)
B1 (Thiamine)	0.6965 mg/kg
B2 (Riboflavin)	92.9696 mg/kg
B3 (Niacin)	0.7877 mg/kg
B4 (Adenine)	0.0047 mg/kg
B5 (Pantothenic acid)	0.6769 mg/kg
B6 (Pyridoxine)	0.6798 mg/kg
B7 (Biotin)	2.1117 mg/kg
B8 (Inositol)	0.2914 mg/kg
B9 (Folacin or Vit M)	0.6897 mg/kg
B10 (Para-aminobenzoic acid)	0.6875 mg/kg
B11 (Folic acid)	0.0534 mg/kg
B12 (Cobalamin)	0.3107 mg/kg

Sources: EFFIONG et al. (2024)

Pleurotus ostreatus is rich in various B vitamins, including Thiamin (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxin (B6), Folate, Biotin (B7), Folic acid (B11), and Vitamin B12 (EFFIONG et al., 2024). Vitamin B2 was reported to be the highest B vitamin in *P. ostreatus* (Table 3), indicating its significant presence in these mushrooms. While some vitamins like B2, B7, B9, and B12 were found to be higher than the RDA (DAILY DIETARY ALLOWANCES: RECOMMENDED DAILY DIETARY ALLOWANCES: RECOMMENDED BY FOOD AND NUTRITION BOARD NATIONAL RESEARCH COUNCIL, U.S.A., 1948), others like B1, B3, B5, and B6 were lower than the recommended values. The rich vitamin B content of *P. ostreatus* enables it to boost the immune system, enhance DNA synthesis, and improve red blood cell production. This makes oyster mushrooms suitable for consumption by pregnant women, menstruating females, and anaemic patients.

Table 4: Amino Acid Characteristics of *Pleurotus ostreatus*



Parameters	Ratio	Total amino acid groups	
		mg/100 g	%
Total amino acids		632.00	100
Total essential amino acids		67.831	10.733
With histidine		–	–
Without histidine		67.831	10.733
Total non-essential amino acids		564.169	89.267
Total acidic amino acids (Asp., Glu)		492.121	77.867
Total essential amino acid/Total amino acid ratio	0.11		
Total basic amino acids (Arg, lys, His)		23.182	3.668
Total basic amino acid/ Total acidic amino acid ratio	0.05	–	–
Total neutral amino acids (Gly, Ala, Leu, Ile, Val, Phe, Pro, Met, Ser, Thr, Tyr, Cys, Gln, Asn, Trp)		106.66	16.877
Total sulphur containing amino acids (Cys, Met)		19.688	3.1152
% Cys in sulphur-containing amino acids		9.3234	47.36
Total branched chain amino acids (Leu, Ile, Val)		13.567	2.1467
Total aromatic amino acids (His, Trp, Tyr, Phe)		10.3028	1.63
% Tyr in total aromatic acids		–	–

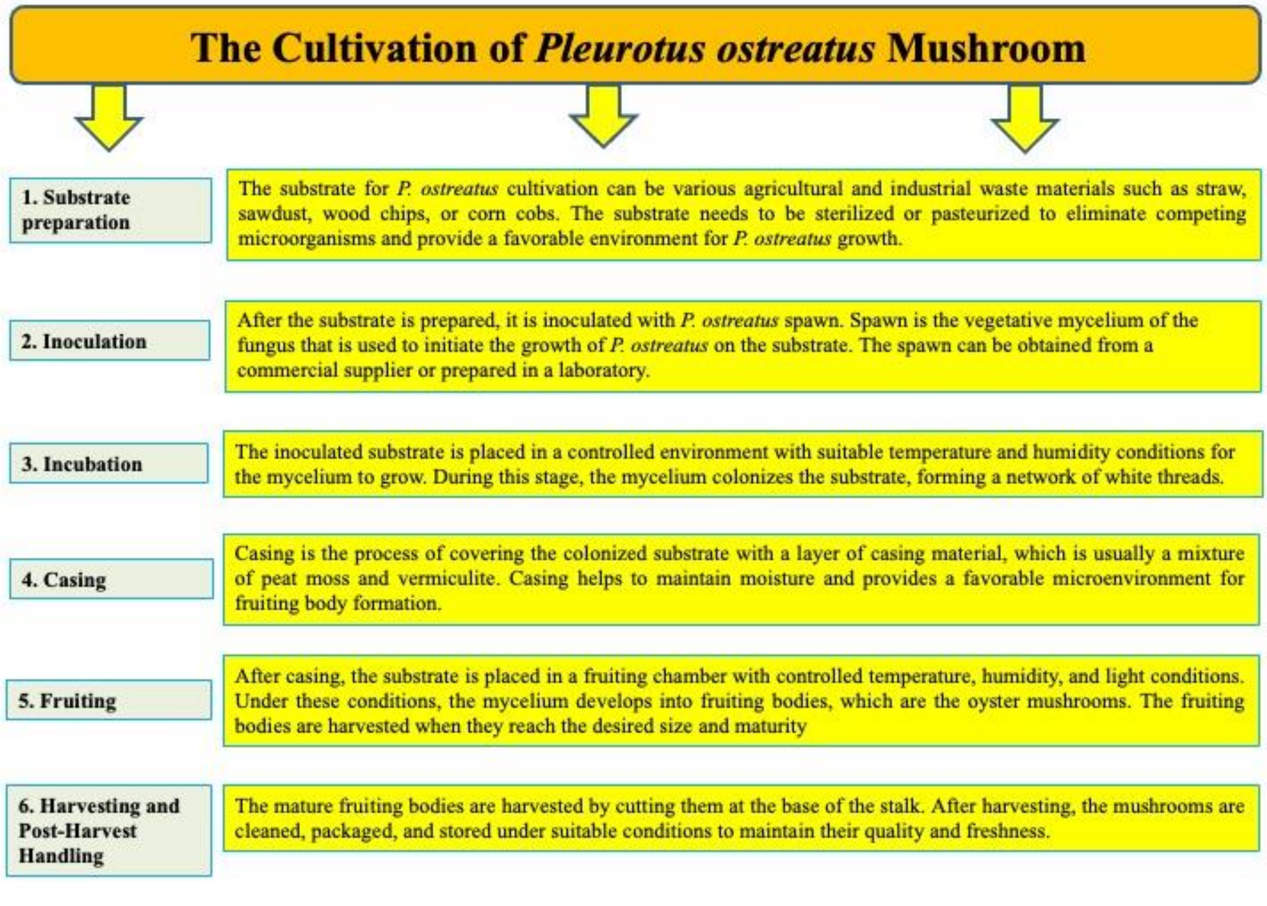
Sources: EFFIONG et al. (2024)

Amino acids are essential for the repair of tissues and various bodily functions. *P. ostreatus* contains essential amino acids such as Threonine, Leucine, Isoleucine, Lysine, Methionine, Phenylalanine, Valine, Histidine, and Tryptophan (Table 4) (LEROY et al., 2023; KUDEŁKA et al., 2021). The mushroom contains amino acids that are not essential for the body, but vital for metabolism, immune function and other physiological activities such as alanine, aspartic acid, asparagine, glutamic acid, glutamine, glycine, proline, cysteine, selinene arginine and tyrosine (CHOI & COLOFF, 2019). The amino acid profile of *P. ostreatus* includes varying quantities of essential and non-essential amino acids. Lysine was found to be the most important essential amino acid and aspartic acid as an example of a completely dependent non-essential amino acids (AL-FARGA et al., 2016).

2.1.2. Health Benefits

According to studies (LESA et al., 2022), *Pleurotus ostreatus*, are valuable for human health because of their many health-promoting qualities and nutritional content: 1. *P. ostreatus* Enhances the Immune Function: *P. ostreatus* are rich in beta-glucans which have been showed to improve the immune system due to its capacity of increase activity by some types of white blood cells that help defend against infections and diseases. 2. Reduces Inflammation: *P. ostreatus* consists of anti-inflammatory substances that could help lower the symptoms from inflammation in your system, which has been linked to a number of long-term disorders. 3. Anti-carcinogenic properties: Research has found positive results that *P. ostreatus* can prevent cancer, and even stop the growth, as well remove free radicals in human body preventing more damaged cells to appear (anti-mutagenic). 4. Reduces Blood Sugar Levels: Research has shown that *P. ostreatus* possesses hypoglycaemic properties and improves insulin sensitivity in humans which can reduce the levels of glucose in human bloodstream. 5. Maintain Heart Health: Beta-glucans from *P. ostreatus* can prevent high cholesterol levels and thus decreased risk of heart-related diseases in an organism. 6. Weight Loss: *P. ostreatus* serves as a weight loss diet because it is both low in fat and calories. 7. Medicinal Properties: The presence of bioactive compounds such as ergothioneine, beta-glucans and polysaccharides in the formulation is responsible for *P. ostreatus* anti-inflammatory, antioxidant and immune-modulating features (SIFAT et al., 2020).

2.2. Steps for Cultivating *Pleurotus ostreatus*



The Cultivation of *Pleurotus ostreatus* Mushroom

1. Substrate preparation

The substrate for *P. ostreatus* cultivation can be various agricultural and industrial waste materials such as straw, sawdust, wood chips, or corn cobs. The substrate needs to be sterilized or pasteurized to eliminate competing microorganisms and provide a favorable environment for *P. ostreatus* growth.

2. Inoculation

After the substrate is prepared, it is inoculated with *P. ostreatus* spawn. Spawn is the vegetative mycelium of the fungus that is used to initiate the growth of *P. ostreatus* on the substrate. The spawn can be obtained from a commercial supplier or prepared in a laboratory.

3. Incubation

The inoculated substrate is placed in a controlled environment with suitable temperature and humidity conditions for the mycelium to grow. During this stage, the mycelium colonizes the substrate, forming a network of white threads.

4. Casing

Casing is the process of covering the colonized substrate with a layer of casing material, which is usually a mixture of peat moss and vermiculite. Casing helps to maintain moisture and provides a favorable microenvironment for fruiting body formation.

5. Fruiting

After casing, the substrate is placed in a fruiting chamber with controlled temperature, humidity, and light conditions. Under these conditions, the mycelium develops into fruiting bodies, which are the oyster mushrooms. The fruiting bodies are harvested when they reach the desired size and maturity

6. Harvesting and Post-Harvest Handling

The mature fruiting bodies are harvested by cutting them at the base of the stalk. After harvesting, the mushrooms are cleaned, packaged, and stored under suitable conditions to maintain their quality and freshness.

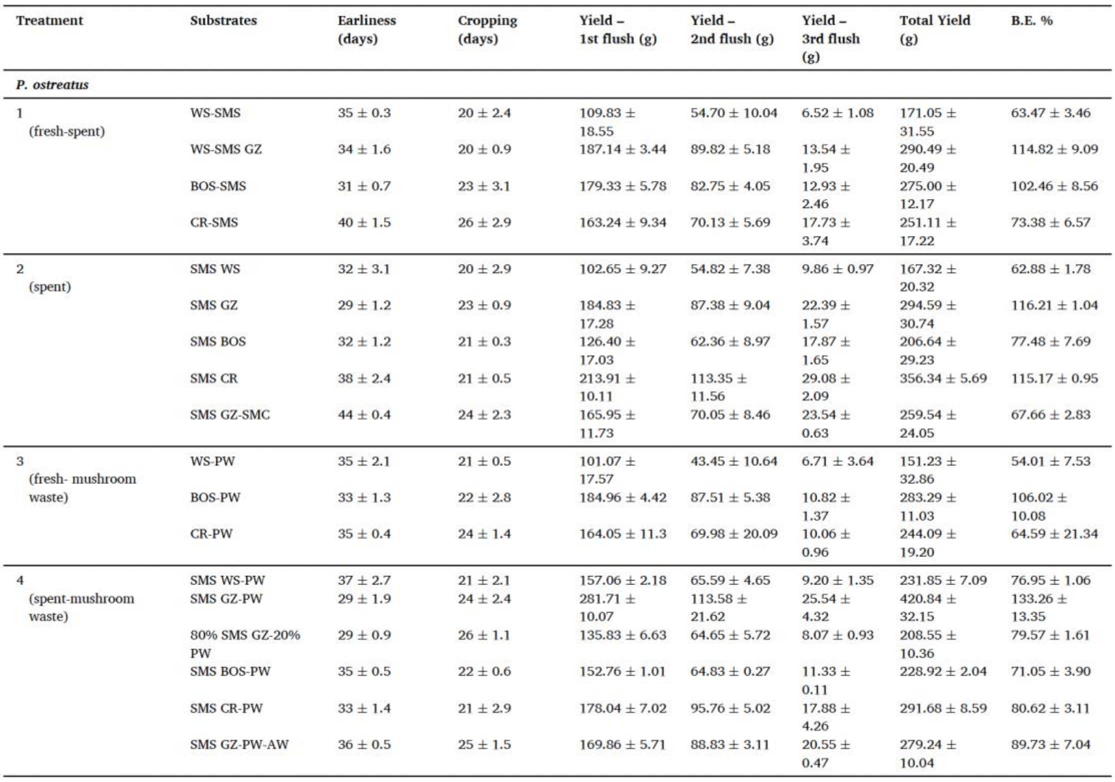
Figure 1: General steps of *Pleurotus ostreatus*

Source: YILDIZ et al. (2022)

2.2.1. New Techniques for *Pleurotus ostreatus* Cultivation

With the global demand for nutritious and flavourful mushrooms on rise, mushroom farming is rising among popular agricultural enterprises evident from significantly increased yields of production over the years. Under this framework, new strategies for growing *Pleurotus ostreatus*, popular cultivated edible mushroom species have emerged as a plausible route to sustainable and inexpensive production. The farmers are expanding the frontiers of mushroom cultivation with spent mushroom substrate (SMS) and agro-industrial wastes as substrates. This not only makes our work easier and faster but also becomes environment-friendly and saves resources. This serves as an introduction that prepares the reader for exploring how these technologies are reshaping *P. ostreatus* cultivation, and assessing their advantages, challenges, and potential to bring about innovative paradigms in mushroom agricultural practices (DEDOUSI et al., 2024).

Table 5: SMSs (spent mushroom substrates), SMC (spent mushroom compost), and mushroom waste affect the production cycle, mushroom yield, and biological efficiency of *Pleurotus ostreatus*



Treatment	Substrates	Earliness (days)	Cropping (days)	Yield – 1st flush (g)	Yield – 2nd flush (g)	Yield – 3rd flush (g)	Total Yield (g)	B.E. %
P. ostreatus								
1 (fresh-spent)	WS-SMS	35 ± 0.3	20 ± 2.4	109.83 ± 18.55	54.70 ± 10.04	6.52 ± 1.08	171.05 ± 31.55	63.47 ± 3.46
	WS-SMS GZ	34 ± 1.6	20 ± 0.9	187.14 ± 3.44	89.82 ± 5.18	13.54 ± 1.95	290.49 ± 20.49	114.82 ± 9.09
	BOS-SMS	31 ± 0.7	23 ± 3.1	179.33 ± 5.78	82.75 ± 4.05	12.93 ± 2.46	275.00 ± 12.17	102.46 ± 8.56
	CR-SMS	40 ± 1.5	26 ± 2.9	163.24 ± 9.34	70.13 ± 5.69	17.73 ± 3.74	251.11 ± 17.22	73.38 ± 6.57
2 (spent)	SMS WS	32 ± 3.1	20 ± 2.9	102.65 ± 9.27	54.82 ± 7.38	9.86 ± 0.97	167.32 ± 20.32	62.88 ± 1.78
	SMS GZ	29 ± 1.2	23 ± 0.9	184.83 ± 17.28	87.38 ± 9.04	22.39 ± 1.57	294.59 ± 30.74	116.21 ± 1.04
	SMS BOS	32 ± 1.2	21 ± 0.3	126.40 ± 17.03	62.36 ± 8.97	17.87 ± 1.65	206.64 ± 29.23	77.48 ± 7.69
	SMS CR	38 ± 2.4	21 ± 0.5	213.91 ± 10.11	113.35 ± 11.56	29.08 ± 2.09	356.34 ± 5.69	115.17 ± 0.95
	SMS GZ-SMC	44 ± 0.4	24 ± 2.3	165.95 ± 11.73	70.05 ± 8.46	23.54 ± 0.63	259.54 ± 24.05	67.66 ± 2.83
3 (fresh- mushroom waste)	WS-PW	35 ± 2.1	21 ± 0.5	101.07 ± 17.57	43.45 ± 10.64	6.71 ± 3.64	151.23 ± 32.86	54.01 ± 7.53
	BOS-PW	33 ± 1.3	22 ± 2.8	184.96 ± 4.42	87.51 ± 5.38	10.82 ± 1.37	283.29 ± 11.03	106.02 ± 10.08
	CR-PW	35 ± 0.4	24 ± 1.4	164.05 ± 11.3	69.98 ± 20.09	10.06 ± 0.96	244.09 ± 19.20	64.59 ± 21.34
4 (spent-mushroom waste)	SMS WS-PW	37 ± 2.7	21 ± 2.1	157.06 ± 2.18	65.59 ± 4.65	9.20 ± 1.35	231.85 ± 7.09	76.95 ± 1.06
	SMS GZ-PW	29 ± 1.9	24 ± 2.4	281.71 ± 10.07	113.58 ± 21.62	25.54 ± 4.32	420.84 ± 32.15	133.26 ± 13.35
	80% SMS GZ-20% PW	29 ± 0.9	26 ± 1.1	135.83 ± 6.63	64.65 ± 5.72	8.07 ± 0.93	208.55 ± 10.36	79.57 ± 1.61
	SMS BOS-PW	35 ± 0.5	22 ± 0.6	152.76 ± 1.01	64.83 ± 0.27	11.33 ± 0.11	228.92 ± 2.04	71.05 ± 3.90
	SMS CR-PW	33 ± 1.4	21 ± 2.9	178.04 ± 7.02	95.76 ± 5.02	17.88 ± 4.26	291.68 ± 8.59	80.62 ± 3.11
	SMS GZ-PW-AW	36 ± 0.5	25 ± 1.5	169.86 ± 5.71	88.83 ± 3.11	20.55 ± 0.47	279.24 ± 10.04	89.73 ± 7.04

Source: DEDOUSI et al. (2024)

A remarkable outcome which has been obtained by employing *P. ostreatus* cultivation on spent mushroom substrate (SMS), and agro-industrial residues as growth substrates through novel techniques is the marked enhancement in biological efficiency (BE%) together along with laccase production. Best substrates were those containing commercial SMS (GZ) that had high yields in the treatment 4, (=133.26%) and BE% values being recorded for *Pleurotus* spp., with simultaneous short earliness and cropping period according to study conducted by. Furthermore, coffee residue (CR) and barley-oats straw (BOS), used as alternative substrates for channels are shown to be promising in sustaining high BE with substantial laccase production. Moreover, the addition of mushroom waste into blended barley and oats straw (BOS) or SMS coffee residues (CR), substrates increased fruit body productivity; thus, using by-products from mushrooms might help to optimize cultivation. Similarly, the positive influence of SMS supplementation in laccase production was also emphasized and this compound largely increased enzyme yield into substrate with commercial SMS demonstrating successfulness of these new methods to upgrade enzyme yields and cultivation efficiency (DEDOUSI et al., 2024).

P. ostreatus cultivation has been practised with a long-established method, started from the soil preparation until harvested as mushrooms suitable for consumption. Nevertheless, novel ways to improve cultivation has been implemented through generation of spent mushroom substrate (SMS) and agro-industrial residues that are currently being widely developed for this purpose. These newly evolved methods for the compromises of substrates together with both SMS and agro-industrial residues are utilized in case of mushroom cultivation. The researchers hope to increase the biomass and biological efficiency of substrates by using mushroom waste (HOA et al., 2015). This not only optimizes mycelial growth and enzyme activity but is also sustainable in that waste reduced (zero-waste) resource utilization increases. These techniques, with revolutionary substrate combinations that include SMS; commercial fresh and spent mushroom composts as well as a variety of agro-industrial residues have shown an encouraging increase in biological efficiency (BE) and enzyme production (MELANOURI et al., 2022). By researching innovative substrate formulations and waste management strategies, the new precision farming methods are much more sustainable and resource-efficient than what we usually do in general cultivation steps of *P. ostreatus*.

2.2.2. Cultivating *Pleurotus ostreatus* in Different Countries

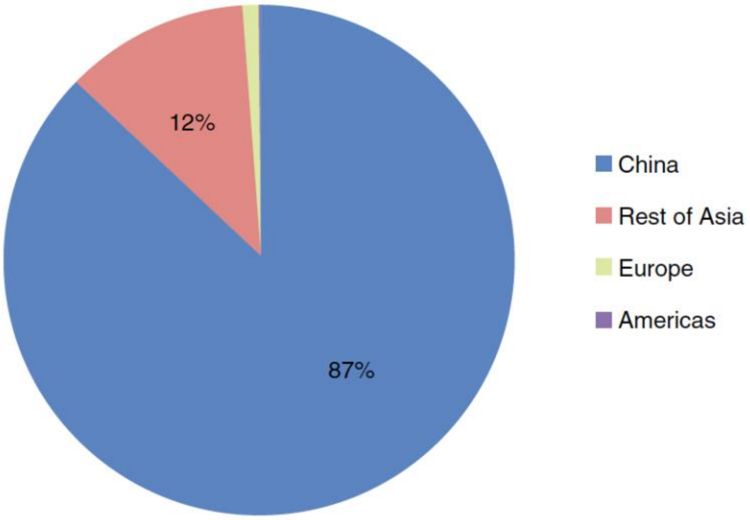


Figure 2: Percentage of total global *Pleurotus* spp. production in specific nations and regions. Source: ROYSE et al. (2017)

Pleurotus ostreatus can be considered an Asian-cultivated edible fungi, of which China is the largest producer. Focused on China, production of *P. ostreatus* was 6.9 M kg/year in 2013 (Figure 2). In addition to Europe, Asian countries lead in the production and consumption of oyster mushrooms, with a big share from Japan but also South Korea (which has an oldest tradition of cultivation), Taiwan, Thailand, Vietnam or India. In Japan, *Pleurotus* spp. increased by 190% from year of 1997 (13.3 million kg/year) to that in 2010 (39.6 million kg/year). In addition, oyster mushroom cultivation is developing in Korea as well and king oyster mushrooms are exported to European countries. The export amount of oyster mushrooms from South Korea has been on the rise over time (YOO et al., 2016). Oyster mushroom is extensively cultivated even in Bangladesh and an estimated figure of 40,000 metric tons has been produced for the year 2018–19. Oyster mushrooms are a popular type (market-oriented), and they also have compatibility with the environment of Bangladesh (FERDOUSI et al., 2020). India contributed 17% of the total mushroom production in *P. ostreatus*, Oyster Mushrooms in 2019 with an annual estimate of 34,170000 kgs (SINGH et al., 2021). In Iran, the production of oyster mushrooms is relatively low compared to button mushrooms. In 2016-17, out of 62,957 tons of edible mushrooms produced in Iran, only 0.4% constituted oyster mushrooms. Despite attempts to cultivate king oyster mushrooms, *Agaricus* *bisporus* (button mushroom) remains more profitable in the Iranian market (NOSTRATABADI et al., 2020). In Vietnam, farmers can cultivate 16 types of mushrooms, yielding 250,000 tonnes annually. The main concentration is on paddy straw mushrooms, oyster mushrooms, and button mushrooms. Since the previous decade, the straw mushroom sector in Vietnam has experienced significant growth, producing around 64,500 tonnes annually. In 2010, the Vietnamese government launched an agricultural development strategy focusing on mushrooms as one of five national strategic commodities (SINGH et al., 2021). Additionally, Malaysia stands out for its high cultivation of oyster mushrooms, accounting for about 94% of the country's mushroom production, particularly focusing on *Pleurotus* species (ROSMIZA et al., 2016). These examples highlight the global cultivation of *P. ostreatus* and the diverse production levels and market dynamics for oyster mushrooms in different regions.

2.3. An overview of Apple Pomace Production

The production of apple pomace is an integral part of the apple processing industry, where apples are cultivated, harvested, and processed to extract juice and other products. Globally, apple pomace is produced at a rate of approximately 4 million tons per year. This substantial amount of waste underscores the importance of finding sustainable solutions for its management and utilization (GOŁĘBIEWSKA et al., 2022). In the 2022/2023 crop year, China was the leading producer of apples worldwide. During that time, China’s apple production amounted to about 44.5 million metric tons. The European Union came in second place with about 12.68 million metric tons of apples (GLOBAL TOP APPLE PRODUCING COUNTRIES 2023 | STATISTA, 2024). Poland stands out as one of the largest producers of apples in the world, alongside China, the United States, and Turkey. The annual production of apples in Poland ranges from 1.877 to 3.9 million tons, with about 50% of the apples processed for juice concentrate production. This processing generates approximately 0.5 million tons of apple pomace in Poland alone. In contrast, Germany produces around 0.25 million tons of apple pomace annually, half the amount generated in Poland. This disparity reflects differences in apple cultivation practices and processing volumes between the two countries. Less apple pomace is produced in countries such as New Zealand, Spain and Brazil — around 20,000 to 13,750 tons annually. Though these amounts in comparison to the major producer are low, they still add up to some amount of global by-product production (VUKUŠIĆ et al., 2020).

The primary apple juice by-product of apple pomace is composed mainly of pulp and skins (95%) with lesser amounts of seeds (2–4%) and stems. Apple pomace contains different proportions of apple peel, seeds, cores, and pulp depending on the fruit cultivar (DIÑEIRO-GARCÍA et al., 2009; AGHILI et al., 2019). According to the data provided by FAO and above-mentioned ratios, apple pomace accounts for 25–30% of mass fresh fruit. In 2020, this will mean that a total of around four MMT of apple pomace is produced worldwide. Similarly, different research has been carried out in order to appraise apple pomace as a valuable natural active ingredient (NAI) (WALDBAUER et al., 2017) and the synthetic methodologies of pectin or fermentable sugars (LUO - XU, 2019) likewise it may find be used also as food complement (AZARI et al., 2020).

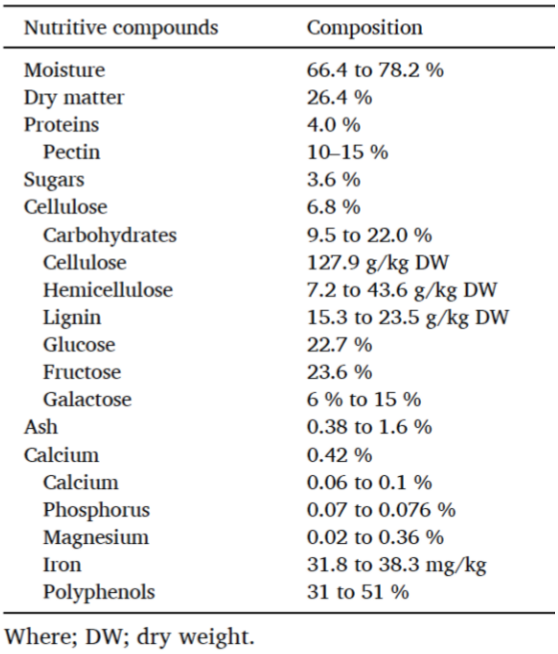
The 2018 publication "What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050" offers an alarming, sobering view into the ever-rising global municipal solid waste issue. The report forecasts that global municipal solid waste generation will be 2.01 billion tons per year, with the number expected to balloon to 3.4 billion tonnes annually by mid-century Hidden in the factory tons, is a scary statistic – almost HALF — that's 44% of all waste produced worldwide — can be lumped into food and green wastes (POVEDARENAN, n.d.).

Apple pomace waste has a high moisture content and biodegradable organic load which makes it environmental challenges if not properly disposed. The disposal of apple pomace can result in the emission of a large amount of greenhouse gases, leading to important environmental problems. For example, apple pomace is often landfilled but can lead to significant greenhouse gas emissions if it decomposes. For example, disposal of apple pomace in landfill has been shown to produce higher greenhouse gas emissions due to waste anaerobic digestion leading the production and release into atmosphere with gases such as methane (CH4) – potent greenhouse gas promoting climate change (GASSARA et al., 2011). Apple pomace as a by-product of apple juice and cider manufacture, is a large volume waste requiring an effective environmentally friendly solution for its disposal.

2.4. Nutritional compounds of Apple Pomace

Apple pomace derived from apple processing, an industrial by-product having rich nutritional potential is in limelight for numerous applications. It is realized as a sustainable resource that encourages microbial growth notably mycelia cultivation. In the present work, apple pomace is highlighted as an ideal substrate due to its complex composition of organic compounds, minerals and fibers that provide a rich media for bioprocesses with potential applications in food industries (processing) and pharmaceutical industries.

Table 6: Nutritional Composition of Apple Pomace



Nutritive compounds	Composition
Moisture	66.4 to 78.2 %
Dry matter	26.4 %
Proteins	4.0 %
Pectin	10–15 %
Sugars	3.6 %
Cellulose	6.8 %
Carbohydrates	9.5 to 22.0 %
Cellulose	127.9 g/kg DW
Hemicellulose	7.2 to 43.6 g/kg DW
Lignin	15.3 to 23.5 g/kg DW
Glucose	22.7 %
Fructose	23.6 %
Galactose	6 % to 15 %
Ash	0.38 to 1.6 %
Calcium	0.42 %
Calcium	0.06 to 0.1 %
Phosphorus	0.07 to 0.076 %
Magnesium	0.02 to 0.36 %
Iron	31.8 to 38.3 mg/kg
Polyphenols	31 to 51 %

Where; DW; dry weight.

Source: KAUSER et al. (2024)

Table 6 shown the chemical composition of apple pomace (dry basis) that includes 46.8% carbon, 6.4% hydrogen, 0.6% nitrogen, and other elements (COSTA et al., 2022). Additionally, it was noted that apple pomace contains some carbon-based (organic) constituents that are important in terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) standards values (SUN et al., 2007). Apple pomace has 70-85% moisture and primarily comprises of lignocellulosic components (7-44% cellulose, 4-24% hemicellulose, and 15-23% lignin). Apple pomace (dry basis) contains 46.8% carbon, 43.6% oxygen, 6.4% hydrogen, 0.6% nitrogen, and 0.3% sulphur (Costa et al., 2022). Apple pomace contains a high concentration of Iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), potassium (K), magnesium (Mg), and calcium (Ca) (BHUSHAN et al., 2008; O'SHEA et al., 2015).

Apple pomace is a valuable source of nutrients for the development of mycelia due to its composition of proteins, carbohydrates, cellulose, hemicellulose, and xyloglucan (ZHENG & SHETTY, 2000). In the survey conducted by research that these nutrients in apple pomace play a role as enhancing treated wastewater system for mycelia growth and metabolism activities are deemed appropriate substrate. Apple pomace has also been employed as a supporting medium for mycelia growth, due to it being rich in nutrients which can enhance the reproduction and development of mycelia (VENDRUSCOLO et al., 2008).

2.5. Mycelial Growth of *Pleurotus ostreatus* on Different Agar Media

The mycelial growth of the *Pleurotus ostreatus* was evaluated in different agar media. Studies reported on comparisons of mycelial growth of P. ostreatus on various agar media (Table 7) (ADITYA et al., 2024). Studies have demonstrated the superiority of Potato Dextrose Agar (PDA) in supporting abundant mycelial growth and maintaining favourable conditions for the *P. ostreatus* development, with Malt Extract Agar (MEA) to be effective in fungi proliferation (LENKA et al., 2022). In order to facilitate growth Corn Meal Agar (CMA) was evaluated for mycelial growth promoting activity of the fungus (ADITYA et al., 2024). Through the comparative studies, it has been validated that PDA and MEA proved to be optimal growth media for mycelium development across various oyster mushroom species. The researchers found that MEA supported abundant mycelial growth, indicating its effectiveness for cultivating oyster mushrooms (LENKA et al., 2022).

Table 7: Mycelial Growth on Different Agar Media

Agar Media	Mycelial Growth (cm)
Potato Dextrose Agar (PDA)	8.38
Malt Extract Agar (MEA)	7.72
Corn Meal Agar (CMA)	6.81

2.6. Potential risk Associated with Apple Pomace

Cultivation of mycelia on apple pomace is an intriguing route for exploitation towards use as agricultural waste, but they must naturally be treated with caution. There are two main issues associated with the risks of consuming apple pomace: phytotoxins, and biotoxin agents including natural toxins derived from plants and pesticides, as well as their influence on mycelial colonization (BHUSHAN et al., 2008). Apple pomace, as a substrate, is rich in natural plant toxins like amygdalin which can be enzymatically broken down into toxic compounds including hydrogen cyanide As it turns out, most studies have indicated that eating traces of amygdalin in apple seeds is generally safe for human consumption. Apple pomace consumption may be associated with little risk of acute cyanide poisoning because to intake as high as approximately 800 g would HAVE TO BE triggered the reference dose (SKINNER et al., 2018). Pesticide residue is another concern, potentially stemming from the use of pesticides in apple orchards. Neonicotinoids, a class of insecticides, have raised concerns, yet studies indicate negligible contents in the majority of apple varieties (CHEN et al., 2014). The poison in some apples, acetamiprid is also of little toxicity and poses a very low risk. Apple fruits have shown an accumulation of fungicides thiophanate, carbendazim and pyrimethanil (TZATZARAKIS et al., 2020). The fungicides used in these peppers are classified as relatively non-toxic by the United States Environmental Protection Agency (EPA), which was confirmed on human less compare to other organisms. However, ongoing research is critical as the field of pesticides and chemical agents changes.

The most bizarre aspect is that apple pomace may be harmful to mycelia colonization. Pesticide residues typically have a negative impact on mycelia growth and development, necessitating more inquiry and management methods. Mycotoxins (toxic secondary metabolites generated by some fungi) would hinder mycelial growth and could have threatened human health if included in a food product (SKOVGAARD, 2010). In addition, there is the possibility that in apple pomace other contaminants are present besides those mentioned above (e.g., heavy metals and pathogens) or foreign material appears which can interfere with mycelia colonization as well be associated to health risks (ŠELO et al., 2021). An in-depth study of this risk factor and contaminants is essential to guarantee the safety, and quality of mycelia colonization. Such an investigation will not only serve to characterize hazards but also enable recommended strategies for mitigation or elimination thereof, providing the successful colonization of mycelia and greater assurance in ensuring safe production practices and high-quality products.

2.7. Beta-Glucan in Mushrooms and Its Role

Beta-Glucans — A Beta-glucan is a type of polysaccharide derived from the cell wall of bacteria, fungi and plants. Glucans are glucose molecules joined along the beta-glycosidic bonds, either in a linear or branched chain. These special properties have been attributed to the beta-glycosidic bonds held by these compounds, which allow them gels (and resist human enzymes). Based on the type of linkage between glucose units, beta-glucans are classified. The beta-glucans found in mushrooms most frequently consist of a long linear chain of glucose units with beta-1,3-glycosidic bonds. Other types of beta-glucans found in mushrooms include beta-1,6-glucan, which has a branched chain of glucose units linked by beta-1,6-glycosidic bonds, and beta-1,3/1,6-glucan, which has a combination of both linear and branched chains (SARI et al., 2017). Many mushrooms are high in beta-glucans; some, like maitake and reishi mushrooms, contain at least 50% of their dry weight as complex polysaccharides (MCCLEARY - DRAGA, 2016). The beta-glucan content of mushrooms is variable, depending on the species, part consumed and cultivar method used. Enzymatic assays for beta-glucan content determination of mushrooms have some issues and there are still no standard methods on quantitative determining the beta-glucans in mushroom extracts (SARI et al., 2017). Beta-glucans also demonstrate a range of health effects, such as immune modulation (boosting and calming the system), anti-inflammatory properties, cholesterol lowering capacity. The immune system identifies them as molecules originating outside the body and can promote immune cells, cytokines that are involved from fighting infections to diseases (SARI et al., 2017). The antimicrobial potential of β-glucans against parasites has led the scientists to conduct research on this. Available evidence has proven their immunomodulatory and antimicrobial potential. In particular, β-glucans have been demonstrated to activate macrophages and natural killer cells through interaction with specific cell surface receptors, increasing overall antimicrobial immunity. Apart their antimicrobial activity, β-glucans have been described as prebiotic agents able to modify the gut microbiota community and metabolic behaviour. Indeed, some studies have also shown that β-glucans can promote the growth of beneficial gut bacteria such as *Prevotella* and *Roseburia* which could bring about a favourable change in microbial composition (FEHLBAUM et al., 2018). Beta-glucans also predicted the propionate, which is a short-chain fatty acid (SCFA) and beta-glucan driven by immunity ability to stimulate this SCFA production in humans has been reported before as well. These SCFAs, especially butyrate, are used as energy by colonic epithelial cells and contribute to multiple gut protective effects including anti-inflammatory and immunomodulatory actions.

2.7.1. Formation of Beta-Glucan

The biosynthetic routes in the β-glucan formation of mycelia are highly intricate within fungal cells. This is when the fungus, as its mycelium grows and expands outwards into new soil layers to colonise them, starts synthesizing β-glucans for structural purposes in their cell wall. It consists of the enzymatic synthesis of β-glucans, where these polysaccharides are produced within fungi cell (SURENJAV et al., 2006). Thus, the generation of biologically active β-glucan in mycelia is a complex and elaborate process, which requires coordination or interaction between various enzymes and metabolic pathways. Additionally, β-glucans are among the most prevalent cell wall components besides chitin and not only ensure biomechanical robustness of fungal cells but also contribute significantly to pathogen-associated molecular patterns (PAMPs) that control interactions with other microorganisms (HOCHSTENBACH et al., 1998). 1. Synthesis: It can be concluded that the synthesis of β-glucan at mycelial level is a well-controlled process which follows an enzymatic pathway. The identification of sequences which encode glucan synthases has been discussed in several species, and this supports the idea that β-glucans may be synthesized rather than regulated. They are catalysed by β-glucosyl (Headrick type) kinases that link glucose monomers via 1,3-β-glycosidic bonds (RUIZ-HERRERA - ORTIZ-CASTELLANOS, 2019). In Fungi, the enzymatic machinery in each cell decides whether a linear or branched β-glucan will be formed. 2. Structure: There are some yeast and mycelial β-glucans with similar linkages, the dimension of chains which compose them might be a major difference or distinguishing feature. The microfibrillar structure of β 1,3 glucan is discussed in the context cell wall architecture and resistance. The composition of branched β-glucans and their variability in structure within fungal species is also analysed to demonstrate the structural diversification that occurs for mycelial cell wall-associated (1→3)-linked-β-glucans (BOWMAN - FREE, 2006). Mycelial β-glucan structure may be influenced by the action of enzymatic machinery, environmental conditions and genetic control. 3. Regulation: The mechanism of synthesis β-glucans in mycelia is also governed by several factors such as environment and genetics. This allusion is consistent with the rarity of β 1,3 glucan linear synthesis observed in specific conditions such as regeneration protoplasmic and soluble extracts from some fungi. It is suggested that this enzyme which synthetizes β-glucans in mycelium formed through its specific regulatory mechanisms. Research demonstrates the significance of regulating β-glucan synthesis in mycelia for maintaining cellular homeostasis and survival during environmental stress. In summary, study demonstrated that β-glucan (1→3)-carbon is as an intermediate in the formation of these compounds and they are synthesized and assembled in a regulated way to form complex structures at specific subcellular locations within fungal cell wall (RUIZ-HERRERA - ORTIZ-CASTELLANOS, 2019).

2.7.2. Distinctive Characteristics and Health Implications in Fungal Mycelia and Fruiting Bodies

Furthermore, there are clear differences in the structure of β-glucans when comparing those produced by mycelium with fruiting bodies. Differences in solubility, branching, molecular weight and shape between these forms can have a remarkable effect on their biological activity (SURENJAV et al., 2006). The differencing glycosidic bonds in the β-glucan structure account for its mycelia and fruiting body-derived fractions. Several studies have demonstrated that the type of beta-glucan found in mushroom fruiting body and mycelia can vary greatly contributing to differing physiological effects. The major structural feature of mushroom beta-glucans is a beta-1,3-D-glucan main chain with single D-glucosyl residues linked beta-1,3 along this main chain (SARI et al., 2017). These beta-glucans are not made by the body and when detected, they prompt a response from the immune system (both innate and adaptive). Fruiting body-derived beta-glucans and cultured mycelia-produced beta-glucans have distinct properties, accessibility and implications. Beta-glucans are present in the fruiting body of the mushroom and also produced from cultured mycelia as soluble beta-glucan (CHANG - WASSER, 2012). Similarly, fruiting body and this growing extracts could ameliorate some conditions that support the bioactivity of beta-glucans from both origins (SARI et al., 2017). On the other hand, for beta-glucans production and extraction process of mushroom fruiting bodies highly time consuming while higher basidiomycetes like mycelia are more efficient by submerged cultivation. These findings may indicate that mycelia could become a more economically feasible source of beta-glucans for agricultural or pharmaceutical applications (KOMURA et al., 2010). In addition, the use of mushroom extracts with soluble beta-glucans versus the consumption of the whole fruiting body is discussed for digestibility and bioactivity (SARI et al., 2017). This suggests that the way in which beta-glucans are ingested can play an important role on their bioavailability and potential health effects.

2.8. Impact of the C/N ratio on Mycelial Growth

The fungi mycelium needs carbon as an essential element for growth. It provides energy and carbon for mycelium metabolism to perform metabolic processes, the synthesis of cellular constituents. Fungi can utilise various carbon sources regardless of whether they are mono-, di- or polysaccharides. Nevertheless, the specific carbon source is dependent upon species and carbohydrate mix in nutrient media on fungal growth. For oyster mushrooms, a study was conducted to identify the best carbon source for mycelial growth so glucose, dextrose, fructose, maltose and also sucrose molasses were tested. For the mycelia of oyster mushroom *Pleurotus ostreatus* (PO), glucose, sucrose and molasses were excellent carbon sources in the composting substrate while for *Pleurotus cystidiosus,* PC tested samples glucose + molasse+ dextrose are good carbon source. Except in the case of oyster mushroom PO, sucrose was demonstrated to be (by far) the best carbon source available with lowest price (HOA - WANG, 2015). Nitrogen is an essential nutrient for all fungi, and they need to produce nitrogen-containing compounds (such as purines, pyrimidines, proteins) or the cell wall constituent chitin (a polymer composed of 3(1-4)-linked units of N-acetylglucosamine (CHANG - MILES, 1992). We use this control as a basic level of reasoning for physiological and metabolic regulation by different microorganisms since it allows to check the nitrogen concentration on which it is based, i.e., nutriment composition (THOMAS et al., 1996). Mycelium growth is greatly influenced by the Carbon to Nitrogen (C/N) ratio. High C/N ratio can inhibit mycelium growth and a low C/N ration limits the energy and carbon available for the mycelium. This is due to the fact that nitrogen element is essential in synthesis of fungi for various nitrogen containing compound, protein and cell wall. Thus, controlling suitable C/N ratio of culture media is required for promoting mycelial growth when cultivating. The study (HOA - WANG, 2015) also pointed out that excess carbon content results in a high C/N ratio which is harmful for metabolite biosynthesis as well as mycelium growth. On the other hand, too little carbon can mean that not enough energy or carbon is present for mycelium to consume. Hence, regulation of C/N ratio in the culture medium becomes essential to attain built-up mycelial growth in oyster mushroom (HOA - WANG, 2015).

2.9. Oyster Mushroom Mycelia-Derived Feed and Food Supplements

There are commercially available feeds and nutritional supplements derived from oyster mushroom mycelia. The mycelium of *Pleurotus ostreatus*, or oyster mushroom, has received interest due to its possible health advantages and nutritional worth. Mycelium-based products are being used as dietary supplements and functional foods due to their bioactive compounds and medicinal properties. A number of studies have demonstrated the possible health beneficial properties overflowing in bioactive compounds from mycelium originated by oyster mushroom, such as immunomodulatory, antioxidant, besides efficacy against tumorigenesis, and antidiabetic effects (TÖRŐS et al., 2022). Studies have investigated a few of these bioactive compounds (e.g., polysaccharides, ergosterol peroxide and proteoglycans) for their possible therapeutic applications.

It's a known fact that oyster mushroom mycelia have numerous benefits and applications right from food to feed, nutraceuticals etc. Unlike the oyster mushroom mycelium, which are the part of the fungus underground system becoming mature largest organ from being vegetative thus giving way to its fruiting body. The mycelia were found to possess a diversity of nutrients, bioactive constituents and functional properties which make them compatible for application in different products.

One of the more interesting aspects regarding oyster mushroom mycelia is their nutrient content. Most grains are low in fat, the major dietary source of energy and good sources of complex carbohydrates; they also provide some proteins and relatively small amounts vitamins, minerals and other nutrients. These are capable of being used in food and feed products which can add nutritional value to the end product (MOON - LO, 2013). There is also potential to extract functional properties from the mycelia of oyster mushrooms. One study published in Pharmaceuticals reveals that they contain active compounds known as polysaccharides, which have been identified for their potential health benefits such as immunomodulatory effects and antioxidant activities to antitumor properties one. The presence of such functional properties in oyster mushroom mycelia could render them useful products for nutraceuticals and health foods, a sector more than simply related to basic nutrition (MANZI, 2000).

Mycelia of oyster mushroom is sustainable as well. Cultivation can be carried out with agricultural waste or other sustainable substrates, hence using organic by-products and encouraging circular economy practices. This makes them eco-friendly for use in food and feed manufacturing (ANTUNES et al., 2020). It's also produced meatless alternatives from oyster mushroom mycelia that resemble muscle tissue fibers found in whole cuts of the meats. These goods are an environmentally friendlier, plant-based choice to conventional meat products that can assist reduce the carbon footprint of manufacturing meats.

Atlast Food (MYFOREST FOODS, 2019) is an American Food technology firm that uses solid-state fermentation techniques to produce mycelium suitable for meatless alternative products. Major application possibilities were in the categories of functional foods, nutraceuticals and animal feed for products produced from oyster mushroom mycelia. Addition of mushroom mycelia to the diet in food products may improve functional and nutritional values into various foods such as fortified, snacks, beverages etc. To build supplements or functional ingredients from the mycelia of oyster mushroom for extra beneficial effects is also very applicable. Their results demonstrated that the utilization of oyster mushroom mycelia as a feed ingredient can be a seemingly promising option to promote the well-being and performance (physical condition) of animals (ANTUNES et al., 2020). Conclusively, the studies included in this review may serve as pioneer evidence for multiple functions and applications of oyster mushroom mycelia including nutritional benefits, sustainable production processes, functional properties available to knowledge-seekers with an eternal thirst.

3. MATERIALS AND METHODS

3.1. Experiment 1: Mycelia growth on Apple Pomace Media with Different Concentration of Apple Pomace

A. Materials and Equipment

Sample of the dried apple pomace (Figure 3) used in the experiment was produced by "Naturpolc" in Debrecen, Hungary, and was packaged in 1 L containers. *Pleurotus ostreatus* (late oyster mushroom) was sourced from Magyar Gomba Kertész Kft., Hungary. The media components consisted of agar-agar, distilled water, and dried apple pomace obtained from apple juice production.

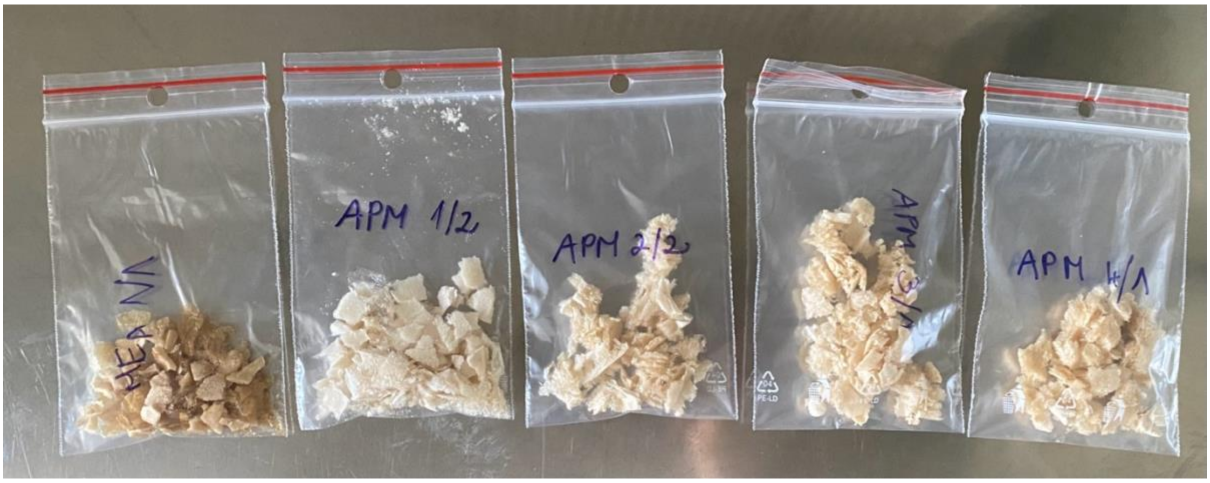


Figure 3: Dried Apple Pomace that were using in the experiment.

B. Malt Extract Agar Preparation

Malt extract medium was prepared by dissolving 20 g of malt extract and 20 g of agar in 1 L of distilled water.

C. Preparation of Apple Pomace Media

Apple pomace was prepared in varying concentrations, as shown in Table 8. The concentration of apple pomace was increased incrementally, while the quantities of agar-agar and distilled water remained constant. The dried apple pomace was boiled in 200 mL of distilled water for 5 minutes. The extract was then filtered using dairy industry filter paper (100 microns). Agar-agar was added to the filtered apple pomace extract before the second boiling step, which lasted for 3 minutes. After boiling, the mixture was transferred into 0.5 L autoclavable flask bottles. The final volume was adjusted to 200 mL using distilled water.

D. Sterilization of Media and Inoculation of the Mycelia

The MEA Media and Apple Pomace Media as presented in Table 8. were sterilized in an autoclave at 121 °C for 25 minutes. Under aseptic conditions, 20 mL of the media were poured into the sterile Petri dishes (diameter= 80 mm) and allowed to be solidified in a horizontal position before cooling to room temperature for inoculation. The prepared media then were exposed to UV light for 30 minutes under laminar flow and then inoculated. A sterile, stainless-steel spatula was used to transfer small discs of 10 mm in diameter from the culture which were then inoculated onto the centre of the culture plate, incubated at room temperature, and protected from light until full growth (8 days).

Table 8: Details of the ingredients (g/L) used in apple pomace-based media preparation for mycelia growth

Components of the media	MEA	AP-20	AP-30	AP-40	AP-50
Malt extract (g)	20	…	…	…	…
Apple pomace (g)	…	20	30	40	50
Distilled water (mL)	1000	200	200	200	200
Agar-agar (g)	20	4	4	4	4

3.2. Experiment 2: Mycelia Growth on Malt Extract Agar with Apple Pomace Liquid in the Centre at Different Concentrations

A. Material and Chemicals.

The dried apple pomace used in the study was sourced from the same manufacturer that was used in the present study. *Pleurotus ostreatus* (late oyster mushroom) was obtained from Magyar Gomba Kertész Kft., Hungary. Malt Extract agar (MEA), chemicals and reagents like dextrose, peptone, distilled water were prepared by the laboratory of University of Debrecen.

B. Preparation of Dextrose, Peptone, and Apple Pomace Concentration solution

1. Dextrose Solution Preparation

Dextrose solution was made by carefully weighing 1 g of dextrose and mixing with 10 mL sterile distilled water in a clean container. After establishing a homogenous mixture by vertexing, the dextrose is completely dissolved. Once homogenized, autoclaved the solution to sterilize it and also pipette tips that were used during liquid handling at 121 °C for 25 minutes.

2. Peptone Solution Preparation

Peptone solution was made by adding 1 g peptone and dissolved in 10 mL of distilled water for preparation. The solution was mixed using a vortex to make sure the peptone is evenly distributed. Finally, the solution was autoclaved at 121 °C for a duration of 25 min with pipette tips to obtain sterility.

3. Apple Pomace Solutions of Various Concentrations

Apple pomace solutions were formulated in four varying concentrations 1 g, 1.5 g, 2 g and 2.5 g apple pomace specific amounts of powdered apple skins (each) mixed with10 mL water in different tubes. Mixtures were homogenized by vortex mixing to form uniform solutions. The solutions as well as the pipette tips were autoclaved at 121 °C for 25 minutes after homogenization to guarantee full sterilization before being used in further experiments.

C. Malt Extract Agar Media Preparation

Malt extract medium was prepared using 20 g of malt extract and 20 g of agar dissolved in 1 L of distilled water. The prepared medium was sterilized in an autoclave at 121 °C for 25 minutes. After sterilization, the bottles were exposed to UV light for 30 minutes before use. Under aseptic condition, the medium was poured into Petri dishes. After solidification, a sample of *Pleurotus ostreatus* mycelia was placed on the surface of the solid medium. Three drops of dextrose, peptone, and apple pomace solutions at different concentrations were added to the centre of the mycelia before incubation.

D. Growth, Biomass Measurement, and Wet Mass

The extent of growth was assessed by measuring the surface area horizontally of the media covered by the mycelia daily for 8 days using ruler, expressed in millimetres (mm). The growth rate was calculated using the following Eq. (1):

$$\text{Growth rate} = \frac{\text{Colony diameter on the last day (mm)}}{\text{Number of days}}$$

The diameter measurements and photographs were taken each day. The wet biomass (g) was measured by scraping the growth from the surface of the media using a sterilized metallic cell scraper. The wet mass of extracted mycelia was then measured with the utmost precision using a sensitive and accurate precision balance.

4. RESULTS & DISCUSSION

4.1. Experiment 1: Mycelia growth on Apple Pomace Media with Different Concentration of Apple Pomace

A. Growth of the Produced Biomass (Daily Growth and Growth Rate)

All living organisms require nutrients for growth and reproduction, and oyster mushrooms are no exception. To cultivate oyster mushrooms in the laboratory, it is essential to provide the necessary compounds in the growth media to support their development and life processes. Five different culture media were tested over six days at room temperature incubation to observe their effects on mycelial growth. The data (Figure 4) show the average colony diameter of *P. ostreatus* mycelia over this period. It was observed that *P. ostreatus* grew particularly well on media containing 200 g/L of apple pomace, producing larger colony diameters. In contrast, media with 250 g/L of apple pomace resulted in smaller colony diameters. These findings align with previous studies by (PICORNELL-BUENDÍA et al., 2015), who found that excessive amounts of nano-amino in the substrate negatively impacted certain growth parameters, such as the pileus length of harvested mushrooms. Each mushroom species requires an optimal carbon-to-nitrogen (C/N) ratio in the substrate used for cultivation to maximize yield in the shortest production period (ZIED et al., 2011). It is also supported that increasing the amount of supplements decreased the growth of mycelium. The slower spawn running at higher concentrations of additives as APM 4 in this study may be due to the excess nitrogen, which is known to inhibit mushroom growth (DEMIRCI, 1998; BAYSAL et al., 2003).

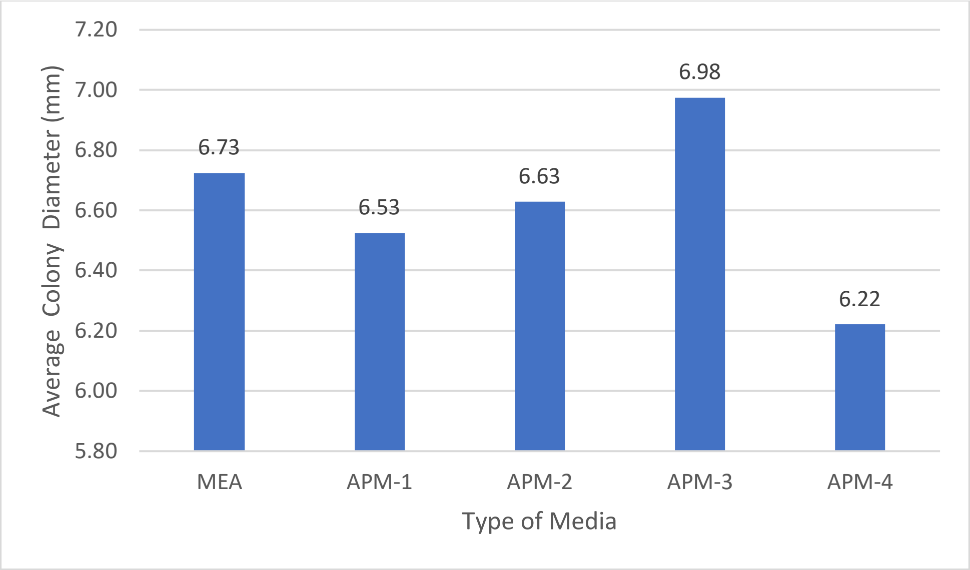


Figure 4: Average (mm) mycelium colony diameter of the *Pleurotus ostreatus* mycelia over 6 days. MEA, Malt Extract Agar; APM-1, Apple Pomace Media 100 g/L; APM-2, Apple Pomace Media 150 g/L; APM-3, Apple Pomace 200 g/L; APM-4, Apple Pomace 250 g/L.

4.2. Experiment 2: Mycelia Growth on Malt Extract Agar with Apple Pomace Liquid in the Centre at Different Concentrations

A. The Growth of the Produced Biomass (Daily Growth and Growth Rate)

Nutrients play a crucial role in the growth of *Pleurotus ostreatus*. While mycelium from many mushroom species can grow across a wide range of nutrient sources, finding the optimal nutrients is key to maximizing growth. In this study, different types of nutrient source liquids, acting as supplements, were applied at varying concentrations to the centre of the *Pleurotus ostreatus* mycelium to determine which were most effective for mycelial growth. Of the six nutrient liquid types tested, data (Figure 5) shown that apple pomace liquid and peptone liquid were the most favourable for enhancing mycelium growth, as measured by colony diameter. The peptone used in this experiment is based on the study by (EDO, 2021), which found that the concentration of peptone increases the growth rate of the mycelial colony. The highest mycelium colony diameters were observed in media containing Apple Pomace 100 g/L liquid, Apple Pomace liquid at a concentration of 150 g/L and peptone liquid. Conversely, the slowest mycelium growth occurred in the medium containing dextrose liquid as the nutrient source. Growing *P. ostreatus* on Malt Extract Agar (MEA) with Apple Pomace liquid concentration in the centre of the mycelia has resulted in a good colony diameter, and it has been stated by (AZIZI et al., 1990) that the addition of nitrogen to the substrate helps in achieving higher mushroom yields (AZIZI et al., 1990; GUPTA – VIJAY, 1991).

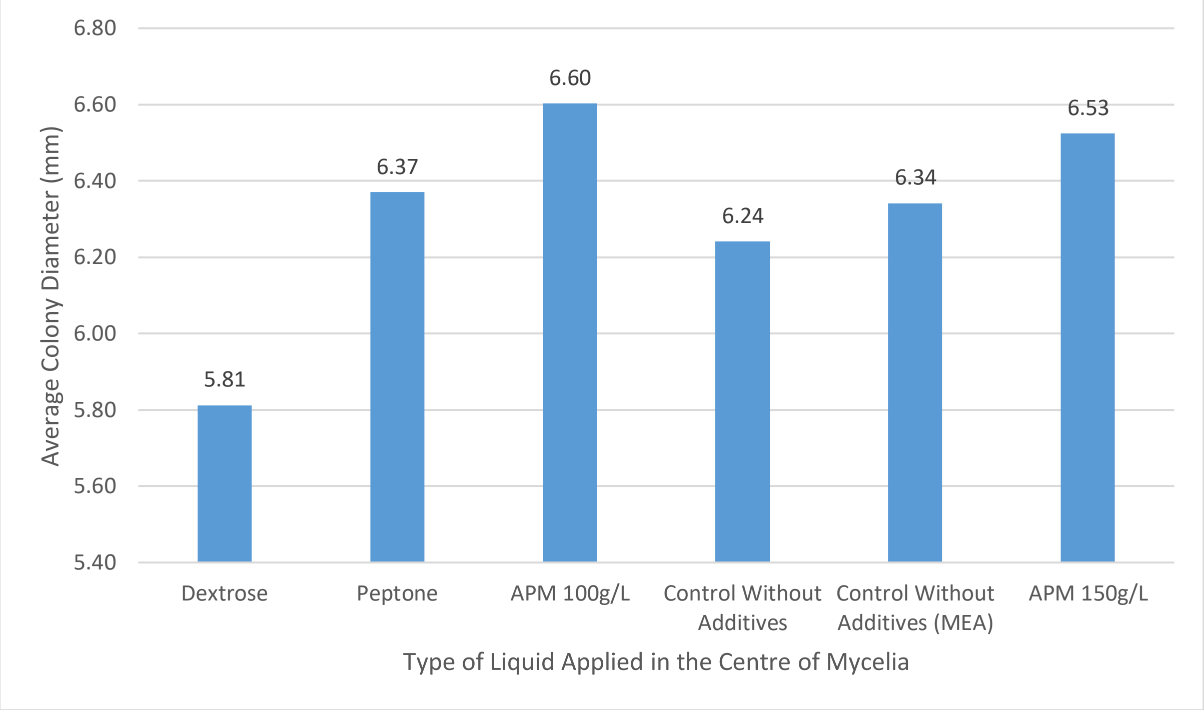


Figure 5: Average mycelial colony diameter (mm) using liquid concentration as a supplement in the centre of the mycelium. APM 100 g/L, Apple Pomace Media liquid 100 g/L; APM 150 g/L, Apple Pomace Media Liquid 150 g/L.

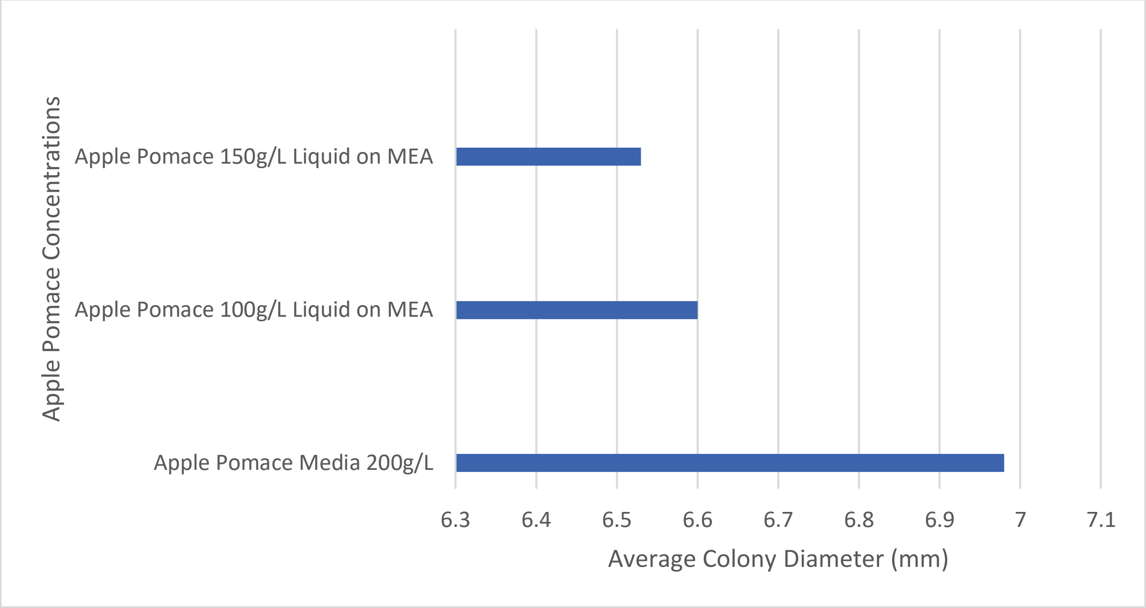


Figure 6: Average colony diameter (mm) growth on media on Apple Pomace Media 200 g/L; using liquid applied in the centre with concentrations of Apple Pomace (100 g/L and 150 g/L) on Malt Extract Agar (MEA).

Figure 6 above indicate that the nutritional composition of apple pomace makes it a promising substrate for mycelial growth. The apple pomace contains 46.8% carbon, 0.6% of nitrogen and other important macronutrients that makes it a valuable supplier to many nutrients necessary for plant metabolic processes. Apple pomace contains cellulose, hemicelluloses and lignin as its main components of lignocellulosic that also help apple pomace to become a good substrate. (COSTA et al., 2022). Studies have revealed that the essential nutrients for *Pleurotus ostreatus* mycelial growth include carbon (C) and nitrogen (N), which are the primary macronutrients required for structural and energy needs. Phosphorus (P), potassium (K), and magnesium (Mg) are also essential macronutrients, while trace elements such as iron (Fe), selenium (Se), zinc (Zn), manganese (Mn), copper (Cu), and molybdenum (Mo) support various physiological functions in mushroom growth and development (WASSER, 2004). Figure 7 shown the best result of the production of mycelia using the Apple Pomace liquid concentration in the centre. The picture was taken on the day 6th.

Figure 7: The best result of production of *Pleurotus ostreatus* on the day 6

4.2.1. Correlation of Apple Pomace Nutrients and Essential Nutrients for Mycelial Development

It is rich in critical nutrients as it contains 46.8% carbon, 0.6% nitrogen and the most essential macronutrients available for plant growth. The lignocellulosic part apple pomace, which includes cellulose and hemicellulose as structural polymers; the possibility of utilization these compounds by mycelial maturation may be expanded due for the developing availability from claiming new substrates (COSTA et al., 2022). Cellulose, the complex carbohydrate that mushrooms are built to consume (whether as mycelium or fruiting body). Another substance is a part of carbohydrates, hemicellulose, which forms the nutritive base for insects. This complex polymer, lignin gives structural support to plants and also a barrier, for decomposition (ADITYA et al., 2024). Lignin is derived from plants and so being an enzyme that breaks down lignocellulose, oyster mushrooms can get energy by this way as well. Thus, apple pomace can serve as the ideal sustainable nutrient substrate for enhancing mycelia growth due to those macronutrients required in essential growth nutrients. The apple pomace provides a suitable environment to develop mycelium due to containing Carbon and Nitrogen which are essential for the synthesis of structural components and proteins. In addition, due to the presence of significant nutrients such as Phosphorus (P), Potassium (K) and Microelements Phosphorus(P) potassium(K) magnesium(Mg), calcium(Ca); essential for plant growth, apple pomace (BHUSHAN et al., 2008; O’SHEA et al., 2015), may be considered an appropriate substrate source provided suitable structural characteristics are satisfied.

The trace elements present in apple pomace (Fe, Zn, Cu and Mn) are essential for the activity of some enzymes functions involved on mushroom growth or development (WASSER, 2004). These play a role in the activation of enzymes and metabolic pathways that mycelia require to thrive. Furthermore, apple pomace is a lignocellulose rich source and hence its cellulose/hemicelluloses components makes it suitable to act as complex substrate for mycelial colonization. The different enzymes included within the apple pomace (xylanase, cellulases) are beneficial to cut complex polysaccharides in substrate and take up necessary nutrients through mycelium. Moreover, the linkage of lignocellulosic fractions in apple pomace to enzymes degrading lignin by mushrooms is serving as a further support for effective absorption of several nutrients (ZHENG & SHETTY, 2000). Various enzymes present in the apple pomace (xylanase, cellulases) helps to degrade complex polysaccharides within substrate and provide essential nutrient uptake through mycelium. Furthermore, the association of apple pomace lignocellulosic fractions to mushroom enzyme works well for reducing dozen or so other nitrogen-containing items are in use regarding uptake vitamins (SÁNCHEZ, 2009; KABEL et al., 2017; VOS et al., 2017). The apple pomace contains the nutritious area in which mycelia grows. Bringing forth the enzymes and presence of carbohydrates most important macronutrients are proteins, trace elements in apple pomace composition creating suitable habitat for such profuse development mycelium. This gives apple pomace a venerable name in mycological cultivation because it grows the perfect conditions to grow, ground and pick mushrooms.

5. CONCLUSION & RECOMMENDATION

We conclude from this experimental thesis paper that apple pomace, a by-product of the apple processing industry is a useful and sustainable substrate for growing mycelium *P. ostreatus.* Not only is this a novel way to utilize agricultural waste, which adds greater sustainability by cutting down on the amount of refuse and increasing mycelium growth as well as nutrition qualities — but it also may have implications for the future production of food products, nutraceuticals, or biotechnological advancements.

The first experiment, mycelial growth was assessed using varying concentrations of apple pomace in the media. Both 200 g/L and even the less detrimental for growth (250 g /L) inhibited further nitrogen application is promoting its control in mycelial colony to lower diameters, which reinforced once lead dry apple pomace over the production of biomass. For the reasons become clear in this study, a critical practice that may lead to maximal propagation of mycelia with correct C/N ratio is essential from natural substrates. Our results are consistent with these reports, that suggested that simultaneous changes to ingredient and nutrient composition may result in an imbalanced nutrients ratio where growth rates were affected i.e. raw materials with overconcentration of nutrients.

The second experiment adding apple pomace liquid to MEA centre demonstrated that these media could be used in combination (synergistic). The solid medium (made up of Malt Extract Agar, MEA) supports fungal growth, and the liquid apple pomace provides an additional nutrient source to encourage even faster rates of grow. The largest colony diameters were observed when 100 g –150 g/L of apple pomace liquid was used, confirming that our apple pomace is indeed rich in the essential nutrients needed for mycelial growth. This study has illustrated that apple pomace and Malt Extract Agar (MEA) is complementary to the growth of mycelia. We demonstrated in two experiments that apple pomace with its nutrient rich nature, especially high level of carbon and nitrogen which are crucial for fungal growth could be a practicable substrate/medium.

Thus, apple pomace for nutrient-supplying ability and MEA as a buffer provide an efficient medium promoting growth of the *Pleurotus ostreatus* mycelium because each component provides different nutrients to stimulate maximum growth. Taken together, the study highlights good agriculture practices for sustainable mushroom cultivation through value-addition of apple pomace as substrate. Apple pomace shows high potential to employ it in biotechnological processes leading toward environmentally cleaner and cost-efficient mycological uses. The results are not only useful for optimizing the cultivation of mushrooms, but it could also be used to further promote bio-circular agriculture by using waste.

In conclusion, this study demonstrated that the application of apple pomace as a substrate for *Pleurotus ostreatus* mycelia production is feasible and offers an environment-friendly alternative to agriculture-oriented business accordingly waste treatment. In doing so, it not only solves environmental qualms by preventing agricultural waste but also maintains a platform for the production of high-quality and nutritionally rich mycelia with wide applications across industries. Research optimal nutrient composition of apple pomace based medium and expand the food & biopharmaceutical industrial application.

SUMMARY

The present study has an eye on the increase in sustainability, reusing agricultural waste such as apple pomace (the residue from making juice) to enhance mushroom production. Focusing on waste minimization, the research looks at ways that this often-overlooked resource could be repurposed as a valuable substrate for growing mushrooms to increase sustainability and produce higher crop yields.

This thesis examines apple pomace as an environmentally sustainable, innovative option for production practice of mushroom. Due to its high organic contents, apple pomace makes environment issues when throws. This technique transforms pollutants into useful substrates for growing the oyster mushroom *P. ostreatus*, and thus avoids pollution by giving an added value to waste products which can function in a circular economy fashion unravelling molecular profiling of apple pomace and how its composition can benefit edible mushroom cultivation, as highlighted by this study.

The literature reports the nutritional qualities of *P. ostreatus* as high protein and fibber content, chitin complexation possibilities; general crop production; improvement techniques through agricultural by-products addition such as apple pomace species. It also covers the global apple pomace production and applications, paying special attention to biotech in food sectors. As apple pomace contains carbons and proteins so, this makes it a perfect substrate for mycelium development. One of the main objectives was to develop and optimise an agar medium comprising apple pomace at different concentrations in order to evaluate mycelial growth efficacy. We also aimed to investigate any potential drawbacks in using apple pomace as a raw material, i.e. contamination risks of heavy metals or pesticides from its source. The first was found in the literature review.

Materials and methods: Two experiments were established in the Food Science Laboratory of University of Debrecen from April to June 2024. Apple pomace obtained by local Magyar Gombar Kertész Ltd., Hungary, in various concentrations were tested for their effects on mycelial growth. One experiment used dried apple pomace added to a nutrient-rich agar medium called Malt Extract Agar (MEA), and the other employed liquid apple pomace. Firstly, the growth rate and colony size were analysed to find conditions which are suitable for

mushroom growing. Analysis showed that growth was faster with lower concentrations than higher apple pomace ones, a parameter likely due to the greater nitrogen content.

A reduced level of apple pomace (200 g/L) resulted in significantly higher conditions for mycelial growth compared to the presence of a greater concentration (250 g/L), as confirmed by our study. The second experiment showed that apple pomace together with other nutrient-rich substances, such as MEA provide fundamental nutrients required to stimulate mycelial growth. This supported the idea that C-N balance on apple pomace is important to nurture high yield of mushrooms.

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