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Sustainable valorization of secondary materials for oyster mushroom (*Pleurotus ostreatus*) Cultivation

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In front of the jury composed of

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بسم الله الرحمن الرحيم

وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ

وَأَنَّ سَعْيَهُ سَوْفَ يُرَىٰ

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ALLAH said: (If you are thanks I will give you more)

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CG	Cofee grounds
Cr	Cardboard
DM	Dry matter
DW	Dry weight
EC	Electrical conductivity
M 3S	Mixture of 3substrates
SD	Sawdust
Vit	Vitamin
TA	Titratable Acidity
WC	Water content

Abstract

The sustainable valorization of secondary materials for the cultivation of oyster mushrooms presents significant potential for improving yields and the nutritional quality of the mushrooms. This study examines the effect of different substrates on oyster mushrooms' growth, life cycle, yield, and nutritional composition. The substrates tested include cardboard (Cr), sawdust (SD), coffee grounds (CG), and combinations of these substrates. The results show that:

The Cr substrate produced oyster mushrooms with the shortest life cycle of 37.33 ± 3.78 days. Their pH is closest to neutrality, with an average value of 6.62 ± 0.03. This same substrate stands out for its contribution to the richness of the mushrooms in vitamin B12 (0.69 ± 0.05 µg/g) and calcium (20 ± 0.49 ppm).

Mushrooms grown on the 70SD30CG substrate (70% sawdust and 30% coffee grounds) showed the highest yield, averaging 137.5 ± 7.5 g. In terms of water content, this substrate also produced mushrooms with the highest water content at 85.6 ± 0.99%, indicating increased freshness of the mushrooms. This same substrate enhances the sweet taste of the mushrooms, with an average content of 0.26 ± 0.009 µg/g DM.

Mushrooms grown on the SD substrate (sawdust) showed the highest concentration in terms of vitamin D content (1.45 ± 0.02 µg/g DW), sodium (1650 ± 45.82 ppm), and lipids (23.93 ± 0.3%).

The combination of the three substrates in equal parts (M3S) produced mushrooms rich in dry matter (27.33 ± 3.9%), vitamin B12 (0.65 ± 0.28 µg/g), ash (3.51 ± 0.37%), potassium (307 ± 2.51 ppm), calcium (20 ± 0.34 ppm), and sugars (0.26 ± 0.04 µg/g DM).

Finally, the 50CGSD substrate (50% sawdust and 50% coffee grounds) favored a higher content of proteins (1.38 ± 0.01 mg/L), polyphenols (0.046 ± 0.007 mg GAE/g), and flavonoids (0.024 ± 0.004 mg QE/g).

These results highlight the importance of carefully selecting and mixing substrates to optimize oyster mushrooms' growth, yield, and nutritional qualities.

keywords: valorization, mushrooms, type of substrate, nutritional composition, life cycle, yield.

الملخص

يظهر تثمين المواد الثانوية المستدامة لزراعة الفطريات إمكانات كبيرة لتحسين المحصول والجودة الغذائية العالية للفطر. تتناول هذه الدراسة تأثير ركائز النمو المختلفة على نمو فطر المحار، دورة حياته، محصوله وتكوينه الغذائي. وتشمل ركائز النمو

التي تم اختبارها ورق الكرتون، نشارة الخشب، تفل القهوة ومزيج من هذه المواد فيما بينها. حيث أظهرت النتائج أن:

الكرتون سجل أقصر دورة حياة للفطريات بمعدل 33.37 ± 78.3 يومًا. الأس الهيدروجيني الأقرب إلى التعادل بمتوسط قيم 62.6 ± 03.0. تساهم هذه الركيزة في ثراء الفطر بفيتامين B 12 (05.0 ± 69.0 ميكروغرام/غرام) والكالسيوم(20±49.0 جزء في المليون (. أظهر الفطر المزروع في ركيزة 7SD30CG (70% من نشارة الخشب و30% من القهوة المطحونة (أعلى

عائد بمتوسط 5.137 ± 7.5 غ. من حيث المحتوى المائي، أنتجت هذه الركيزة فطرًا يحتوي على أعلى محتوى مائي بمعدل 6.85 ± 99.0%، مما يشير إلى زيادة نضارة الفطر. تعزز هذه الركيزة نفسها من حيث المذاق الحلو للفطر، بمتوسط محتوى26.0 ± 009.0 ميكروغرام/غرام من المادة الجافة. أظهر الفطر المزروع في الركيزة (SDنشارة الخشب) أعلى تركيز

من حيث محتوى فيتامين D (45.1 ± 02.0 ميكروغرام/غرام من وزن الجسم)، الصوديوم (1650 ±82.45 جزء في

المليون) والدهون (93.23 ± 3.0%). أنتج الجمع بين ركائز النمو الثلاث بقيم متساوية(M3S) فطرًا غنيا بالمادة الجافة (27.33 ± 9.3%)، فيتامين B12 (65.0 ± 28.0 ميكروغرام/غرام)، رماد(51.3 ± 37.0%) البوتاسيوم(307 ±51.2 جزء في المليون)، كالسيوم (20 ± 34.0 جزء في المليون) والسكريات (0.26 ± 04.0 ميكروغرام/غرام من المادة الجافة). وأخيرًا،

أنتجت ا لركيزة CGSD50 (50% من نشارة الخشب و50% من نشارة الخشب و50% من القهوة المطحونة) محتوى أعلى

من البروتينات(1.38 ± 01.0 ملغم/لتر)، البوليفينول(046.0 ± 007.0 ملغ من مكافئ AG/غرام)،ومركبات الفلافونويد(024.0 ± 004.0 ملغ EQ/غرام.) تسلط هذه النتائج الضوء على أهمية اختيار الركائز وخلطها بعناية لتحسين نمو فطر المحار، الإنتاجية، والصفات الغذائية.

الكلمات المفتاحية: التثمين، الفطر، نوع وسط النمو، التركيب الغذائي، دورة الحياة، المحصول.

Résumé

La valorisation durable des matières secondaires pour la culture des pleurotes présente un potentiel significatif pour l'amélioration des rendements et de la qualité nutritionnelle des champignons. Cette étude examine l'effet de différents substrats sur la croissance, le cycle de vie, le rendement et la composition nutritionnelle des pleurotes. Les substrats testés incluent le carton (Cr), la sciure de bois (SD), le marc du café (CG) et des combinaisons de ces substrats. Les résultats montrent que :

Le substrat Cr a produit des pleurotes avec le cycle de vie le plus court avec 37.33 ± 3.78 jours. Leur pH est le plus proche de la neutralité avec une valeur moyenne de 6.62 ± 0.03. Ce même substrat se démarque par sa contribution à la richesse des pleurotes en vitamine B12 (0,69 ± 0,05 µg/g) et en calcium (20 ± 0,49 ppm).

Les champignons cultivés sur le substrat 70SD30CG (70 % de sciure de bois et 30 % de marc de café) ont montré le rendement le plus élevé avec une moyenne de 137.5 ± 7.5 g. En termes de teneur en eau, ce même substrat a également produit des champignons avec la plus haute teneur en eau à 85.6 ± 0.99 %, indiquant une fraîcheur accrue des champignons. Ce même substrat favorise le goût sucré des pleurotes, avec une teneur moyenne de 0,26 ± 0,009 µg/g DM.

Les champignons cultivés sur le substrat SD (sciure de bois) ont montré la plus haute concentration en termes de teneur en vitamine D (1.45 ± 0.02 µg/g DW), en Na (1650±45.82 ppm) et en lipides (23.93±0.3%).

La combinaison des trois substrats à parts égales (M3S) produit des pleurotes riches en matière sèche (27.33±3.9 %), en vitamine B12 (0.65±0.28 µg/g) en cendre (3.51±0.37), en K (307±2.51 ppm), en Ca (20 ±0.34 ppm) et en sucres (0.26 ±0.04 µg/g DM).

Enfin, le substrat 50CGSD (50 % de sciure de bois et 50 % de marc de café) favorise une teneur plus élevée en protéines (1.38 ± 0.01 mg/L), en polyphénoles (0.046 ± 0.007 mg EAG/g) et en flavonoides (0.024 ± 0.004 mg EQ/g).

Ces résultats soulignent l'importance de choisir et de mélanger judicieusement les substrats pour optimiser la croissance, le rendement et les qualités nutritionnelles des pleurotes.

Mots clés: valorisation, champignons, type de substrat, composition nutritionnelle, cycle de vie, rendement

Introduction

Introduction

The increasing demand for food, coupled with growing concerns about environmental sustainability, calls for exploring innovative approaches in food production. Mushrooms play important dietary, medicinal, recreational, and socio-economic roles worldwide (Mlambo and Maphosa, 2022). Many mushrooms are scavengers that can survive in diverse environments and are found in habitats considered unfavorable for other eukaryotes (Jamir et al., 2024). They belong to a vast and varied group of heterotrophic organisms present on dead and decaying wood and other organic matter (Yap et al., 2023). Mushrooms have garnered significant attention due to their high nutritional value (Antunes et al., 2020; Guo et al., 2023) and therapeutic benefits (Bolaniran et al., 2021; Abdelshafy et al., 2021; Visvanathan et al., 2022; Koreti et al., 2022; Saldanha et al., 2023). Mushroom cultivation aligns perfectly with sustainable agriculture and offers several advantages (Randive, 2012) as it embodies the principles of circular economics, creating a closed-loop system where secondary materials are transformed into growth substrates.

In the spirit of promoting resource efficiency and sustainability, used mushroom substrates are considered valuable resources after harvesting. Indeed, several studies collectively highlight the potential of mushroom cultivation in the circular economy, particularly through the utilization of spent mushroom substrates. Grimm et al. (2021) indicate that resource use efficiency could be maximized by using spent mushroom substrate as food for invertebrates, such as insects or earthworms, which produce high-quality compost and can serve as food or feed for other livestock. Mohd Hanafi et al. (2018) reported that the main applications of exhausted mushroom substrate are animal feed, fertilizer, energy production, and wastewater treatment. The use of spent mushroom substrate in new cultivation cycles has already been reported due to its economic and environmental viability (Cunha Zied et al., 2020), thus contributing to sustainable agricultural practices.

This symbiotic relationship offers numerous benefits for both environmental sustainability and economic development. It is with this objective in mind that this work is carried out, aiming to explore the potential of using various secondary materials, namely cardboard, sawdust, and coffee grounds, as well as the combination of these three substances, as a growth substrate for edible mushrooms. Our hypothesis is that the growth substrate composed of the combination of the three secondary materials will induce the best yield and the highest nutritional quality.

It is important to note that mushroom cultivation on waste requires adhering to specific conditions and following specific protocols to ensure food safety.

This manuscript is structured into three main parts, in addition to the introduction and conclusion.

The first part is a literature review, in which we discuss information regarding secondary materials and their reuse, firstly, and secondly, information about edible mushrooms.

The second part presents the method for producing oyster mushrooms (*Pleurotus ostreatus*) on different types of substrates, followed by the protocols used to analyze the various parameters of the mushrooms' nutritional quality.

The third part focuses on the presentation, analysis, and discussion of the results.

Literature review

Chapter 1. Secondary materials recoverable in mushroom cultivation

1.1. Recycling secondary materials and the circular economy

Every year, approximately 2 billion tons of municipal solid waste are generated worldwide, representing everything considered worthless (Thakur et al., 2020). Managing this waste by landfilling and uncontrolled incineration leads to soil, air and water pollution, and this mismanagement of municipal solid waste is detrimental to the environment and human health, so several approaches have been developed to solve the municipal solid waste problem (Balet, 2023). More recently, waste has become an opportunity to generate valuable compounds due to its rich chemical composition (Kouassi et al., 2022).

Taking into account the limits and benefits of waste recovery and management helps us realize the future of a circular economy while creating a sustainable system that reduces waste and transforms it into new products (Khaire et al., 2024). This helps reduce our impact on the environment, create jobs, and support the growth of environmentally friendly industries (Aggeri et al., 2021). The three principles of the circular economy: reduce, reuse and recycle are fundamental principles that guide this activity (Collard, 2020). We can effectively reduce our environmental impact (Dejous et al., 2022).

Waste management and the circular economy are two key concepts that promote sustainable environmental management (Dorbane et al., 2023). By adopting these principles, we can create a more sustainable future for ourselves and future generations (Abbas et al., 2020).

1.2. Secondary materials used as substrates for mushroom cultivation

1.2.1. Olive pomace

Olive pomace is the main co-product of olive oil extraction process (Mansour-Benamar et al., 2016). Although harmful to the environment, olive pomace is a potential source of natural molecules beneficial to human health (Belghith, 2023).

The chemical composition of olive pomace varies according to olive variety, growing conditions, oil extraction process, and degree of olive ripening (Benamar et al., 2013).

Table 1. Chemical characteristics of olive pomace (Benamar et al., 2013).

Components	Percentage in pomace
Moisture	70.20 ±0.25
Dry matter	29.80 ±0.25
Ash content	1.95 ±0.09
pH	6.80 ±0.06
Carbon	56.39 ±0.08
Organic matter	97.23 ±0.13
Cellulose	33.42
Hémicellulose	15.12
Lignin	22.1
Nitrogen	1.06
Phosphorus	0.113
Potassium	0.833
Calcium	0.820

Olive pomace is a waste product of the olive industry, and its recovery helps solve environmental problems and meet national economic needs on the other (Mansour-Benamar et al., 2016).

1.2.2. Corn cobs

Corn cobs (Fig. 1) are the aerial part of the plant excluding the kernel, called residue, obtained during the recovery of kernels (Lizotte, 2013).

Figure 1. Corn cobs (Pinto et al., 2011)

A considerable quantity of maize residues is formed, which can be used for several purposes (Barry, 2023). For example, they can be used as a crop substrate after grinding and as a raw material for the production of biogas and liquid biofuels (Bimenyimana et al., 2023). Today, maize residues constitute an important renewable resource that could contribute to energy and climate achievement while promoting rural development and the circular economy (AILE., 2020).

Table 2 shows the chemical composition of maize cobs according to two references.

Table 2. Chemical composition of corn cobs (Zhang, 2012)

Hemicelluloses (DM%)	**Cellulose (DM%)**	**Lignin (DM%)**	**Protein (DM%)**	References
34,7	31,7	20,3	8	Aliakbarian et al., 2008
41,4	40,0	5,8	2,5	Kaliyan and Morey, 2010

1.2.3. Coffee grounds

Coffee grounds are the residue from the consumption of soluble coffee obtained after roasting merchant coffee beans (Mansour-Benamar et al., 2016).

Table 3. The main compounds in coffee grounds (Limousy et al., 2013).

Elements	**Quantities (%)**
Carbohydrate	45.3
Lipides	9.3-16.2
Protein	14
Minerals	6800 mg/kg of dry matter
Polyphenols	13-18 mg gallic acid eq

Coffee grounds are essentially composed of polysaccharides, lipids, proteins, polyphenols, and minerals and are rich in protein; however, their high lignin content can be a limiting factor for exploitation (Mansour-Benamar et al., 2016). Large quantities of coffee grounds are wasted, which has prompted the exploration of different methods of valorizing this waste in several fields (Belabed et al., 2020). Coffee grounds have many possible uses in the food and beverage industry, energy and biofuel production, cosmetics and pharmaceuticals, corrosion inhibition, and wastewater treatment (Bensaada et al., 2022).

1.2.4. Sawdust

Sawdust (Fig. 2) is a by-product generated by the industrial processing of wood and can be used for several purposes (Benyoucef and Harrache, 2015).

Figure 2. Sawdust

According to the chemical composition analysis of wood shavings determined by TAPPI standards, it is mainly composed of 39.7% cellulose, followed by hemicellulose at 26.9%, determined by the difference between the holocellulose and cellulose contents, and then lignin at 25.4% (Benyoucef and Harrache, 2015). Holocellulose, a mixture of cellulosic and hemicellulosic constituents, is present at 66.59%, and its content is determined by sodium chlorite ($NaClO_2$) dosage catalyzed by acetic acid (Benyoucef and Harrache, 2015). With 4.32% extractable matter, Pinus sylvestris contains an amount that remains within the normal range (about 5% by mass) for coniferous woods andfinally, the ash content was estimated at 4.92% (Benyoucef and Harrache, 2015).

According to Mwango et al. (2019), there are four potential avenues for the valorization of wood shavings: **(1)** as culture substrates, **(2)** in wastewater treatment as clean, natural, and low-cost adsorbents, **(3)** in composting, and **(4)** in industry.

1.2.5. Cardboard

Cardboard is mainly composed of cellulose and can also contain variable amounts of hemicellulose, lignin, mineral fillers, ash, dyes, and various additives (Mazaherifaret et al., 2024). Cardboard fibers can be recycled (Comère and Giuliani, 2013).

Cardboard is lightweight, making it easy to handle and suitable for producing value-added materials with efficient storage (Ghennaiet and Hannachi, 2022). It is also inexpensive and can be recycled efficiently (Carton, 2022). Cardboard can serve as a raw material for a wide range of applications in different fields (Mazaherifar et al., 2024).

Chapter 2. General Overview of Mushrooms

2.1. Edible Mushrooms

2.1.1. Definition

Mushrooms belong to a significant and diverse group of heterotrophic organisms frequently found on dead and decaying wood and other organic matter (Yap et al., 2023).

Edible mushrooms form a distinct group of organisms that includes species with large bodies and visible sporophores (macrofungi or macro-mycetes) (Boa, 2006).

Boletes have pores instead of gills on the underside of their caps, while truffles grow underground and lack a stem or cap (Boa, 2006). There are approximately 2,500 species of mushrooms recognized as edible, with the most sought-after and expensive ones belonging to the mycorrhizal group. This group includes *Tuber melanosporum Vitt.* (Perigord black truffle), Tuber magnatum Pico & Vitt. (Italian white truffle), Tricholoma matsutake (Ito & Imai) Sing. (matsutake), *Boletus edulis BFull:* Fr. sensu lato (porcini), *Cantharellus cibarius* Fr. (chanterelle), and *Amanita caesarea* (Scop.:Fr.) Pers: Schw. (Caesar's mushroom) (Yun and Hall, 2004). Figure (3) shows the morphology of a fresh mushroom.

2.1.2. Habitat

Mushrooms exhibit high variability in their morphology and habitat; they can be found in nature as wild fungi or cultivated on farms (Chauhan et al., 2023). The natural habitat of edible mushrooms includes forests with ectomycorrhizal trees, such as those found in Central and East African forests (Ducousso et al., 2003). These mushrooms are collected by local people (Ndong et al., 2011).

According to Mlambo and Maphosa (2022), most edible mushroom species worldwide are still gathered from the wild, a practice that is unsustainable and does not contribute to global food security.

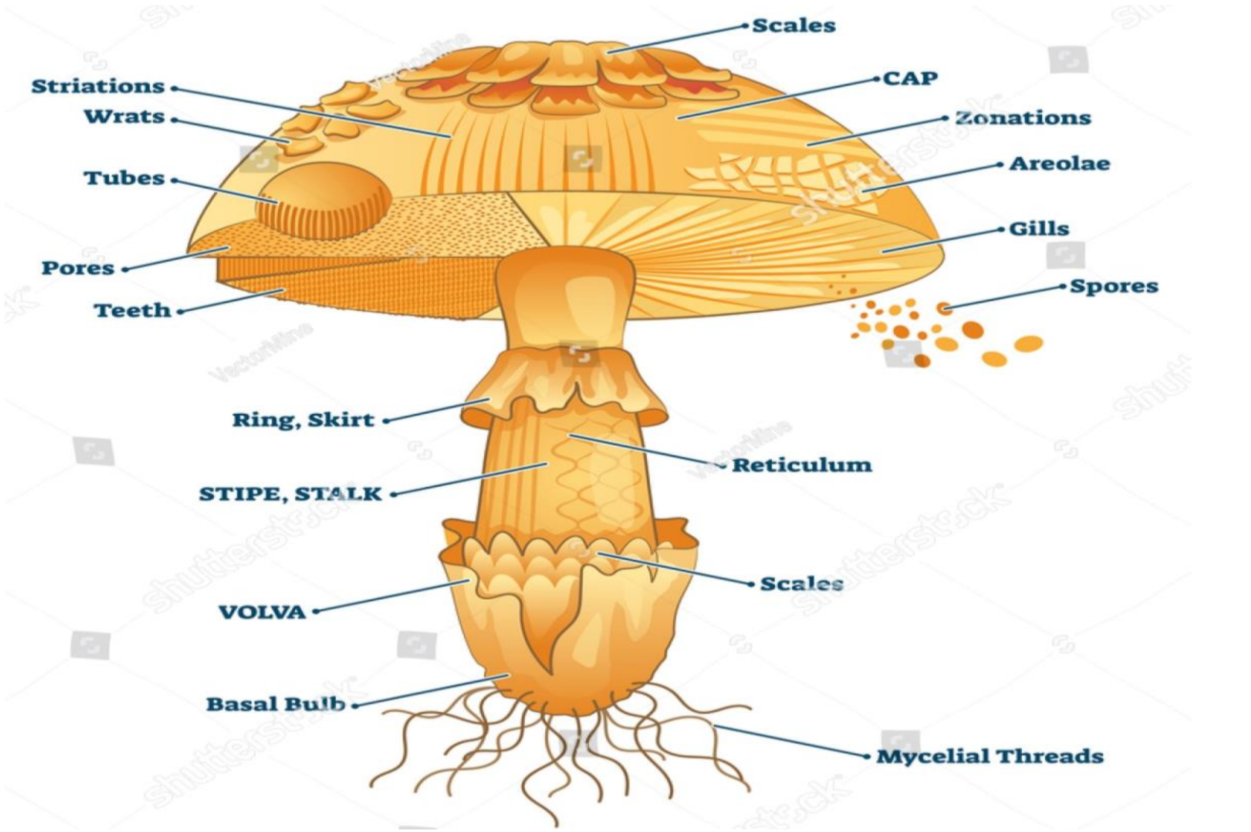


Figure 3. The morphology of a fresh mushroom (www.shtterstock.com)

Over the past century, the harvesting of several edible mushroom species has declined significantly, prompting the search for cultivation methods. Thus far, only a few truffle species have been produced in commercial quantities, although techniques have been developed that could lead to the cultivation of species such as *Cantharellus cibarius*, *Lyophyllum shimeji*, and *Lactarius deliciosus* (Yun and Hall, 2004). Nevertheless, cultivating several mycorrhizal mushrooms, such as *Tuber magnatum* and *Tricholoma matsutake*, remains a challenge (Yun and Hall, 2004). Generally, the growth and appearance of macrofungi are closely linked to environmental factors (Wang et al., 2021).

2.1.3. Life cycle of Mushrooms

The inability of mushrooms to photosynthesize means they must obtain their nutrients from organic matter produced by other organisms (Ndong et al., 2011). Mushrooms are thus ecologically subdivided into three groups: **(i)** parasites, **(ii)** saprophytes, and **(iii)** symbionts.

Parasitic mushrooms include numerous pathogenic species that cause significant damage to agriculture, forestry, livestock, and human health (Garcia-Hermoso, 2013).

Saprophyte mushrooms play a crucial role in the decomposition of organic matter in natural environments (Zhang et al., 2016). They are essential contributors to the functioning and balance of ecosystems (Ndong et al., 2011).

Symbiotic mushrooms benefit from the association they form with another organism. They obtain nutrients from their "partner" in exchange for certain benefits, such as improved mineral nutrition or protection against pathogenic microorganisms (Luo et al., 2016). Two types of symbiosis are particularly interesting (Ndong et al., 2011):

- The association of Termitomyces fungi with termites: The mycelium of Termitomyces grows on combs within underground cultivation chambers, and the fruiting bodies appear periodically on or near termite mounds. All described species in the genus Termitomyces in tropical Africa are edible (Ndong et al., 2011).

- Ectomycorrhization of trees in the families Caesalpiniaceae (including *Gilbertiodendron dewevrei*), Euphorbiaceae (mainly *Uapaca spp*.), and Gnetaceae (only *Gnetum africanum*) by fungi: Their mycelium forms sheaths around the tree's rootlets, increasing their absorption capacity (Ndong et al., 2011).

2.1.4. Importance of Mushrooms

Mushrooms are universally valued for their nutritional and therapeutic importance (Bolaniran et al., 2021; Koreti et al., 2022).

a) Therapeutic Importance

Macrofungi, due to their abundance and diverse range of bioactive metabolites, are considered an important reservoir of bioresources (Zhou et al., 2022). They are regarded as a functional food that helps prevent various deadly diseases such as cancer, stroke, and heart disease (Ogidi et al., 2023). According to Cheng et al. (2023), mushrooms provide numerous types of natural metabolites for drug development, with penicillin being the most well-known example.

The fruiting bodies of mushrooms are particularly utilized in Asia for their biological activities, including antioxidant, neuroprotective, anti-inflammatory, hepatoprotective, immunostimulatory, hypoglycemic, and anticancer properties (Fons et al., 2018). "Sanghuang" is one of the most important groups of medicinal macrofungi and has been used in traditional Chinese medicine for two centuries (Zhou et al., 2022).

b) Nutritional Importance

Edible macrofungi are nutrient-rich and versatile as substitutes for meat, fish, fruits, and vegetables. They are excellent sources of protein with essential amino acids, vitamins (B1, B2, niacin, C, B9, and provitamin D), dietary fiber, and minerals (P, K, Ca, and Fe) while being low in fat and Na (Das et al., 2021).

Total protein content varies from 7 to 48% of dry weight depending on the species, making them a food of choice in tropical Africa (Ndong et al., 2011). According to Fons et al. (2018), mushrooms are high in water content (50-90%) and have a low nutritional value (20-80 kcal/100 g). They provide approximately 0.5-7% protein, 0.5-2% minerals (K, P, Se, Fe, Zn, and trace elements), 2-13% carbohydrates, and 0.05-2% lipids. They are a good source of B-group vitamins but tend to absorb fat during cooking due to their lamellae and tubes.

c) Socio-economic Importance

Edible ectomycorrhizal mushrooms represent a potential wealth in Central, East, and Southern Africa (Ducousso et al., 2003; Ndoye et al., 2007). They are the subject of significant trade (Ducousso et al., 2003).

In many regions of tropical Africa, mushrooms are abundantly consumed and are the focus of substantial local trade (De Kesel et al., 2017).

2.1.5. Major Edible Species Cultivated Worldwide

The global market for edible mushrooms is divided into two categories: fresh and dried mushrooms. Approximately 35 species of edible mushrooms are widely cultivated worldwide, including *Agaricus bisporus*, *Lentinula edodes*, *Pleurotus ostreatus*, *Flammulina velutipes*, *Auricularia auricula-judae*, and *Grifola frondos* (Guo et al., 2023).

The fresh mushroom sector dominated the global market for edible mushrooms in 2020, accounting for more than 50% of total sales (Guo et al., 2023).

2.2. Oyster Mushrooms: *Pleurotus ostreatus*

2.2.1. Definition

Mushrooms are an integral part of the normal human diet, and recently, the consumption quantities have significantly increased, including a variety of species (Deepalakshmi and Sankaran, 2014). The genus *Pleurotus* comprises approximately 40 different species commonly known as oyster mushrooms. Among the species in this genus, *Pleurotus ostreatus* is popularly consumed worldwide due to its taste, flavor, nutritional value, and medicinal properties (Ficior et al., 2006; Adebayo and Oloke, 2017). Due to the presence of numerous nutritional compositions and diverse active ingredients in *P. ostreatus*, it exhibits reported antidiabetic, antibacterial, anticholesterolic, anti-inflammatory, anticholesterolic, antiarthritic, antioxidant, anticancer, ocular, and antiviral properties (Deepalakshmi and Sankaran, 2014).

2.2.2. Clasification

According to Deepalakshmi and Sankaran (2014), *Pleurotus ostreatus* is a macrofungus belonging to the phylum Basidiomycetes:

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

2.2.3. Description

Both the scientific name and the common name refer to the shape of the fruiting body. The Latin word "Pleurotus" (lateral) refers to the lateral growth of the stem relative to the cap, while the Latin "ostreatus" and the English common name, "Oyster," refer to the cap's shape, resembling the bivalve of the same name (Hassen et al., 2011).

The cap of *P. ostreatus* is broad, fan-shaped or oyster-shaped, and expands to 5-25 cm. Natural specimens range in color from white to gray or beige to dark brown. The margin is curled when young and smooth, often somewhat lobed and wavy (Hassen et al., 2011).

Figure (4) shows the fruiting bodies of *P. ostreatus*, with white, firm flesh of varying thickness due to the arrangement of the stipes (Deepalakshmi and Sankaran, 2014).

Stipe (stem): It is an eccentric or lateral peduncle attached to the pileus (cap), opening like an oyster shell during morphogenesis, hence the common name "oyster mushrooms" (Rajarathnam et al., 1987).

Gill (lamellae): The gills of the mushroom are white to cream and descend onto the stem. Their spores are white to gray-lilac and are best observed on a dark surface (Hassen et al., 2011).

Figure 4. Morphological appearance of the fruit of *Pleurotes ostereatus*

https://www.interflora.fr/blog/culture-pleurotus-ostreatus/

2.2.4. Life Cycle of *Pleurotus ostreatus*

Pleurotus mushrooms exhibit the typical life cycle of basidiomycetes (Martínez-Carrera, 1999). It begins with the germination of a basidiospore in a suitable substrate, giving rise to a monocaryotic mycelium containing genetically identical nuclei (n) and capable of indefinite independent growth (Adebayo and Martinez-Carrera, 2015). When two compatible monocaryotic mycelia come into close contact, they can establish a fertile dikaryon through hyphal fusion or plasmogamy (Adebayo and Martinez-Carrera, 2015). This dikaryon (n+n), with clamp connections and binucleate in each hyphal compartment, contains two genetically different nuclei (one from each monokaryon) throughout the mycelium (Martínez-Carrera, 1999). When environmental conditions are favorable (temperature, light, relative humidity), the dikaryotic mycelium differentiates into fruiting bodies with specialized structures called basidia. Within these binucleate, club-shaped cells in the lamellae (hymenium) of each fruiting body, karyogamy (fusion of paired nuclei; 2n) and meiosis (recombination and segregation) occur (Adebayo and Martinez-Carrera, 2015) (Fig. 5).

2.2.5. Growth and Production of Oyster Mushrooms

This mushroom is produced on a variety of lignocellulosic substrates (Ficior et al., 2006).

Substrate Preparation: The substrate is chopped to a length of about 2 to 6 cm (Sanchez, 2010). One of the most commonly used substrates for this mushroom is a mixture of cottonseed hulls and wheat straw (high water-holding capacity) (Martínez-Carrera, 1999). This mixture is pasteurized (65°C for 45 minutes) with steam (Royse, 2002).

Inoculation: After pasteurization, the substrate is cooled and inoculated with the desired strain.

Bag Filling: Transparent or black perforated plastic (polyethylene) bags are filled with the substrate. The bags are then incubated for 12 to 14 days at 25°C and subsequently transferred to the production room (Sanchez, 2010).

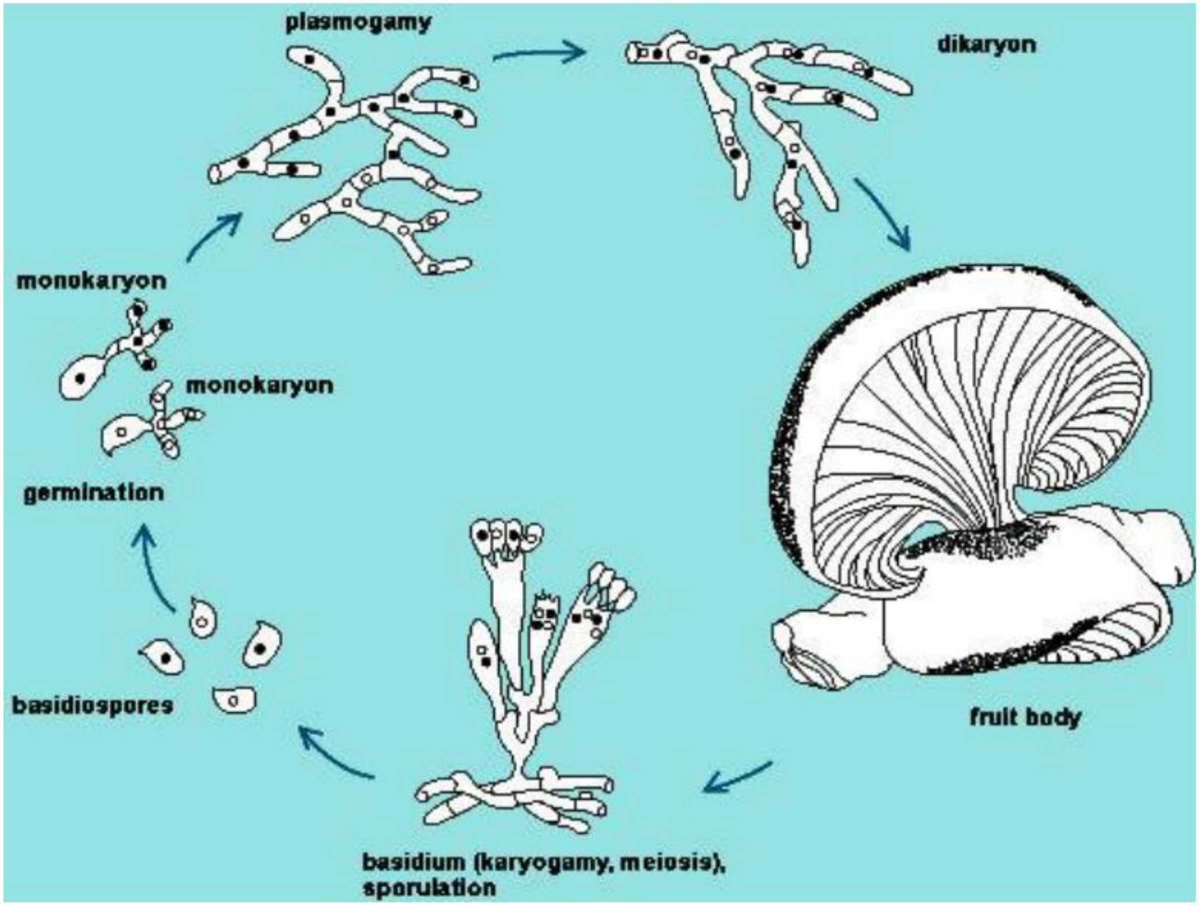


Figure 5. Oyster mushroom (*Pleurotus ostreatus*) life cycle (Martínez-Carrera, 1999)

Mushroom Production: The mushrooms start forming on the edges of the bag perforations placed on mushroom shelves and suspended systems (the main systems used for Pleurotus cultivation) under optimal conditions (Sanchez, 2010).

Harvesting: The mushrooms are harvested from the substrate approximately 3 to 4 weeks after spawning, depending on the strain, the amount of supplement used, and the temperature (Sanchez, 2010).

2.2.6. Factors Influencing the Growth and Fruiting of Oyster Mushrooms

Several factors influence mushroom growth, including **(1)** strains, **(2)** different lignocellulosic substrates, **(3)** different types of inoculum, **(4)** moisture, **(5)** temperature, and **(6)** physicochemical conditions (Sanchez, 2010).

Yield increases can be attributed to several factors, mainly **(1)** the increase in available nutrients in the inoculum, **(2)** a larger number of inoculation points, and **(3)** increasing the amount of inoculum, which allows for faster colonization of the substrate and, consequently, faster completion of mycelial growth (Sanchez, 2010).

2.2.7. Parasites and Contaminants

Oyster mushroom yield is susceptible to various biotic agents, including bacterial, fungal, viral, and nematode diseases, which can cause significant losses of up to 20% in quantity and quality (Potočnik et al., 2015).

a) Fungi

Competitive fungi are among the most important of these agents. Aspergillus and Fusarium deplete nutrients and produce metabolites that inhibit mushroom growth, leading to reduced mycelial growth and yield (Hassan, 2013). Pathogenic fungi, such as Trichoderma, cause direct infections on the mycelium and fruiting bodies, resulting in green rot (Mwangi, 2016). Other destructive fungi in the mushroom industry include *Lecanicillium fungicola*, which causes dry bubble disease, and *Cladobotryum sp*., which causes spider web disease, leading to significant losses in commercial mushroom production (Carrasco-Cabrera et al., 2019).

b) Nematodes

Nematodes are tiny white worms that feed on mycelium, leading to significant yield reduction or even total loss (Demers, 2015).

c) Mites

Mites are also mycophagous, causing yield losses (Demers, 2015).

d) Sciarid flies

These small flies are common in indoor mushroom farms. The females are attracted to the smell of mushrooms, where they lay their eggs, which develop by feeding on the mushrooms (Demers, 2015).

e) Slugs

Slugs are fond of mushrooms and are mainly a problem for outdoor mushroom cultivation (Demers, 2015).

2.2.8. Control Methods

The best prevention methods include proper sterilization of substrates, materials, and tools, as well as strict hygiene for individuals and facilities (Demers, 2015).

Several methods have been employed to combat pathogens and competitors of oyster mushrooms, including chemical methods using chlorothalonil, prochloraze, thiabendazole, and carbendazim (Potočnik et al., 2015); biological methods such as the use of *Bacillus subtilis* and *B. amylofaciens* bacteria (Nagy et al., 2012); and physical methods involving sterilization and pasteurization of culture media (Mahmoud and Hassan, 2008). Chemical fungicides are the fastest and most effective means of controlling these pathogens, but they have negative effects on humans, the environment, and the mushroom product itself due to the rapid absorption of these pesticides by mushrooms (Hassan and Ibrahim, 2022).

Material and Methods

Material and Methods

1. Fungal material

The study focused on oyster mushrooms *(Pleurotus ostreatus)*. The mycelium used was purchased from Aures Champignons in Batna, Algeria. It consists of pure mycelium growing on a sterilized barley grain medium (Fig. 6).

Figure 6. Spawn (seed, mycelium) grown on barley grain substrate

2. Culture Substrates

The substrate is the material on which mushrooms grow. It can be composed of straw, wood chips, coffee grounds, horse manure, etc. In order to valorize several types of secondary materials and test their effect on yield and mushroom quality, we chose to work with several types of waste used as fruiting substrates. The substrates used are summarized in Table 4.

Table 4. Main substrates used

Substrate	Origine	Preparation
Coffee grounds (CG)	Coffee shop in the town of Dhalaa in the wilaya of Oum El Bouaghi.	Sieved and sun-dried immediately after collection.
Sawdust (SD)	Carpentry workshop in the town of Dhalaa in the wilaya of Oum El Bouaghi.	Sorted to get rid of pieces of wood.
Cardboard (Cr)	Food packaging boxes recovered from a supermarket.	Food packaging boxes recovered from a supermarket.
33% CG+33%SD+ 33% Cr	Coffee shop and carpentry workshop in Dhalaa and food packaging boxes recovered from a supermarket.	Coffee grounds, sawdust and cardboard are sorted and thoroughly mixed to ensure homogenization of the final substrate.
50% CG+50%SD	Coffee shop and carpentry workshop in Dhalaa town.	The coffee mars and sawdust are sorted and mixed to ensure homogenization of the final substrate.
70% CG+30% SD	Coffee shop and carpentry workshop in Dhalaa town.	The coffee mars and sawdust are sorted and mixed to ensure homogenization of the final substrate.

Figure 7. The three substrates used: a: sawdust, b: dry coffee grounds, c: cardboard.

3. Trial Conduct

3.1. Substrate Preparation

Coffee grounds and wood shavings were used as-is, while the cardboard was cut into small pieces, prioritizing the inner part untouched by ink.

Coffee grounds were moistened with tap water, while cardboard and wood shavings were soaked separately for 5 minutes and then drained to remove excess water while retaining some moisture.

3.2. Substrate Sterilization

The moistened substrates were sterilized in glass jars using a pressure cooker to eliminate any pathogenic organisms (Fig. 8).

These pre-sterilized glass jars were filled with moist substrates and placed in a pressure cooker filled with water. Sterilization lasted for 30 minutes at a high temperature.

3.3. Inoculation and Spawning

Once cooled, the substrates were inoculated with mushroom spawn (mycelium) homogeneously.

Calcium carbonate was added to adjust the pH of the substrates.

The substrates were introduced into perforated plastic bags using a sterile needle to ensure optimal aeration.

3.4. Incubation

The inoculated bags were placed in a dark closet and maintained at a constant temperature of 25°C for 25 days.

This incubation period allowed the mycelium to completely colonize the substrate.

3.5. Fruiting

Once the substrates exhibited a uniform white surface and primordia (precursors of mushrooms) appeared, the bags were placed in a ventilated chamber.

Optimal humidity (the room is cooled and humidified daily by watering the floor and walls) and a temperature between 16°C and 18°C were maintained.

Additional pores were created in the bags for better air circulation.

3.6. Harvesting

At the end of fruiting, mushrooms were manually harvested by gently detaching them from the substrate with a circular motion.

The trial was conducted with six treatments and three replicates for each treatment.

Figure 8. Some stages in mushroom cultivation: a. substrate sterilization, b. incubation, c. fruiting, and d. harvested mushrooms.

4. Mushroom analysis

All analyses were carried out at the Faculty of Natural and Life Sciences laboratory of the university.

4.1. Number of Days

The life cycle duration of oyster mushrooms was determined by counting the number of days between inoculation and harvest.

4.2. Yield

The yield of each treatment was measured by weighing the harvested mushrooms on an analytical balance.

4.3. Dry Matter and Water Content

The dry matter content of the samples was determined after drying them in an oven at 105°C for 48 hours. The water content was calculated using the following formula: WC = (Fresh weight - Dry weight / Fresh weight) * 100

4.4. pH, Electrical Conductivity (EC), and Titratable Acidity

The pH was determined by preparing a solution of 1 g of mushroom powder and 10 mL of distilled water. The solution was agitated for 20 minutes, and the pH was measured using a pH meter (AOAC., 2016).

In the same solution, we performed a reading with a conductivity-meter to measure the electrical conductivity (EC) (AOAC., 2016).

Titratable acidity was determined by mixing 2.5 g of mushroom powder with 25 mL of distilled water. After agitation for 10 minutes, the mixture was filtered through filter paper. One drop of phenolphthalein (0.1%) was added to the filtrate, and the solution was titrated with NaOH (0.1 N) until a color change occurred. The volume of NaOH required for neutralization was noted (V). Acidity was expressed as citric acid using the following formula: Acidity (%) = V x 0.9 (Capita et al., 2006).

4.5. Ash Content

The total ash content was determined following the method described by Rao and Xiang (2009). This method involves incinerating the sample until white ashes are obtained. One gram of pre-dried sample is placed in a pre-weighed porcelain crucible and incinerated in a muffle furnace at a temperature of 500°C for 3 hours. The loss of mass observed during incineration corresponds to the fraction of organic matter, while the remaining residue represents the ashes.

4.6. Sodium (Na), potassium (K) and calcium (Ca) Content

The ashes obtained from the incineration of mushrooms were placed in 2.5 ml of 0.5N hydrochloric acid, and the container was adjusted with bi-distilled water to a final volume of 5 ml. From this solution, we performed sodium (Na), potassium (K) and calcium (Ca) analysis by flame photometer.

4.6. Total Sugars

Soluble sugars were assayed according to the method of Dubois et al. (1956), cited by Boudjabi et al. (2019), where sugar extraction was performed on 0.1 g of mushrooms placed in test tubes containing 3 ml of ethanol (80%). The tubes were left at room temperature for 48 hours and then placed in a water bath at 80°C to evaporate the alcohol. In each tube, 20 ml of distilled water was added, and then 1 ml of this solution was mixed in new test tubes with 1 ml of phenol (5%) and 5 ml of sulfuric acid. After cooling, the optical density of the samples was measured at a wavelength of 496 nm.

4.7. Lipid Quantification

The lipid quantification protocol involves solvent extraction followed by evaporation (AOAC., 2016).

Main steps:

- Sample preparation: Grind or homogenize the sample into a fine powder and weigh an accurate amount.

- Lipid extraction: Add a solvent mixture (chloroform: methanol) to the tube, vortex, and let it stand for 30 minutes.

- Centrifugation and phase separation: Centrifuge the mixture, collect the solvent phase (containing lipids), and repeat the extraction once or twice.

- Lipid evaporation: Combine the solvent phases in an evaporation dish and allow the solvent to evaporate completely.

- Lipid quantification: Weigh the dish with the lipid residue, subtract the weight of the empty dish, and calculate the lipid content as a percentage.

4.8. Proteins

The method used for protein quantification is the method of Lowry et al. (1951) cited by Gouzi, (2014). It is a colorimetric technique widely employed for protein measurement in plant samples. This method is based on the Folin-Ciocalteu reaction with reactive groups of proteins, such as tyrosine, tryptophan, and cysteine residues, resulting in the formation of an intense blue compound. The intensity of the blue color is directly proportional to the protein concentration in the sample.

4.9. Polyphenols

The total polyphenol content was evaluated by spectrophotometry using the Folin-Ciocalteu colorimetric method. This method quantifies the overall concentration of hydroxyl groups present in the analyzed extract. Extraction is performed by macerating 10 g of mushrooms with ethanol.

The determination of total polyphenols in the fresh mushroom extract is based on a colorimetric method derived from the work of Singleton and Ross (1965). This method involves diluting 200 µL of mushroom extract ten times with distilled water, then mixing 800 µL of sodium carbonate (Na2CO3) at 7.5% with 100 µL of Folin-Ciocalteu reagent diluted ten times. The mixture is incubated at room temperature for 30 minutes. Absorbance is measured at 765 nm using a spectrophotometer.

A calibration curve is established using a gallic acid solution of known concentration. The determination of gallic acid equivalents (GAE) in the sample is based on the calibration curve and the measured absorbance value. The results are expressed as mg GAE per gram of fresh mushroom.

4.10. Flavonoids

Flavonoids in fresh mushrooms were analyzed using a colorimetric protocol described by Kim et al. (2003). The analysis consists of the following steps:

-Dilute the fresh mushroom extract ten times.

- Mix the diluted extract with 2% aluminum chloride ($AlCl_3$).
- Incubate for 30 minutes.
- Measure absorbance at 430 nm.
- Determine flavonoid concentration using a quercetin calibration curve.

Flavonoid content is expressed as mg quercetin equivalents (QE) per gram of fresh mushroom.

4.11. Vitamin C

Vitamin C content is determined according to the RSCGE (2013) protocol. This protocol describes a two-step method to determine the vitamin C content of a food sample. The first step involves calibrating the iodine solution using a vitamin C solution of known concentration. The second step involves analyzing the food sample by extracting vitamin C, using starch solution as an indicator, and titrating with the calibrated iodine solution. The vitamin C concentration of the sample is calculated based on the volume of iodine used and the calibration value.

4.12. Vitamin B12

For vitamin B12 analysis, fresh, crushed mushrooms are extracted with sodium acetate. The extract is treated with a series of reagents to develop a colored compound, and its absorbance is measured at 450 nm. Vitamin B12 concentration is calculated using a formula that takes into account the measured absorbance, dilution factor, and molar extinction coefficient of vitamin B12. Triplicate analyses are performed for better precision.

4.13. Vitamin D

Mushrooms are ground and extracted with ethanol. The extract is then treated with a series of reagents to develop a colored compound, and its absorbance is measured at 420 nm. Vitamin D concentration is calculated using a formula that takes into account the measured absorbance, dilution factor, and molar extinction coefficient of vitamin D. This protocol enables precise quantitative analysis of vitamin D in edible mushrooms. Triplicate analyses are performed for better precision.

5. Statistical Analysis

Data were analyzed using ORIGIN PRO software. Results are presented as mean ± standard deviation (SD). Statistically significant differences between substrates were determined by a one-way ANOVA, followed by a Tukey post-hoc test. The statistical significance threshold was set at $p < 0.05$. Significance levels were indicated by asterisks (**ns** for $p > 0.05$ * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$), while ns indicates no significance.

Correlation analysis between the measured parameters on oyster mushrooms was performed using the same statistical software. Pearson correlation coefficients were calculated to quantify the strength and direction of the relationships between variables. A significance threshold of $\alpha = 0.05$ was used to determine the statistical significance of correlations.

Additionally, a principal component analysis (PCA) was performed to identify relationships between the measured parameters and substrate types. This analysis helped to highlight discriminate parameters associated with each substrate type.

Results and Discussion

3. Results and Discussion

The use of coffee grounds, rich in nitrogen, is expected to increase the availability of this crucial element for mushroom growth, as confirmed by Karunarathna and Rajapaksha (2021). However, coffee grounds lose a significant portion of their moisture during the brewing process, resulting in a relatively dry substrate for mushroom cultivation (Karunarathna and Rajapaksha, 2021). Our trials demonstrated that this substrate alone did not allow mushroom fruiting, despite the observed mycelial growth. This experiment, repeated twice with identical results, led us to exclude this substrate from the study.

3.1. Number of days in the life cycle of oyster mushrooms

The analysis of variance of the results for the number of days in the life cycle of oyster mushrooms between inoculation and harvest does not show a significant effect ($P = 0.112$) of the type of culture substrate on the duration of the life cycle of oyster mushrooms (Table 5).

Among the tested substrates, those based on Cr and M3S resulted in the shortest life cycles, with respective averages of 37.33 ± 3.78 days and 37 ± 4 days (Fig. 9). These results are similar to those of Östbring et al. (2023), who obtained oyster mushrooms within periods ranging from 33 to 38 days.

The substrate 50CGSD, on the other hand, resulted in the longest life cycle, with an average of 45 ± 6.08 days (Fig. 9).

Our results confirm the findings of Assan and Mpofu (2014), who reported that the life cycle of mushrooms is closely linked to the type of substrate used for cultivation.

Cardboard, being rich in carbon, provides a substantial energy source for mushrooms, promoting their growth (Sanchez, 2010).

Despite a carbon-to-nitrogen ratio of wood chips favorable for fungal growth and fruiting (Grimmett and Jones, 2019), the mushrooms cultivated on SD in our study exhibited a longer life cycle compared to those grown on Cr. In their study, Sharma et al. (2013) reported that oyster mushrooms cultivated on SD were harvested after only 37.80 ± 1.48 days. In our study, the harvest was carried out after 42 days.

Our results revealed that the combination of the three substrates can create a synergistic effect, providing a complete range of nutrients that promote the growth and fruiting of oyster mushrooms. This synergy could explain the hypothesis that a mixture of substrates would induce higher yield and superior nutritional quality.

3.2. Physical parameters

Although the analysis of the averages of the **yield** of the harvested mushrooms does not reveal any statistically significant difference between the substrates used (P = 0.098) (Table 5), a trend emerges. The substrate 70SD30CG stands out for producing the highest average yield (137.5 ± 7.5 g), followed by the substrate M3S (129.66 ± 61.8 g). In contrast, the SD substrate exhibits the lowest yield, with an average of 67.33 ± 18 g (Fig. 9). However, it is important to highlight that the significant variability in yields observed within the substrates does not allow for definitive conclusions regarding significant differences between them.

Variations in substrate composition can affect various aspects, such as mycelial growth, fruiting, and the nutritional composition of mushrooms (Suwannarach et al., 2022).

Our study demonstrates that the combination of the three substrates, as well as 70SD30CG, generates a synergistic effect, providing a complete range of essential nutrients that stimulate the growth and fruiting of oyster mushrooms.

The low nitrogen content, high lignin content, and tendency to dry out make wood a suboptimal substrate for oyster mushroom cultivation (Mercy et al., 2011; Decena and Del Rosario, 2022). Mixing it with other substrates can help improve the yields of SD-based substrates.

Inconsistencies in the literature regarding the effectiveness of wood chips for oyster mushroom cultivation warrant further research. Khan et al. (2012) identified Kikar wood chips as the most suitable substrate, offering the best yield. In contrast, Ponmurugan et al. (2007) reported disappointing results with wood chips, which aligns with our own observations.

The analysis of **dry matter** and **water content** in the mushrooms reveals a highly significant effect of the substrates used (P= 0.0007) (Table 5).

Regarding **water content** (**WC)**:

The substrate 70SD30CG has the highest water content, with 85.6 ± 0.99%, indicating high moisture content (Fig. 9). This water content contributes to the freshness of the mushrooms (Agarwal et al., 2017).

In contrast, the Cr substrate stands out for having the lowest water content, at 66.51 ± 6.75%, suggesting more significant dehydration of the mushrooms. This indicates that mushrooms grown on Cr substrate were less fresh. Agarwal et al. (2017) reported that the water content of the mushroom Pleurotus varies depending on the species and ranges from 85 to 90%. Akyuz and Kirbag (2010) reported water content of 90 %.

As for **dry matter (DM):**

The cardboard substrate exhibits the highest dry matter value, with 33.49 ± 6.75% (Fig. 9), indicating a more significant concentration of non-aqueous compounds in the mushrooms.

In contrast, the substrate 70SD30CG has the lowest dry matter value (14.4 ± 0.99%) (Fig. 9), suggesting a higher proportion of water in the mushrooms.

These results suggest that the type of substrate significantly influences the composition of the mushrooms in terms of water and dry matter content. The substrate 70SD30CG favors high water content and low dry matter, while the Cr substrate produces mushrooms with lower water content and higher dry matter.

The variable capacity of substrates to retain water directly influences the water content of the mushrooms, affecting their growth and development. In this regard, Sanchez (2010) highlighted the role of cardboard, whose structure and composition promote moisture retention, creating a humid microclimate conducive to oyster mushroom cultivation. This observation contrasts with our results, underscoring the complexity of the relationship between substrate properties and mushroom growth. Due to their porous nature, wood chips have an increased water retention capacity, creating a humid environment favorable for mushroom growth (Grimmett and Jones, 2019). Our findings corroborate this observation, emphasizing the importance of substrate properties in mushroom cultivation.

Table 5. One-way ANOVA testing the effects of substrate types on variations in the number of days of the life cycle of oyster mushrooms and physical parameters in oyster mushroom fruiting bodies.

Factor	DF	MS	P-value	Sig.	MS	P-value	Sig.
		Days			**Yield**		
Substrate	4	33.76	0.11	ns	2529	0.098	Ns
Error	10	13.66			962.9		
		WC			**DM**		
Substrate	4	190.9	0.0007	***	190.9	0.0007	***
Error	10	15.58			15.58		

DF: degrees of freedom, MS: Mean Square, Sig: statistical significance, ***: $p < 0.001$, **: $p < 0.01$, *: $p \leq 0.05$, ^{ns}: $p > 0.05$)

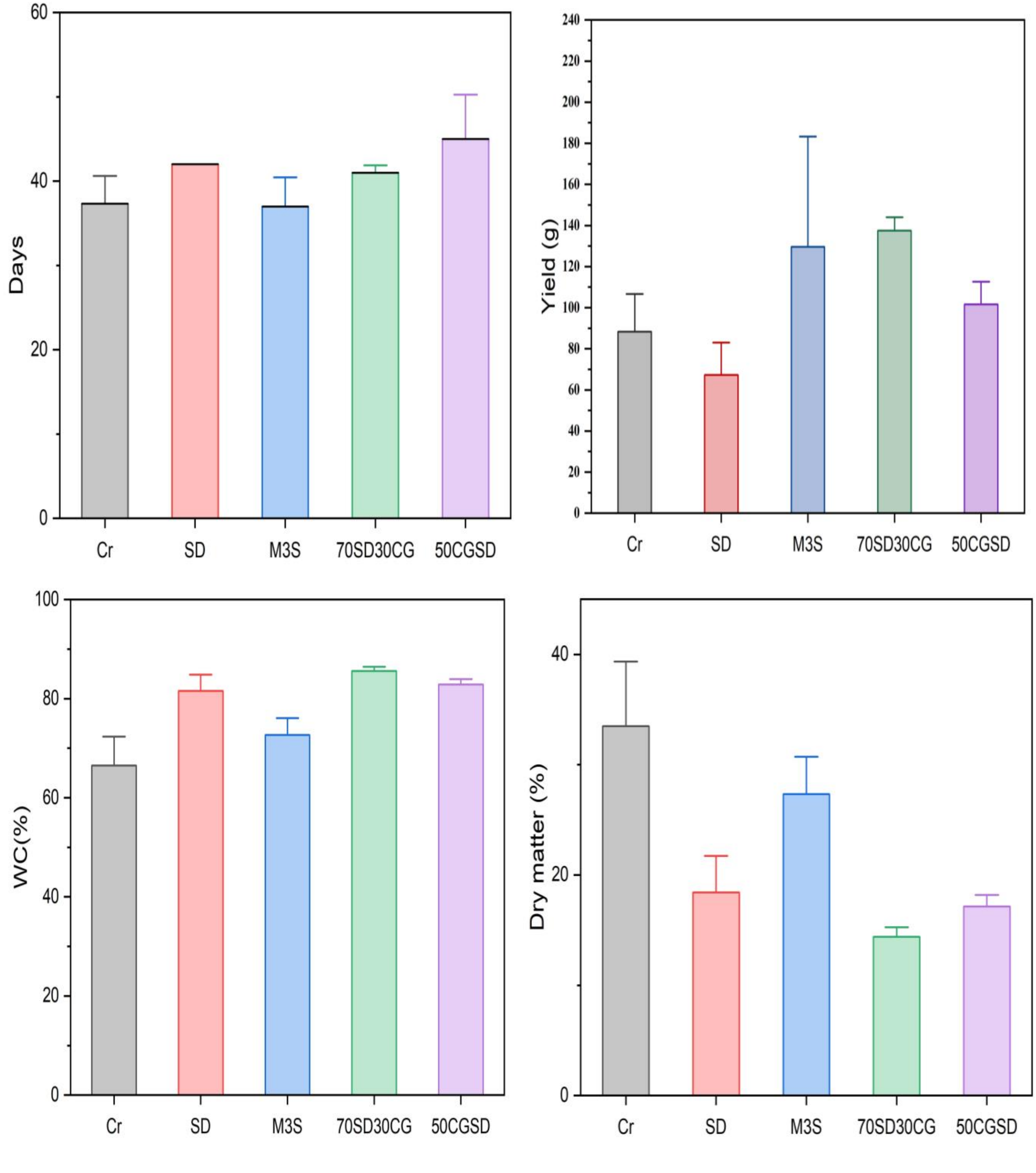


Figure 9. Histograms (mean±SD) showing the variation in the number of days in the life cycle of oyster mushrooms and physical parameters in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a percentage of 33%, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.3. Physicochemical properties

The type of substrate influences **the pH** of the mushrooms. The analysis of the averages of the pH of mushrooms cultivated on the different substrates reveals a highly significant effect of the substrate type ($P<0.0001$) (Table 6). Notable differences in pH are observed between the mushrooms grown on the various substrates.

The SD substrate stands out for producing mushrooms with the most acidic pH, reaching an average of 5.97 ± 0.23. In contrast, the substrates 50CGSD and Cr favor a pH closer to neutrality, with respective average values of 6.63 ± 0.01 and 6.62 ± 0.03 (Fig. 10).

Our results clearly demonstrate that the type of substrate used for mushroom cultivation significantly influences their pH. The SD substrate, rich in organic matter, particularly lignin and phenolic compounds, creates a more acidic environment conducive to the degradation of lignin into phenolic derivatives, thus lowering the pH of the fungal environment (Grimmett and Jones, 2019). In contrast, the substrates 50CGSD and Cr, more balanced in terms of nutrients, promote a more neutral pH.

Cr, on the other hand, exhibits variable pH depending on its composition. Made from naturally acidic wood fibers, it often contains bleaching agents and alkaline additives that can neutralize this acidity, explaining the more neutral pH of the mushrooms grown on this substrate (Sanchez, 2010).

Nevertheless, these preliminary results suggest that the choice of substrate can play an important role in regulating the pH of cultivated mushrooms, with potential implications for their nutritional and sensory qualities.

The statistical analysis of the **electrical conductivity (EC)** of the mushrooms reveals a highly significant effect of the substrates used ($P = 0.0046$).

There is an increase in EC with the substrates 50CGSD and 70SD30CG, reaching respective values of 3.2 ± 0.02 mS/cm and 3.2 ± 0.06 mS/cm.

In contrast, the substrate M3S exhibits the lowest EC, with a value of 2.36 ± 0.28 mS/cm.

These results suggest that the type of substrate significantly influences the EC of the mushrooms. The substrates 50CGSD and 70SD30CG favor a higher EC, while the substrate M3S produces mushrooms with lower EC.

EC is an indicator of mineral ion concentration. Higher EC may reflect better nutrient availability in mushrooms, which could explain the high yields observed with the 70SD30CG substrate.

The analysis of **titratable acidity (TA)** in the mushrooms reveals a highly significant effect of the substrates used ($p < 0.0001$) (Table 6).

The substrate 70SD30CG has the highest titratable acidity, with an average value of 19.26 ± 0.54%.

The substrates M3S and SD exhibit the lowest titratable acidity, with respective average values of 8.4 ± 1.03% and 8.07 ± 0.09% (Fig. 10).

These results indicate that the type of substrate significantly influences the titratable acidity of the mushrooms. The substrate 70SD30CG favors higher acidity, while the substrates M3S and SD produce mushrooms with lower acidity.

Titratable acidity is an indicator of organic acid concentration in mushrooms. Indeed, mushrooms contain various organic acids such as citric, ketoglutaric, malic, succinic, and fumaric acids, and some also contain oxalic, ascorbic, quinic, and shikimic acids (Valentão et al., 2005).

Citric acid is known for its role in preventing the browning of mushrooms (Barros et al., 2013).

Titratable acidity is mentioned as a parameter that can be influenced by biocontrol agents such as *Trichoderma* (Malviya et al., 2022).

Organic acids play a crucial role in the flavor profile of mushrooms (Gąsecka et al., 2018; Jabłońska-Ryś et al., 2022), they are responsible for the taste and flavor of mushrooms and may also have a biological impact due to their antioxidant properties (Gąsecka et al., 2018).

Jabłońska-Ryś et al. (2022) reported that among the organic acids present in mushrooms, malic acid stands out as the dominant element, conferring a characteristic flavor to the fruiting bodies.

Indeed, organic acids play a vital role in maintaining the quality and stability of mushrooms, and their antioxidant properties may have biological implications (Gąsecka et al., 2018; Wang et al., 2016).

Table 6. One-way ANOVA testing the effects of substrate types on variations in physicochemical properties in oyster mushroom fruiting bodies.

Factor	DF	MS	P-value	Sig.	MS	P-value	Sig.
		pH			**EC**		
Substrate	4	0.222	0.0000	***	0.486	0.0046	**
Error	10	0.011			0.063		
		Titratable acidity			DF: degrees of freedom, MS : Mean Square, Sig : statistical significance, ***: p < 0.001, **: p < 0.01, *: $p \le 0.05$, ^{ns}: p >0.05)		
Substrate	4	71.47	1.12E-7	***			
Error	10	0.842					

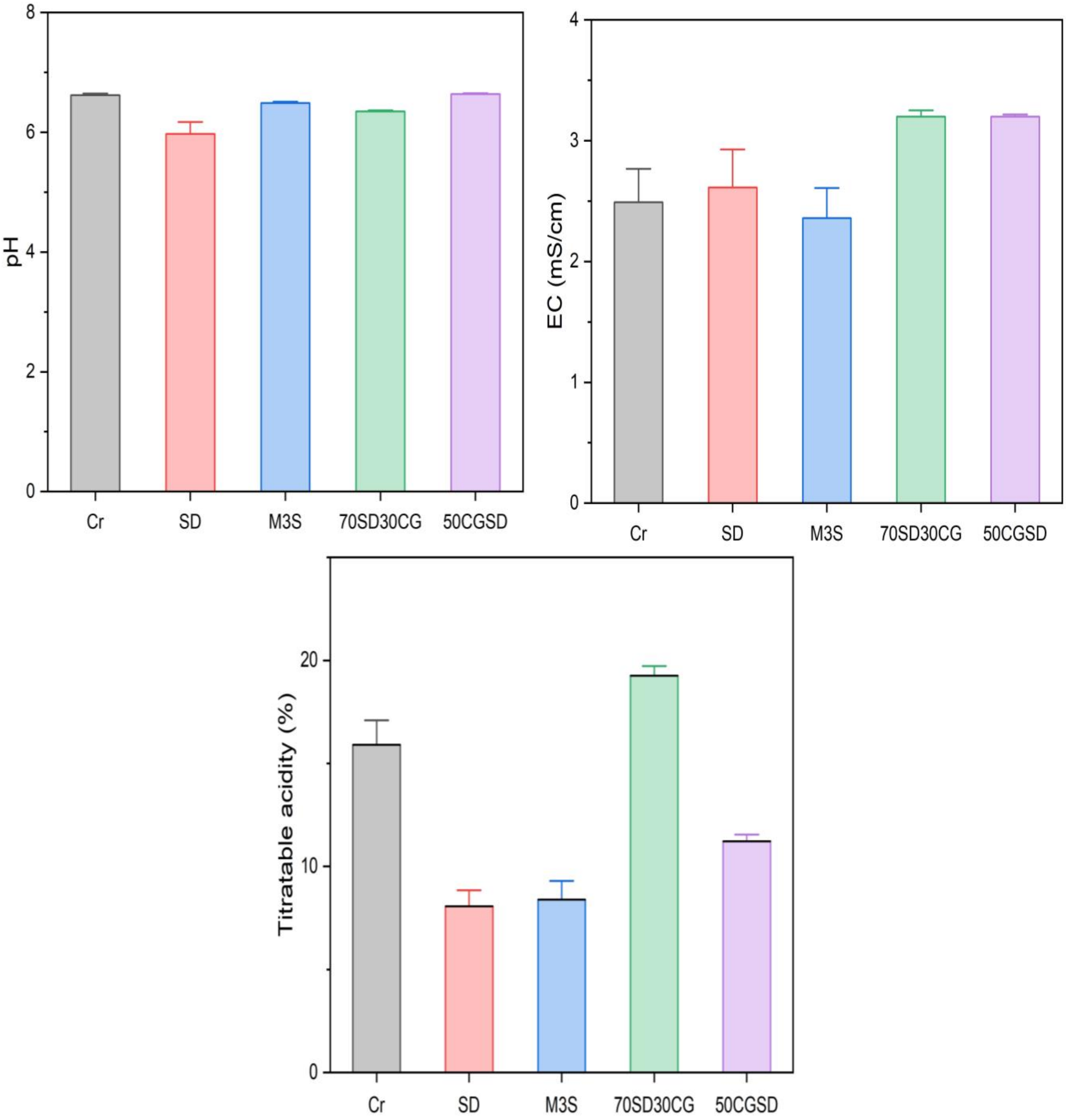


Figure 10. Histograms (mean±SD) showing the variation in physicochemical properties of oyster mushrooms in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a 33% ratio, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.4. Ash and minerals

The analysis of variance reveals a highly significant effect ($P < 0.0001$) of the culture substrate type on **the ash content** of oyster mushrooms (Table 7).

Mushrooms cultivated on the M3S substrate exhibit significantly higher average ash content (3.51 ± 0.37%) compared to the other substrates. Indeed, the substrate 70SD30CG produced oyster mushrooms with an ash content not exceeding 1.62 ± 0.18% (Fig. 11).

Regarding ash content in oyster mushrooms, low values (0.64 and 0.62-0.68 g/100g) have been reported by Reis et al. (2012) and Mattila et al. (2002). In contrast, other studies (Alam et al., 2008; Johnsy et al., 2011) have revealed higher values (9.36 and 10.17 g/100g, respectively).

Mushrooms are rich in several essential mineral elements, including potassium (K), phosphorus (P), sodium (Na), calcium (Ca), and magnesium (Mg), as confirmed by Agarwal et al. (2017).

The analysis of variance reveals a highly significant effect of the substrate type on **the sodium** content of oyster mushrooms ($P < 0.0000$) (Table 7). Indeed, the highest sodium content is observed in mushrooms cultivated on the SD substrate (1650 ± 45.8 ppm), while those grown on the M3S substrate exhibit a significantly lower sodium content (196.66 ± 25.16 ppm) (Fig. 11).

Ibe et al. (2013) reported that mushrooms generally have low sodium content. Other studies have shown that the sodium content of oyster mushrooms varies between 0.3 mg/100 g (Akyuz and Kirbag, 2010) and 4.39 mg/100 g (Oyetayo and Ariyo, 2013). Gogavekar et al. (2014) described a sodium content of 70 mg/100 g in the species *Pleurotus sajor-caju*.

ANOVA reveals a highly significant effect of the substrate type on **the potassium** content of oyster mushrooms ($P < 0.0000$) (Table 7). Indeed, the highest potassium content is observed in mushrooms cultivated on the M3S substrate (307.66 ± 2.5 mg/kg), while those grown on substrates 70SD30CG and 50CGSD exhibit significantly lower potassium contents, with respective averages of 221.5 ± 1.5 mg/kg and 229.66 ± 1.52 mg/kg (Fig. 11).

These results confirm the influence of the substrate type on the nutritional composition of oyster mushrooms. The M3S substrate, rich in potassium, favors a more significant accumulation of this element in the mushrooms. Conversely, the substrates 70SD30CG and 50CGSD, less rich in potassium, lead to lower potassium contents in the cultivated oyster mushrooms.

Scientific literature reports variable potassium contents in oyster mushrooms depending on species, culture conditions, and analysis methods. Notably, Deepalakshmi and Mirunalini (2014) described a potassium content of 1400 mg/100 g in *Pleurotus ostreatus* cultivated on a rice straw-based substrate. In contrast, Oyetayo and Ariyo (2013) observed a lower potassium content of 9.42 mg/100 g in the same species cultivated on a wood chip-based substrate.

ANOVA reveals a highly significant effect of the substrate type on **the calcium** content of oyster mushrooms ($P < 0.0000$) (Table 7). Indeed, the highest calcium content is observed in mushrooms cultivated on substrates M3S, Cr, and 50SDCG, with respective averages of 20.00 ± 0.34 ppm and 19.96 ± 0.49 ppm. These contents represent approximately twice those observed in mushrooms grown on substrates SD and 70SD30CG, with respective averages of 10.00 ± 0.30 ppm and 10.00 ± 0.31 ppm (Fig. 11).

These results confirm the influence of the substrate type on the nutritional composition of oyster mushrooms. The substrates M3S, Cr, and 50SDCG, rich in calcium, favor a more significant accumulation of this element in the mushrooms. Conversely, the substrates SD and 70SD30CG, less rich in calcium, lead to lower calcium contents in the cultivated oyster mushrooms.

Scientific literature reports variable calcium contents in oyster mushrooms depending on species, culture conditions, and analysis methods. Alam et al. (2008) described calcium contents of 35.9 mg/100 g in *Pleurotus ostreatus* cultivated on a rice straw-based substrate, while Ahmed et al. (2013) observed a higher calcium content of 327 mg/100 g in *Pleurotus florida* cultivated on a wood chip-based substrate. In contrast, Mattila et al. (2001) reported a very low calcium content of 0.01 mg/100 g in *Pleurotus ostreatus* cultivated on a wood shavings-based substrate, while Akyuz and Kirbag (2010) described a calcium content of 0.2 mg/100 g in the same species cultivated on a wheat straw-based substrate.

Table 7. One-way ANOVA testing the effects of substrate types on variations in ash and mineral content in oyster mushroom fruiting bodies.

Factor	DF	MS	P-value	Sig.	MS	P-value	Sig.
		Ash			**Na**		
Substrate	4	1.79	2.2E-5	***	997343.3	2.2E-13	***
Error	10	0.065			825		
		K			**Ca**		
Substrate	4	3481.35	8.3E-11	***	89.98	1.6E-12	***
Error	10	9.45			0.11		

DF: degrees of freedom, MS: Mean Square, Sig : statistical significance, ***: $p < 0.001$, **: $p < 0.01$, *: $p \leq 0.05$, ^{ns}: $p > 0.05$)

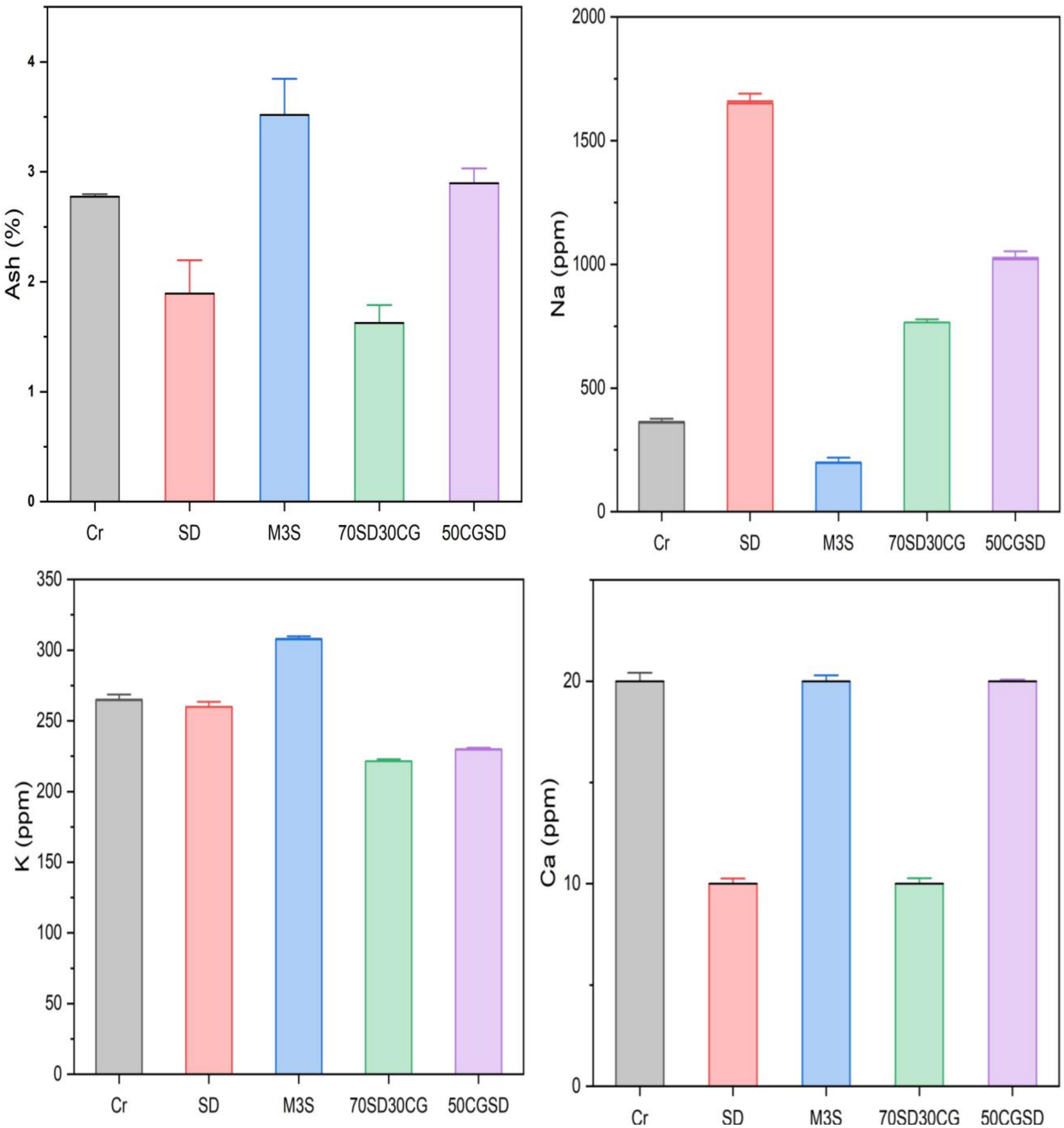


Figure 11. Histograms (mean±SD) showing the variation in ash and mineral content analyzed in oyster mushrooms in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a 33% ratio, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.5. Biochemical parameters

The **total sugar content** of oyster mushrooms is also strongly influenced by the type of substrate used and reveals a highly significant effect ($P < 0.0001$) (Table 8). The highest contents are observed in mushrooms cultivated on the substrates 70SD30CG and M3S, with respective averages of 0.268 ± 0.009 and 0.263 ± 0.04 µg/g DM. In contrast, the lowest contents are recorded for mushrooms grown on the SD and Cr substrates, with respective averages of 0.112 ± 0.05 and 0.117 ± 0.03 µg/g DM (Fig. 12). Turfan et al. (2019) reported that mushrooms have a low sugar and calorie content, which aligns with our findings.

The results of the study by Miettinen et al. (2020) affirm that mushrooms cultivated on wood-based substrates, such as wood shavings, wood chips, or agricultural by-products, tend to have higher levels of certain sugars, which does not align with our results.

Several studies have reported that substrate composition affects sugar content in mushroom fruiting bodies, and sugar content varies depending on the substrate composition used in mushroom cultivation (Sarris et al., 2020; Chang, 1976; Ortega et al., 1992; Assan and Mpofu, 2014; Bandura et al., 2021). However, Rajarathnam et al. (1986) reported that substrate type affects mushroom growth but not sugar content.

For lipids, the analysis of variance indicates a highly significant effect ($P<0.0000$) (Table 8) and a wide variation in lipid content in oyster mushrooms cultivated in the different substrates used.

Mushrooms grown on the SD substrate exhibit the highest lipid content, with an average of 23.93±0.3% (Fig. 12), while the substrate 70SD30CG produced oyster mushrooms with low lipid content (3.8±0.2%).

Mattila et al. (2002) and Reis et al. (2012) reported very low lipid values (0.35 and 0.16 g/100g), while other studies (Alam et al., 2008; Bonatti et al., 2004) reported higher values (4.6 and 6.32 g/100g).

The type of substrate significantly influences **the protein** content of oyster mushrooms, with a highly significant effect ($P < 0.0000$) (Table 8). Mushrooms cultivated on the 50CGSD substrate display the highest protein content (1.38 ± 0.01 mg/L), while those grown on the M3S substrate exhibit the lowest content (0.63 ± 0.02 mg/L).

The protein contents recorded in our study are low and align with the results of Reis et al. (2012) and Mattila et al. (2002), who reported values ranging from 0.76 to 1.21 g/100 g and 1.97 g/100 g, respectively. However, other studies (Akyuz and Kirbag, 2010; Gogavekar et al., 2014) have described much higher protein contents, ranging from 23.91 to 41.6 g/100 g.

Diamantopoulou et al. (2023) classify oyster mushrooms as a good source of protein, reporting a content of 37.04% in mushrooms cultivated on beech wood chips. Turfan et al. (2018) studied the quantity of total soluble proteins in various wild and cultivated mushrooms, with contents ranging from 33.57 mg to 126.57 mg g-1.

Table 8. One-way ANOVA testing the effects of substrate types on variations in biochemical properties in oyster mushroom fruiting bodies.

Factor	DF	MS	P-value	Sig.	MS	P-value	Sig.
		Sugars			**Lipid**		
Substrate	4	0.017	4.5E-4	***	183.6	7.6E-11	***
Error	10	0.001			0.49		
		Proteins			DF: degrees of freedom, MS : Mean Square, Sig : statistical significance, ***: p < 0.001, **: p < 0.01, *: $p \leq 0.05$, ^{ns}: p >0.05)		
Substrate	4	0.256	2.33E-10	***			
Error	10	8.5E-4					

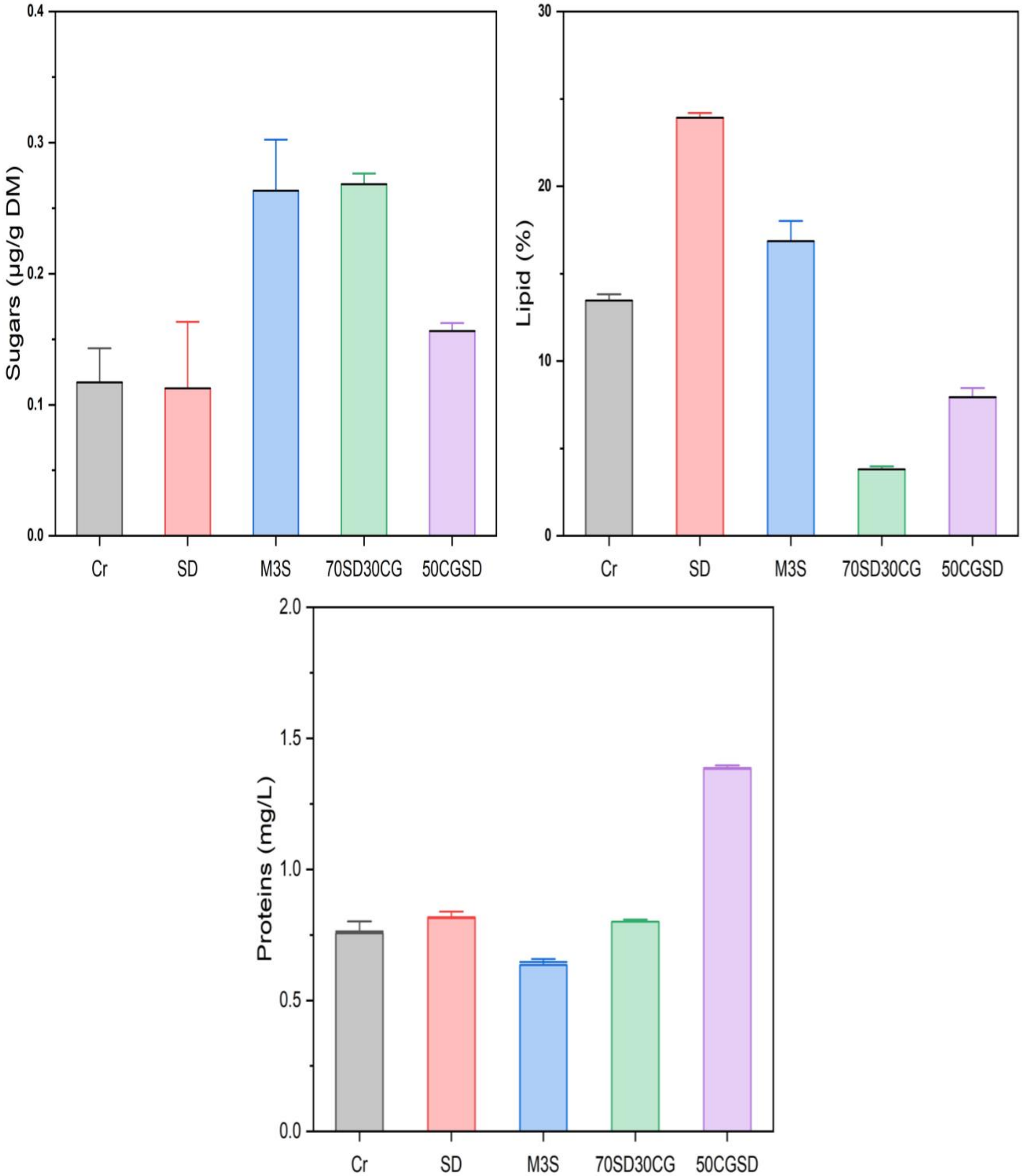


Figure 12. Histograms (mean±SD) showing the variation in biochemical parameters analyzed in oyster mushrooms in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a 33% ratio, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.6. Vitamins

The **vitamin C** content of oyster mushrooms is strongly influenced by the type of substrate used ($P < 0.0001$) (Table 9). The substrate based on Cr allows for the highest vitamin C content, with an average of 1.74 ± 0.09 mg/g, while the SD substrate gives the lowest content, with an average of 0.72 ± 0.02 mg/g (Fig. 13).

In their study on vitamin C content and antioxidants in edible and wild mushrooms, Bano and Rajarathnam (1986) reported vitamin C values ranging from 3.9 to 28.1 mg per 100 grams of fresh weight, with *Pleurotus ostreatus* exhibiting the highest concentration.

Kozarski et al. (2015) found 100 mg/100 g of ascorbic acid in the methanolic extract of the wild edible mushroom *Cantharellus cibarius*. Similarly, Grangeia et al. (2011) found high concentrations of ascorbic acid in methanolic extracts of various wild edible saprotrophic and mycorrhizal mushrooms (81.32-400.36 mg/100 g DW). A mean content of 0.7+_0.01 mg/100g of vitamin C was found by Ibe et al. (2013) in *Pleurotus florida*.

The **vitamin B12** content of oyster mushrooms is also influenced by the type of substrate used ($P = 0.0059$) (Table 9). Mushrooms cultivated on the Cr substrate exhibit the highest vitamin B12 content, with an average of 0.69 ± 0.05 µg/g, while those grown on the 50 CGSD substrate have the lowest content, with an average of 0.23 ± 0.002 µg/g (Fig. 13).

Essential for the brain, nervous system, and red blood cell formation, vitamin B12, a water-soluble vitamin, is predominantly found in animal-based products (meat, fish, dairy, eggs), but some mushrooms and other vegetables also contain it (Carmel, 2008; Watanabe et al., 2014).

The type of substrate on which mushrooms are cultivated can influence their vitamin B12 content (Royse, 2017). Mattila et al. (2001) reported B12 values ranging from 0.05 to 0.6 µg/100g.
Effiong et al. (2024) and Piska et al. (2017) reported values of 0.31 mg/kg and 0.5 mg/100g, respectively.

Studies have revealed that mushrooms cultivated on animal by-product-enriched substrates, such as manure or bone meal, generally display higher vitamin B12 contents than those grown on vegetable-based substrates. This phenomenon is likely due to the presence of vitamin B12-

producing bacteria in animal by-products, capable of synthesizing and transferring this vitamin to mushrooms (Watanabe et al., 2014).

Null or trace concentrations (0.01-0.09 µg/100 g dry weight) of vitamin B12 have been determined in porcini mushrooms (*Boletus spp*.), parasol mushrooms (*Macrolepiota procera*), oyster mushrooms (*Pleurotus ostreatus*), and black morels (*Morchella conica*) (Watanabe et al., 2012). In contrast, black trumpet mushrooms (*Craterellus cornucopioides*) and golden chanterelle (*Cantharellus cibarius*) contained considerable levels (1.09-2.65 µg/100 g dry weight) of vitamin B12 (Watanabe et al., 2012).

The analysis of variance **of vitamin D** results reveals a highly significant effect of the substrate type (P < 0.0000) (Table 9). Mushrooms cultivated on SD display the highest vitamin D content (1.45 ± 0.02 µg/g DW), while the substrate 70SD30CG presents the lowest content (0.406 ± 0.007 µg/g DW) (Fig. 13).

In *Agaricus bisporus* (button mushroom), contents ranging from 0 to 3.75 µg/100 g fresh weight have been reported by several authors (Mattila et al., 1994; Souci et al., 1986; Holland et al., 1991). In contrast, *Lentinula edodes* (shiitake) exhibits much higher concentrations, with values ranging from 21.8-109.6 µg/100 g fresh weight, as demonstrated by the results of Takamura et al. (1991).

Tableau 9. One-way ANOVA testing the effects of substrate types on variations onvitamin content in oyster mushroom fruiting bodies.

Factor	DF	MS	P-value	Sig.	MS	P-value	Sig.
		Vit C			**Vit B12**		
Substrate	4	0.492	1.4E-7	***	0.121	0.005	**
Error	10	0.006			0.017		
		Vit D			DF: degrees of freedom, MS : Mean Square, Sig : statistical significance, ***: $p < 0.001$, **: $p < 0.01$, *: $p \le 0.05$, ^{ns}: $p > 0.05$)		
Substrate	4	0.726	3.3E-15	***			
Error	10	2.5E-4					

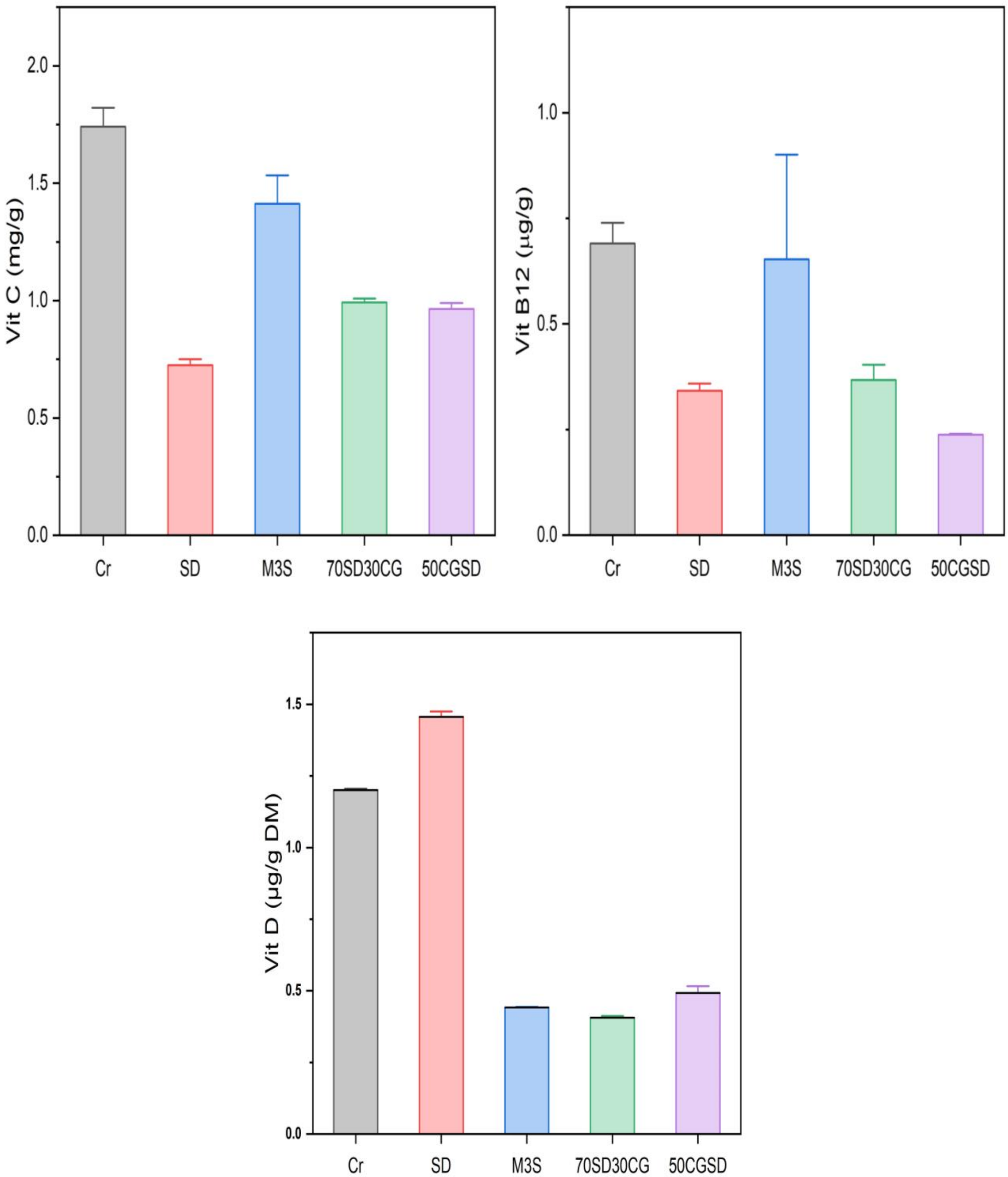


Figure 13. Histograms (mean±SD) showing the variation in vitamin content analyzed in oyster mushrooms in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a 33% ratio, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.7. Secondary metabolites

The **polyphenol** content of oyster mushrooms is also influenced by the type of substrate used and presents a highly significant effect (P = 0.0086) (Table 10). The highest contents are observed in mushrooms cultivated on the 50 CGSD substrate, with an average of 0.046 ± 0.007 mg EAG/g (Fig. 14).

In contrast, the lowest contents are recorded for mushrooms grown on the M3S substrate, with an average of 0.028 ± 0.004 mg EAG/g.

Phenolic compounds contribute to the biochemical profile and antioxidant properties of the mushroom (Silva et al., 2024).

For the same species of oyster mushrooms, Yilmaz et al. (2017) reported significantly higher polyphenol contents than those observed in this study. They measured polyphenol contents ranging from 2.672 ± 0.003 mg EAG/g in oyster mushrooms cultivated on walnut sawdust to 1.073 ± 0.028 mg EAG/g in those cultivated on peanut waste.
Studies conducted by Barros et al. (2007) and Cheung (2003) revealed that the polyphenol content of different Pleurotus species ranged from 6.14 to 10.93 mg EAG/g and reached approximately 9.6 mg GAE/g dry weight for *Pleurotus ostreatus*, respectively.

The flavonoid content of oyster mushrooms is also influenced by the type of substrate used and shows a highly significant effect (P = 0.0041) (Table 10).

The highest contents are observed in mushrooms cultivated on the 50 CGSD substrate, with an average of 0.024 ± 0.004 mg EQ/g. In contrast, the lowest contents are recorded for mushrooms grown on the 70 SD30CG substrate, with an average of 0.011 ± 0.0009 mg EQ/g (Fig. 14)

Studies conducted by Jayakumar et al. (2006) and Heleno et al. (2010) revealed that the flavonoid content of *Pleurotus ostreatus* and *Pleurotus eryngii* varied respectively from approximately 1.5 mg EQ/g and 0.7 mg EQ/g.

Tableau 10. One-way ANOVA testing the effects of substrate types on variations on polyphenols and flavonoids in oyster mushroom fruiting bodies.

Factor	DF	MS	p-value	Sig	MS	p-value	Sig
		Polyphenols			**Flavonoids**		
substrate	4	1.31E-4	0.0086	**	8.1E-5	0.0041	**
error	10	2.09E-5			1.05E-5		

DF: degrees of freedom, MS : Mean Square, Sig : statistical significance, ***: $p < 0.001$, **: $p < 0.01$, *: $p \leq 0.05$, ^{ns}: $p > 0.05$)

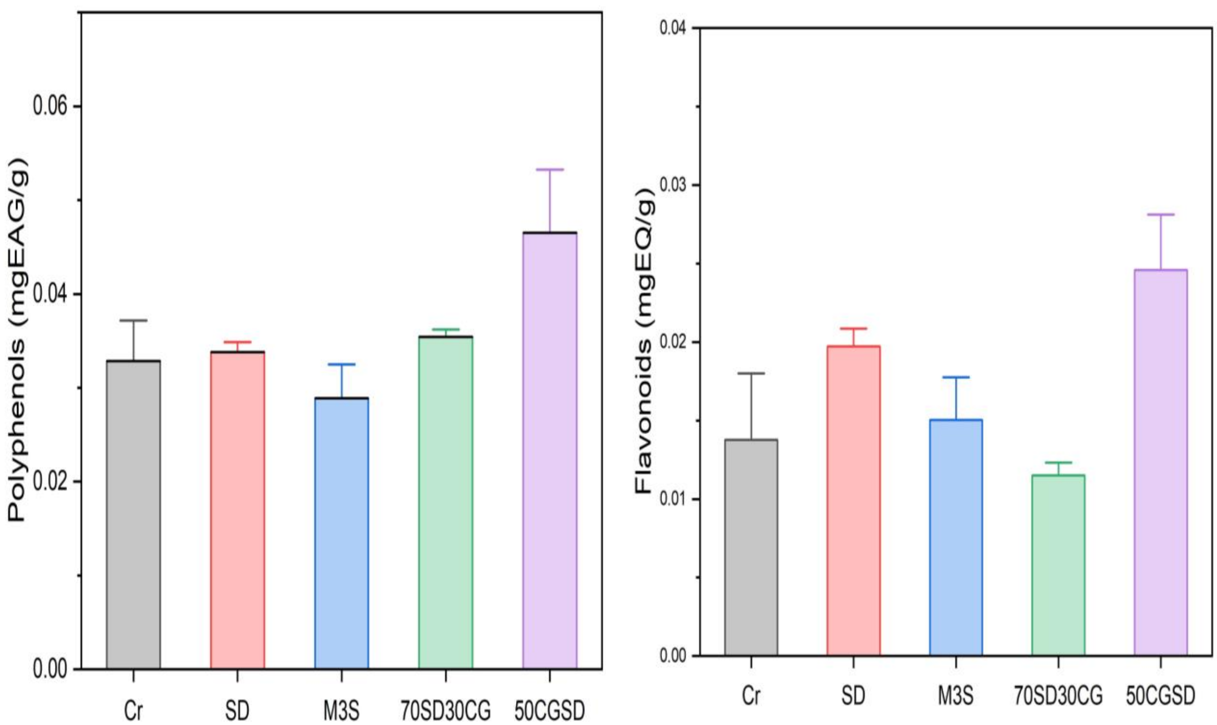


Figure 14. Histograms (mean±SD) showing the variation in secondary metabolites analyzed in oyster mushrooms in relation to the type of substrate (Cr: cardboard, SD: sawdust, M3S: mixture of 3 substrates with a 33% ratio, 70SD30CG: 70% sawdust and 30% coffee grounds, 50SDCG: 50% sawdust and 50% coffee grounds).

3.8. Multivariate analysis of the relationship between substrates and measured variables

3.8.1. Correlations

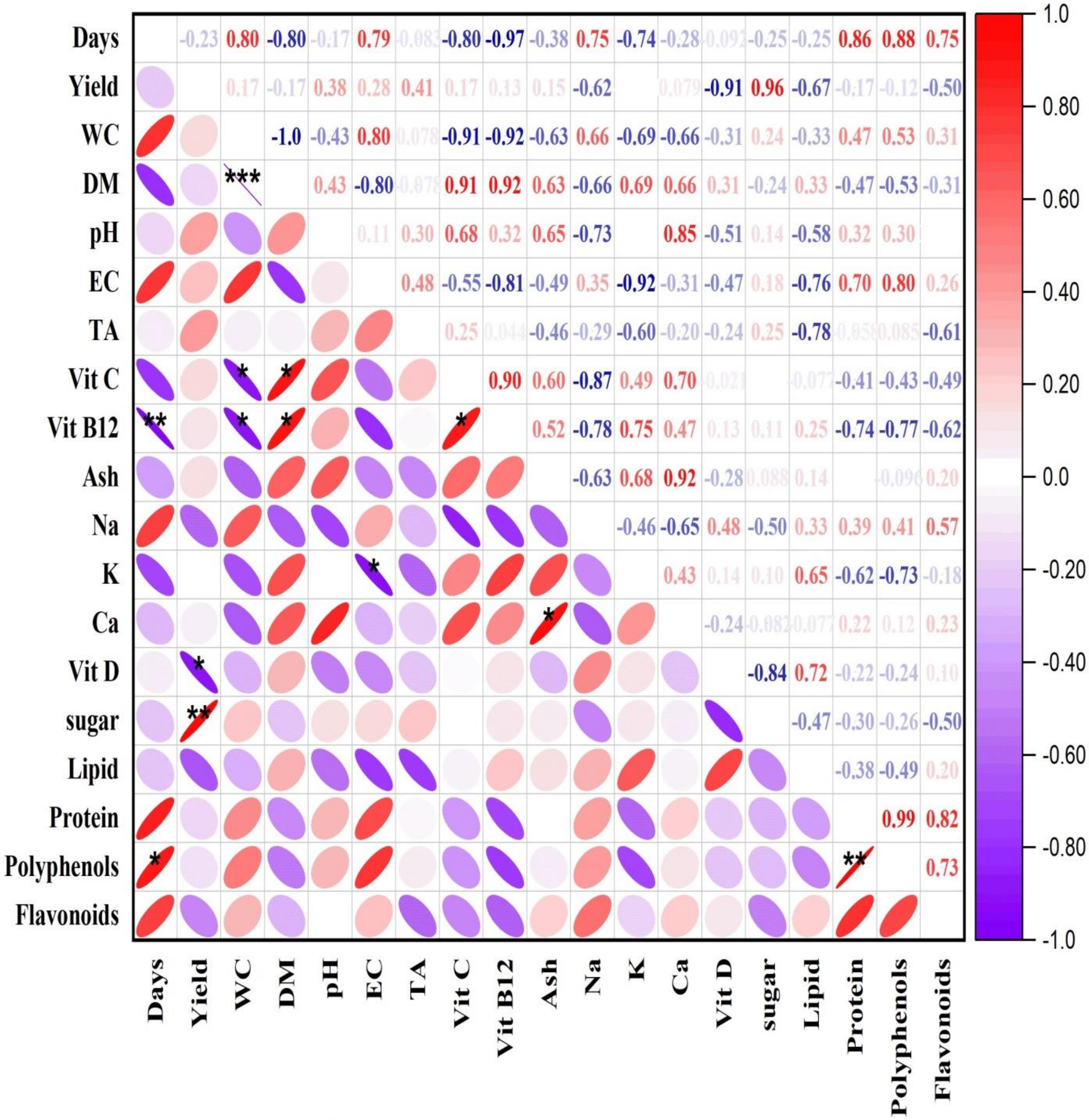
The correlation matrix (Fig. 15) shows the correlation coefficients between all pairs of variables in the dataset. In this matrix, the correlation coefficients are color-coded to indicate the strength of the correlation. Red indicates a positive correlation, blue indicates a negative correlation, and white indicates no correlation. The darker the color, the stronger the correlation.

Based on the correlation matrix, we can make the following observations:

Variables related to growth (life cycle and WC) are positively correlated with each other. This means that mushrooms with longer life cycle tend to have higher water content.

Variables related to nutritional composition are generally positively correlated with each other, for example, very strong positive correlations are observed between Vit C and Vit B12 (r= 0.90) and DM (r= 0.91), between ash and Ca (r= 0.92), between protein and polyphenols (r= 0.99) and flavonoids (r= 0.82), and between sugars and yield (r= 0.96) (Fig. 15).

There are some negative correlations between variables related to growth and yield and variables related to nutritional composition, for example, a negative correlation between yield and Vit C (r= -0.91) and Vit B12 (r= -0.92), and between EC and K (r= -0.92) and Vit B12 (r= -0.81). Thus, a negative correlation between yield and Vit D (r= -0.90) is revealed by the correlation matrix. This means that mushrooms with higher yields tend to have lower vitamin D content.



* p<=0.05 ** p<=0.01 *** p<=0.001

Figure 15. Correlation matrix showing Pearson correlation tests between the parameters measured on mushroom fruiting bodies under different substrate types. The correlation coefficient values are color-coded and represented in circular diagrams (above the diagonal), and the correlation test values are indicated above the diagonal. Significant correlations ($p < 0.05$) are expressed by asterisks in bold. Red indicates a positive correlation, blue indicates a negative correlation, and white indicates no correlation. The darker the color, the stronger the correlation.

3.8.2. Principal Component Analysis (PCA)

Figure (16) shows a biplot of a principal component analysis (PCA) performed on the 19 measured variables.

The biplot consists of two axes, PC1 (principal component 1) and PC2 (principal component 2), which represent the two most significant sources of variability in the data. Each point in the plot represents an individual mushroom sample, and the position of each point is determined by its scores on PC1 and PC2. The arrows represent the loadings of the variables on the principal components. The length of an arrow indicates the importance of the variable in defining the component, and the direction of the arrow indicates the correlation between the variable and the component.

Based on the positions of the points and the directions of the arrows, we can interpret the relationships between the variables and the principal components.

PC1 (45.42% of variance): This component is primarily associated with variables related to growth and mineral content, such as life cycle, yield, WC, DM, ash, Ca, K, Vit C, and Vit B12. The positive loadings of these variables on PC1 suggest that they are positively correlated with each other. In other words, mushrooms with higher yields and longer life cycle tend to have higher water content. Negative loadings of variables on this axis indicate that as one increases, the other decreases, for example, between WC and DM.

PC2 (25.76% of variance): This component is primarily associated with variables related to nutritional composition, such as Vit D, sugars, lipids, TA, pH, and yield. The positive loadings of these variables on PC2 suggest that they are positively correlated with each other (pH, TA, sugars, and yield). In other words, mushrooms with pH values closer to neutrality and higher levels of TA and sugars tend to have higher yields and lower contents of Vit D and lipids.

The positions of the substrate labels in the biplot provide insights into the effects of different substrates on the characteristics of the mushrooms.

Mushrooms grown on the Cr substrate are located in the lower-left quadrant of the biplot, suggesting that they have lower yields, shorter life cycle and lower water content compared to mushrooms grown on other substrates. However, they have higher vitamin B12 and calcium content.

Mushrooms grown on the 70SD30CG substrate are located in the upper-right quadrant of the biplot, suggesting that they have the highest yields, the highest water content, and the highest sugar content.

Mushrooms grown on the SD substrate are located in the lower-left quadrant of the biplot, suggesting that they have lower yields, slightly shorter life cycle, and lower water content compared to mushrooms grown on other substrates. However, they have the highest vitamin D and sodium content.

Mushrooms grown on the M3S substrate are located in the upper-right quadrant of the biplot and close to the positioning of the Cr substrate, suggesting that these two substrates have intermediate values for Vit B12 and Ca. However, mushrooms grown on this substrate have the highest contents of K, ash, Ca, DM, and sugars.

Mushrooms grown on the 50CGSD substrate are located in the upper-right quadrant of the biplot, suggesting that they have the longest life cycle and the lowest vitamin contents compared to mushrooms grown on other substrates. However, they have the highest contents of Ca, protein, polyphenols, and flavonoids.

The multivariate analysis reveals that the choice of substrate significantly influences the growth, yield, and nutritional composition of oyster mushrooms. Cultivated on varied substrates, the mushrooms exhibit distinct characteristics, offering a range of options for consumers and food producers.

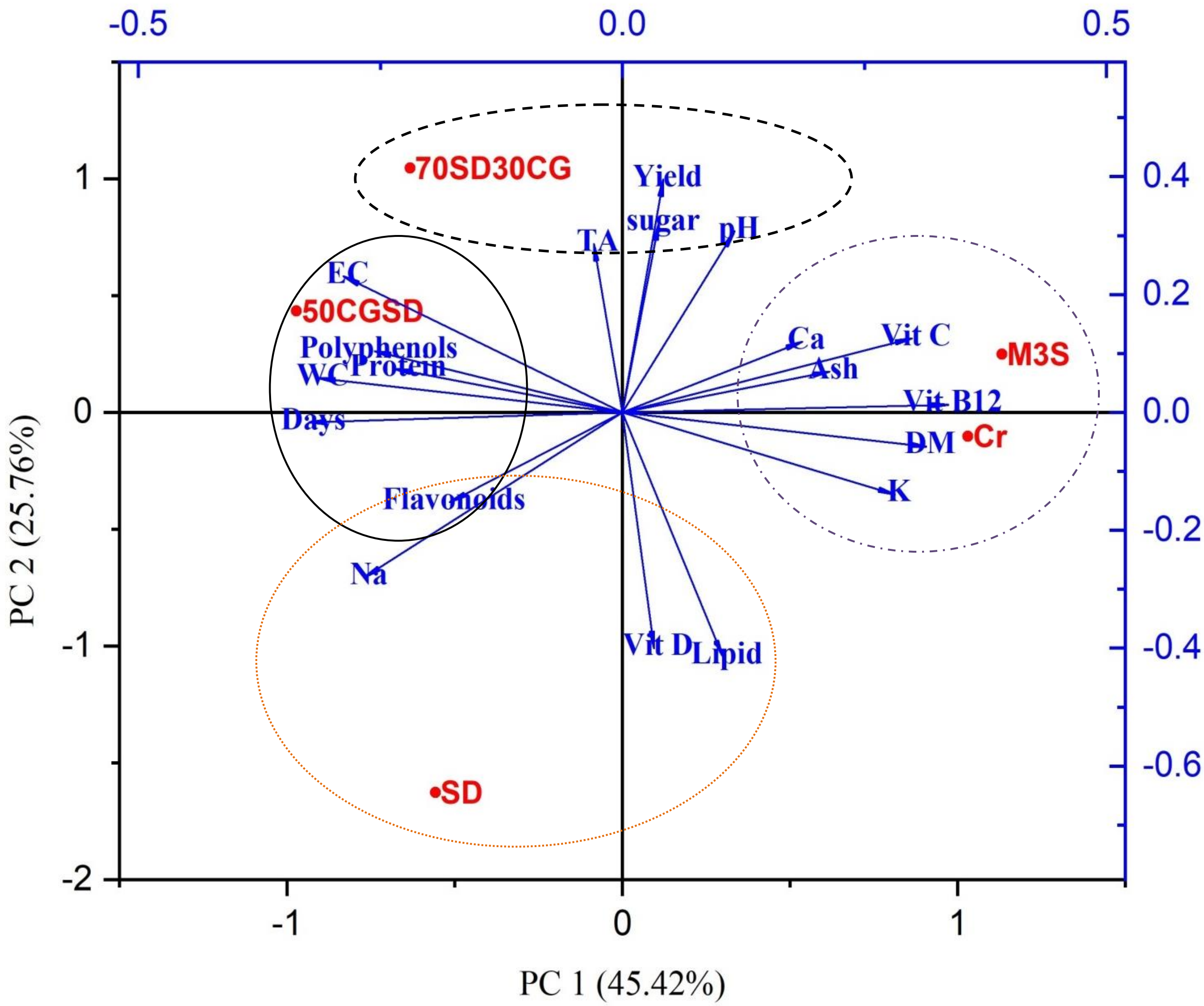


Figure 16. Biplots of principal component analysis (PCA) displaying the relationships between the measured parameters and used substrates.

Conclusion

General conclusion

Mushrooms are the second most cultivated mushrooms in the world, as they adapt to a variety of substrates, from straw to sawdust.

It was in this situation that our objective of finding a substrate that improves the yield and nutritional quality of oyster mushrooms developed.

Various substrates have been used to grow oyster mushrooms, such as cardboard (Cr), sawdust (SD), coffee grounds (CG) and combinations of these. Quantitative (yield, dry matter and water content) and qualitative (pH, B12, Vitamin D, Na, K, C, sugar content, lipids, proteins, polyphenols and flavonoids) parameters were evaluated. The conclusions obtained showed that each substrate has an impact on one or more parameters, both quantitative and qualitative.

Mushrooms grown on mixed substrates (sawdust and coffee grounds) showed the highest yields, water content and sugar content. Mushrooms grown on sawdust substrate (SD) showed the highest vitamin D, sodium and lipid content. Oyster mushrooms obtained by combining the three substrates in equal proportions (M3S) were extremely rich in dry matter, vitamin B1, ash, K, Ca and sugars. Proteins, polyphenols and flavonoids are more present in the 50CGSD substrate (50% sawdust and 50% coffee grounds).

Based on the results of this study, future research should focus on optimizing substrate blends to maximize the yield and nutritional qualities of oyster mushrooms. A thorough exploration of various cultural parameters, such as temperature, humidity, and incubation duration, would help assess the impact of these factors on the performance of secondary substrates. Furthermore, a rigorous analysis of the economic and environmental impact of using these recycled substrates would provide valuable insights to promote more sustainable and profitable farming practices.

In conclusion, the sustainable valorization of secondary materials for oyster mushroom cultivation offers promising prospects for a more eco-friendly and economically viable agriculture. The results of this study underscore the critical importance of substrate selection and optimization to enhance the growth, yield, and nutritional quality of mushrooms while contributing to the sustainable management of organic waste. By adopting an innovative and sustainable approach to oyster mushroom cultivation, we can not only meet the growing demand for nutritious food but also reduce our environmental footprint and promote a more environmentally friendly circular economy.

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Annexes

Annexes

- **Annexe 01: Vitamin B12**

Materials:

- Fresh edible mushrooms
- UV-Visible spectrophotometer
- Spectrophotometer cuvettes
- 0.1 M sodium acetate ($CH3COONa$) solution
- 0.1 M hydrochloric acid (HCl) solution
- 0.1% cobalt chloride solution ($CoCl2$)
- 0.1% sodium nitrite solution ($NaNO2$)
- Ammonium acetate solution ($NH4CH3COO$) 0.1 M

1. Sample preparation:

- Clean the mushrooms and cut into small pieces.
- Weigh 1 g of mushrooms and grind finely in a mortar.
- Add 10 ml of 0.1 M sodium acetate and mix vigorously.
- Centrifuge the mixture at 4000g for 10 minutes.
- Remove the supernatant and store for analysis

2. Determination of vitamin B12:

- Pipette 1 ml of the supernatant into a spectrophotometer cell.
- Add 1 ml of 0.1 M hydrochloric acid and mix.
- Leave to stand for 10 minutes.
- Add 1 ml of 0.1% cobalt chloride solution and mix.
- Leave to stand for 5 minutes.
- Add 1 ml of 0.1% sodium nitrite solution and mix.
- Leave to stand for 2 minutes.
- Add 2 ml of 0.1 M ammonium acetate solution and mix.
- Measure the absorbance of the mixture at 450 nm.

3. Calculation of vitamin B12 concentration:

- The vitamin B12 concentration (in mg/g) is calculated using the following formula:

Vitamin B12 concentration = $(A \times F) / (\varepsilon \times m)$

Where:

- A is the absorbance measured at 450 nm
- F is the dilution factor (10 in this case)
- ε is the molar extinction coefficient of vitamin B12 at 450 nm (6600 L/mol*cm)
- m is the mass of the mushroom sample (1 g)

- **Annexe 02: Vitamin D**

Materials:

- Fresh edible mushrooms
- UV-Visible spectrophotometer
- Spectrophotometer cuvettes
- 95% ethanol solution
  - Methanol solution
  - Dichloromethane solution
  - 0.1 N potassium hydroxide solution
  - Hydrochloric acid solution 0.1 N
  - Sodium acetate solution 3 M
  - 0.2% 2,4-dinitrophenylhydrazine (DNPH) solution

Method:

1. Sample preparation:
   - Clean the mushrooms and cut into small pieces.
   - Weigh out 1 g of mushrooms and grind finely in a mortar.
   - Add 10 ml of 95% ethanol and mix vigorously.
   - Centrifuge the mixture at 4000g for 10 minutes.
   - Remove the supernatant and store for analysis.

2. Determination of vitamin D:

Pipe 1 ml of the supernatant into a spectrophotometer cell.

- Add 1 ml of methanol solution and mix.
- Leave to stand for 10 minutes.
- Add 1 ml of dichloromethane solution and mix.
- Leave to stand for 5 minutes.
- Add 2 ml of 0.1 N potassium hydroxide solution and mix.

- Leave to stand for 20 minutes.
- Add 1 ml of 0.1 N hydrochloric acid solution and mix.
- Add 2 ml of 3 M sodium acetate solution and mix.
- Add 1 ml of 0.2% DNPH solution and mix.
- Leave to stand for 20 minutes.
- Measure the absorbance of the mixture at 420 nm.

3. Calculation of vitamin D concentration:

o The Vitamin D concentration (in mg/g) is calculated using the following formula

formula: Vitamin D concentration = (A x F) / (ε x m)

Where:

- A is the absorbance measured at 420 nm
- F is the dilution factor (10 in this case)
- ε is the molar extinction coefficient of vitamin D at 420 nm (6600 L/mol*cm)
- m is the mass of the mushroom sample (1g)

عنوان المذكرة: التثمين المستدام للمواد الثانوية لزراعة فطر المحار(*Pleurotus ostreatus*)
الاسم واللقب: بن منصور اية
المؤطر: عبابسة نوال/ بركاني شريفة
ملخص:

تثمين المواد الثانوية المستدامة لزراعة الفطريات يظهر إمكانات كبيرة لتحسين المحصول والجودة الغذائية للفطر. تتناول هذه الدراسة تأثير الركائز المختلفة على نمو فطر المحار، دورة حياته، محصوله وتكوينه الغذائي. وتشمل الركائز التي تم اختبارها ورق الكرتون ، نشارة الخشب، تفل القهوة و مزيج من هذه الركائز فيما بينها. حيث أظهرت النتائج: أن : ركيزة الكرتون سجلت أقصر دورة حياة للفطريات بمعدل 37.33 ± 3.78 يو ًم. أما الأس الهيدروجيني الأقرب إلى التعادل بمتوسط قيم 6.62 ± 0.03. تبرز هذه الركيزة نفسها لمساهمتها في ثراء الفطر بفيتامين B 12 (0.05 ± 0.69 ميكروغرام/غرام) والكالسيوم (20± 0.49 جزء في المليون). أظهر الفطر المزروع على ركيزة 70SD30CG (70% من نشارة الخشب و30% من القهوة المطحونة) أعلى عائد بمتوسط 137.5 ± 7.5 غ. من حيث المحتوى المائي، أنتجت هذه الركيزة أيضًا فطرًا يحتوي على أعلى محتوى مائي عند 85.6 ± 0.99%، مما يشير إلى زيادة نضارة الفطر. تعزز هذه الركيزة نفسها من المذاق الحلو للفطر، بمتوسط محتوى 0.26 ± 0.009 ميكروغرام/غرام من DM. أظهر الفطر المزروع على ركيزة SD (نشارة الخشب) أعلى تركيز من حيث محتوى فيتامين D (1.45 ± 0.02 ميكروغرام/غرام من وزن الجسم) ، الصوديوم (1650 ± 45.82 جزء في المليون) والدهون (23.93 ± 0.3%). أنتج الجمع بين الركائز الثلاث في أجزاء متساوية (M3S) فطرًا غنيًا بالمادة الجافة (27.33 ± 3.9%)، فيتامين B12 (0.65 ± 0.28 ميكروغرام/غرام), رماد (3.51 ± 0.37%) البوتاسيوم (307 ± 2.51 جزء في المليون), كالسيوم (20 ± 0.34 جزء في المليون) والسكريات (0.26 ± 0.04 ميكروغرام/غرام من DM). وأخيرًا، فإن الركيزة CGSD50 (50% من نشارة الخشب و50% من القهوة المطحونة) فضلت محتوى أعلى من البروتينات (1.38 ± 0.01 ملغم/لتر), البوليفينول (0.046 ± 0.007 ملغم من مكافئ غازي/غرام) ، ومركبات الفلافونويد (0.024 ± 0.004 مجم كيو إي/غرام). تسلط هذه النتائج الضوء على أهمية اختيار الركائز وخلطها بعناية لتحسين نمو فطر المحار؛ والنمو، والإنتاجية، والصفات الغذائية.

الكلمات المفتاحية: التثمين، الفطر، نوع الركيزة، التركيب الغذائي، دورة الحياة، المحصول.

Title of the dissertation: Sustainable valorization of secondary materials for oyster mushroom *(Pleurotus ostreatus*) cultivation
Full name: Aya Ben Mansour
Directed by: Nawal ABABSA/Cherifa BERKANI
Abstract:

The sustainable valorization of secondary materials for the cultivation of oyster mushrooms presents significant potential for improving yields and the nutritional quality of the mushrooms. This study examines the effect of different substrates on oyster mushrooms' growth, life cycle, yield, and nutritional composition. The substrates tested include cardboard (Cr), sawdust (SD), coffee grounds (CG), and combinations of these substrates. The results show that:
The Cr substrate produced oyster mushrooms with the shortest life cycle of 37.33 ± 3.78 days.
Their pH is closest to neutrality, with an average value of 6.62 ± 0.03. This same substrate stands out for its contribution to the richness of the mushrooms in vitamin B12 (0.69 ± 0.05 µg/g) and calcium (20 ± 0.49 ppm).
Mushrooms grown on the 70SD30CG substrate (70% sawdust and 30% coffee grounds) showed the highest yield, averaging 137.5 ± 7.5 g. In terms of water content, this substrate also produced mushrooms with the highest water content at 85.6 ± 0.99%, indicating increased freshness of the U Gmushrooms. This same substrate enhances the sweet taste of the mushrooms, with an average content of 0.26 ± 0.009 µg/g DM. Mushrooms grown on the SD substrate (sawdust) showed the highest concentration in terms of vitamin D content (1.45 ± 0.02 µg/g DW), sodium (1650 ± 45.82 ppm), and lipids (23.93 ± 0.3%).
The combination of the three substrates in equal parts (M3S) produced mushrooms rich in dry matter (27.33 ± 3.9%), vitamin B12 (0.65 ± 0.28 µg/g), ash (3.51 ± 0.37%), potassium (307 ± 2.51 ppm), calcium (20 ± 0.34 ppm), and sugars (0.26 ± 0.04 µg/g DM).
Finally, the 50CGSD substrate (50% sawdust and 50% coffee grounds) favored a higher content of proteins (1.38 ± 0.01 mg/L), polyphenols (0.046 ± 0.007 mg GAE/g), and flavonoids (0.024 ± 0.004 mg QE/g).
These results highlight the importance of carefully selecting and mixing substrates to optimize oyster mushrooms' growth, yield, and nutritional qualities.

Key words : valorization, mushrooms, type of substrate, nutritional composition, life cycle, yield

Titre du mémoire : Valorisation durable de matières secondaires pour la culture de pleurotes (*Pleurotus ostreatus*).
Nom et prénom : Aya Ben Mansour
Encadreur : Nawal ABABSA /Cherifa BERKANI

Résumé :

La valorisation durable des matières secondaires pour la culture des pleurotes présente un potentiel significatif pour l'amélioration des rendements et de la qualité nutritionnelle des champignons. Cette étude examine l'effet de différents substrats sur la croissance, le cycle de vie, le rendement et la composition nutritionnelle des pleurotes. Les substrats testés incluent le carton (Cr), la sciure de bois (SD), le marc du café (CG) et des combinaisons de ces substrats. Les résultats montrent que :
Le substrat Cr a produit des pleurotes avec le cycle de vie le plus court avec 37.33 ± 3.78 jours.
Leur pH est le plus proche de la neutralité avec une valeur moyenne de 6.62 ± 0.03. Ce même substrat se démarque par sa contribution à la richesse des pleurotes en vitamine B12 (0,69 ± 0,05 µg/g) et en calcium (20 ± 0,49 ppm).
Les champignons cultivés sur le substrat 70SD30CG (70 % de sciure de bois et 30 % de marc de café) ont montré le rendement le plus élevé avec une moyenne de 137.5 ± 7.5 g. En termes de teneur en eau, ce même substrat a également produit des champignons avec la plus haute teneur en eau à 85.6 ± 0.99 %, indiquant une fraîcheur accrue des champignons. Ce même substrat favorise le goût sucré des pleurotes, avec une teneur moyenne de 0,26 ± 0,009 µg/g DM.
Les champignons cultivés sur le substrat SD (sciure de bois) ont montré la plus haute concentration en termes de teneur en vitamine D (1.45 ± 0.02 µg/g DW), en Na (1650±45.82 ppm) et en lipides (23.93±0.3%).
La combinaison des trois substrats à parts égales (M3S) produit des pleurotes riches en matière, sèche (27.33±3.9 %), en vitamine B12 (0.65±0.28 µg/g) en cendre (3.51±0.37), en K (307±2.51 ppm), en Ca (20 ±0.34 ppm) et en sucres (0.26 ±0.04 µg/g DM).
Enfin, le substrat 50CGSD (50 % de sciure de bois et 50 % de marc de café) favorise une teneur plus élevée en protéines (1.38 ± 0.01 mg/L), en polyphénols (0.046 ± 0.007 mg EAG/g) et en flavonoïdes (0.024 ± 0.004 mg EQ/g).
Ces résultats soulignent l'importance de choisir et de mélanger judicieusement les substrats pour optimiser la croissance, le rendement et les qualités nutritionnelles des pleurotes.

Mots clés : valorisation, champignons, type de substrat, composition nutritionnelle, cycle de vie, rendement