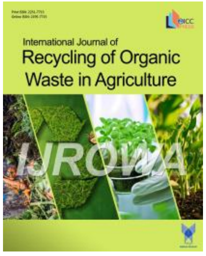

Int. J. Recycl. Org. Waste Agric 2026; 15(1): 62-70

International Journal of Recycling of Organic Waste in Agriculture (IJROWA)

https://doi.org/10.57647/ijrowa-2026-17443

Research Article

The Impact of Lignocellulosic Wastes and Industrial by-Products on Some Chemical Properties of Shiitake Mushroom (*Lentinula Edodes*)

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Article History:

Received:
06 January 2025

Revised:
03 May 2025

Accepted:
21 August 2025

Published Online:
07 September 2025

Published in Issue:
31 March 2026

Abstract

Purpose: The edible and medicinal Shiitake mushroom is one of the most well-known mushrooms worldwide. Mushroom cultivation reduces the environmental impact of agricultural waste used as substrates and provides an economically viable alternative for producing food with high flavor and quality, as well as valuable metabolites.

Method: The present study was conducted in 2023 at the Islamic Azad University, Isfahan branch, using a split-plot design arrangement. The main plots included sawdust from oak, poplar, mulberry, and corn wood, while the subplots included wheat bran (0 and 25%), oat seeds (0 and 30%), gypsum ($CaSO_4 \cdot 2H_2O$) (0 and 30 mmol per kilogram), whey powder (0 and 1%), dairy factory wastewater (0 and 50%), and sugar factory wastewater (0 and 50%).

Results: The highest average carbohydrate content in the Shiitake mushroom was observed in the oak sawdust treatment with wheat bran supplement (29.71%), while the lowest was found in the poplar sawdust treatment with dairy wastewater (25.54%). Additionally, the highest average protein content in the Shiitake mushroom was observed in the oak sawdust treatment with dairy wastewater supplement, while the lowest was found in the poplar and corn sawdust treatments without any supplements (15.34 and 15.37 g per kg dry weight, respectively).

Conclusion: The highest average carbohydrate content in Shiitake mushrooms was observed in the oak sawdust treatment with wheat bran supplementation. The highest fiber content in Shiitake mushrooms was observed in the oat treatment, while the lowest content was found in treatments with whey, dairy wastewater, control, and gypsum.

Keywords: Shiitake mushroom, Soluble solids, Fiber, Carbohydrates, Protein

Cite this article: Lotfi, A., Jafarpour, M., & Khalili, F., (2026). The impact of lignocellulosic wastes and industrial by-products on some chemical properties of Shiitake mushroom (*Lentinula edodes*). *International Journal of Recycling of Organic Waste in Agriculture, 15(1),* 62-70. https://doi.org/10.57647/ijrowa-2026-17443

1. Introduction

Mushrooms are considered one of the most important food and medicinal sources globally. Their antimicrobial, antitumor, and antioxidant properties have made them useful in drug production. In addition to their medicinal properties, mushrooms, due to their high protein content, low calories, and mineral content, are a staple in the diets of people worldwide (Chen et al., 2021). Among the vast resources of mushrooms, basidiomycetes, particularly macroscopic mushrooms, contain significant medicinal resources and biologically active elements (Royse et al., 2017).

The edible Shiitake mushroom, scientifically known as Lentinula edodes, belongs to the Basidiomycetes class. This mushroom ranks second in global production after the button mushroom (Liu et al., 2022). Shiitake is one of the most well-known mushrooms worldwide and accounts for a quarter of global mushroom production. In China, it is known as Xiang Gu (fragrant or aromatic mushroom), while in Japan, it is associated with the Shia tree, earning it the name "Shiitake." The mushroom is valued for its high nutritional content and excellent flavor, making it suitable for various culinary applications (Zhang et al., 2021). Shiitake mushrooms contain a variety of compounds, including 13-18% protein, 55 mg niacin per 100 grams, 8.7 mg thiamine per 100 grams, 5 mg riboflavin per 100 grams, 3.5-5.6% ash, 6-15% fiber, and 2-5% fat. Additionally, they are rich in various vitamins and minerals, such as iron and calcium, and are a good source of vitamin D precursors (Marlina et al., 2015). Key secondary metabolites in Shiitake include beta-D-glucans (lentinan) and heteroglycan-protein complexes (LEM), known for their antitumor and immune-boosting effects, eritadenine (cholesterol-lowering), ergosterol (precursor to vitamin D2), and antiviral RNA fragments (Qiu et al., 2022). The chitosan produced by Shiitake mushrooms inhibits hydroxyl radicals and chelates iron ions. Hot water extracts of Shiitake mycelium contain the polysaccharide KS-2, an alpha-mannan peptide with amino acids such as serine, threonine, alanine, and proline. The polysaccharides in Shiitake are primarily responsible for its anticancer activity (Hu et al., 2021). Shiitake mushrooms are particularly used to treat hypertension and reduce blood cholesterol levels (Liu et al., 2022). Other therapeutic effects include antibacterial, antiviral, antifungal, antidiabetic, antioxidant, cholesterol-lowering, cardiovascular protection, and liver protection, attributed to its bioactive compounds (Zhang et al., 2021; Gaitán-Hernández et al., 2011).

Mushroom cultivation not only reduces the environmental impact of agricultural waste used as substrates but also provides a cost-effective alternative for producing high-quality food and valuable metabolites such as enzymes or polysaccharides (Qiu et al., 2022; Royse and Sanchez, 2007). Access to inexpensive and uniform substrates is a key factor in successful Shiitake production. Shiitake mushrooms are native to Japan, China, and other Asian countries with temperate climates and grow naturally on fallen deciduous trees, particularly chestnut, beech, oak, and Shia trees (Parola et al., 2017).

Based on previous studies, no research has simultaneously investigated various substrate combinations, including sawdust from oak, poplar, mulberry, and corn, along with wheat bran, oat seed powder, gypsum, dairy factory wastewater, sugar factory wastewater, and whey powder, on the quantitative and qualitative properties of the edible-medicinal Shiitake mushroom. Therefore, this research aims to identify and select an appropriate substrate for enhancing the quantitative and qualitative properties and to evaluate factors influencing secondary metabolite production in Shiitake mushrooms. The aim of this study was to examine the impact of oak, poplar, mulberry and corn wood sawdust as substrate treatment levels, combined with wheat bran, oat seed powder, gypsum, whey powder, dairy waste water and sugar factory effluent as supplements on the ash content, soluble solids, fiber, carbohydrates and protein properties of the edible-medicinal Shiitake mushroom.

2. Materials and methods

The present study was conducted in 2024 at the mushroom production hall of the Islamic Azad University of Isfahan (Khorasgan). The experimental design was split-plot in the randomized complete block design with three replications. The different substrates, including oak, poplar, mulberry, and corn wood sawdust, were considered as the main factor levels in the main plots, while various supplements—including wheat bran (0% and 25%), oat seed powder (0% and 30%), gypsum ($CaSO_4 \cdot 2H_2O$) (0 and 30 mmol per kilogram), whey powder (0% and 1%), dairy wastewater (0% and 50%), and sugar factory effluent (0% and 50%)—were assigned as sub-factor levels in the subplots (Thi Bitch et al., 2025).

The moisture content of the substrate treatments was adjusted to 55-65% (Royse and Sanchez, 2007). Two thousand grams of substrate were placed into polypropylene bags and sterilized in an autoclave for 1.5 hours at 121°C and 1.5 atmospheres (Thi Bitch et al., 2025). The inoculation of the substrate was carried out at a 3% rate (based on fresh substrate weight) with Shiitake mushroom spawn at 25°C (Baktemur et al., 2022). For the dairy and sugar factory waste water treatments, the substrates were moistened to a 50% concentration (10 liters of fresh water + 10 liters of wastewater from the respective factories). After moistening, the substrates were placed on perforated trays at room temperature allowing excess water to drain completely. Then, 2% gypsum by weight was added. The prepared substrates were placed into polypropylene bags and sterilized again in an autoclave at 121°C and 1.5 atmospheres for 1.5 hours (Kumar et al., 2022). From the first day of cultivation until the completion of the vegetative growth stage, the temperature was maintained at 24 ± 2°C under dark conditions. After full white mycelial colonization of the substrate, the bags were partially opened to induce the browning stage (Atila, 2019). During this stage, the moisture content was adjusted to 85-90%. Once the substrate had browned (the mycelium completely adhered to the substrate, forming a firm mass resembling cracked tree bark), the temperature was lowered to 18 ± 2°C for the fruiting phase, and the plastic covering was fully removed (Thi Bitch et al., 2025). Carbon dioxide levels were reduced to below 1200 ppm during the fruiting stage. The relative humidity was increased to 90% using a misting system, and four 40-watt fluorescent lamps were used for fruiting body development. The lights were on for 16 hours daily until harvest. Harvesting of Shiitake mushrooms was considered when the secondary veil of the basidiocarp ruptured, and the gill edges had not yet deformed, with the gills fully expanded (Atila et al., 2018). The sustainable management of agro-industrial waste is a critical challenge, with solutions ranging from direct biological utilization, such as mushroom cultivation, to thermochemical processes like composting. Wieczorko Barán et al. (2024) demonstrated that composting various agro-wastes produces a stable, nutrient-rich soil amendment, highlighting a key alternative valorization pathway. The ash content, soluble solids, fiber content, carbohydrates, and protein were measured. Ash content was determined by burning Shiitake mushroom samples in a furnace at 550°C (Shams et al., 2022). To determine the soluble solids content, Shiitake mushroom samples were homogenized using a mixer, filtered through paper, and measured using a refractometer calibrated with distilled water. The soluble solids were expressed as a percentage (Brix degree) (Jiang et al., 2012). The crude fiber content was measured by digesting defatted samples with diluted sulfuric acid and sodium hydroxide, followed by filtration using a Buchner funnel (Shams et al., 2022).

The carbohydrate content of the dried mushroom samples was measured according to the formula:

Carbohydrate Content = 100 - (Crude Protein + Crude Fat + Ash + Water) (Hu et al., 2020)

Crude protein content was determined using the Kjeldahl nitrogen analyzer. The dried mushrooms were finely powdered, and 2 grams of each sample were weighed and placed into special tubes. A mixture of 8 grams of catalyst (96% potassium sulfate, 3.5% copper sulfate, and 0.5% selenium oxide) was added to each tube, followed by 20 ml of concentrated sulfuric acid to increase the boiling point. The tubes were heated for 1.5–2 hours until the organic materials were digested, leaving a nearly colorless liquid in the bottom of the flask. After cooling, the liquid was repeatedly washed with 400 ml of distilled water and distilled in a Kjeldahl distillation apparatus with 50 ml of boric acid solution and a few drops of methyl red. Sodium hydroxide (50%) was added until the solution became alkaline. Distillation was continued until all ammonia was captured in the receiving flask. About 200-250 ml of the distilled solution was collected and titrated with normal sulfuric acid. A control sample was also considered, and all procedures were repeated without the mushroom sample to serve as a blank. The nitrogen percentage was measured, and the crude protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25 (Hu et al., 2021). Finally, the data were analyzed using SAS software version 9.2, and means were compared using Least significant difference test at the 5% probability level. Graphs were created using Excel software.

3. Results and discussion

3.1. Analysis of variance

The results of the analysis of variance indicated that the effect of the substrate, treatment, and the interaction between substrate and treatment on the ash content of Shiitake mushrooms were not significant at the 5% probability level (Table 1). The results of the mean comparison showed that the effect of the substrates on the ash content of Shiitake mushrooms was not significant at the 5% level based on LSD test. Similarly, the results of the mean comparison revealed that the effect of the supplements on the ash content of Shiitake mushrooms was not significant at the 5% level in LSD test.

The analysis of variance results showed that the effect of the substrate, treatment, and the interaction between substrate × treatment on the soluble solids content of Shiitake mushrooms was not significant at the 5% probability level (Table 1). The mean comparison results showed that the effect of the substrates on the soluble solids content of Shiitake mushrooms was not significant at the 5% level in LSD test. Likewise, the mean comparison results indicated that the effect of the supplements on the soluble solids content of Shiitake mushrooms was not significant at the 5% level using LSD test. The results of the analysis of variance showed that the effect of the treatment was significant on the fiber content of Shiitake mushrooms at the 1% probability level. However, the effect of the substrate and the interaction between substrate × treatment was not significant at the 5% probability level for this trait (Table 1).

3.2. Mean comparison test

The results of the mean comparison indicated that the effect of the substrates on the fiber content of Shiitake mushrooms was not significant at the 5% level in LSD test (Fig. 1). Furthermore, the results of the mean comparison showed that the effect of the supplements on the fiber content of Shiitake mushrooms was significant at the 5% level in LSD test. The highest fiber content of Shiitake mushrooms (86.93 g /kg) was observed in the oat treatment, while the lowest content was found in the whey, dairy wastewater, control, and gypsum treatments (Fig. 1). The results of the analysis of variance showed that the effect of the treatment was significant on the carbohydrate content of Shiitake mushrooms at the 1% probability level, and the interaction between substrate × treatment was significant at the 5% probability level. However, the effect of the substrate was not significant at the 5% probability level for this trait (Table 1). The results of the mean comparison indicated that the interaction between substrate × treatment was significant at the 5% level in LSD test for the carbohydrate content of Shiitake mushrooms. According to the results, the highest carbohydrate content of Shiitake mushrooms (71.29%) was found in the oak sawdust treatment with wheat bran supplement (Fig. 2). The lowest content (54.25%) was observed in the poplar sawdust treatment with dairy wastewater supplement. No significant differences were observed between some treatments at the 5% level of LSD test (Fig. 3).

Table 1. Results of analysis of variance for the effect of treatments on some chemical properties of shiitake mushroom

Sources of Variation	Degree of Freedom	Average Squares				
		Protein	Carbohydrate	Fiber content	Soluble solids	Ash Contents
Substrate	3	7.825 ^{ns}	62.176 ^{ns}	56.57 ^{ns}	0.3374 ^{ns}	0.7188 ^{ns}
Ea	8	23.384	837.48	33.37	7.6319	12.9642
Treatment	6	**613.121	**282.191	**2777.42	0.8586 ^{ns}	1.8144 ^{ns}
Substrate × Treatment	18	**150.589	*59.236	1.90^{ns}	0.0187 ^{ns}	0.0103 ^{ns}
Eb	48	39.214	26.145	55.87	1.8267	7.4457
Coefficient of Variation (%)		24.11	8.16	12.33	24.07	22.34

ns, * and **: non-significant and significant at 5% and 1% probability levels, respectively

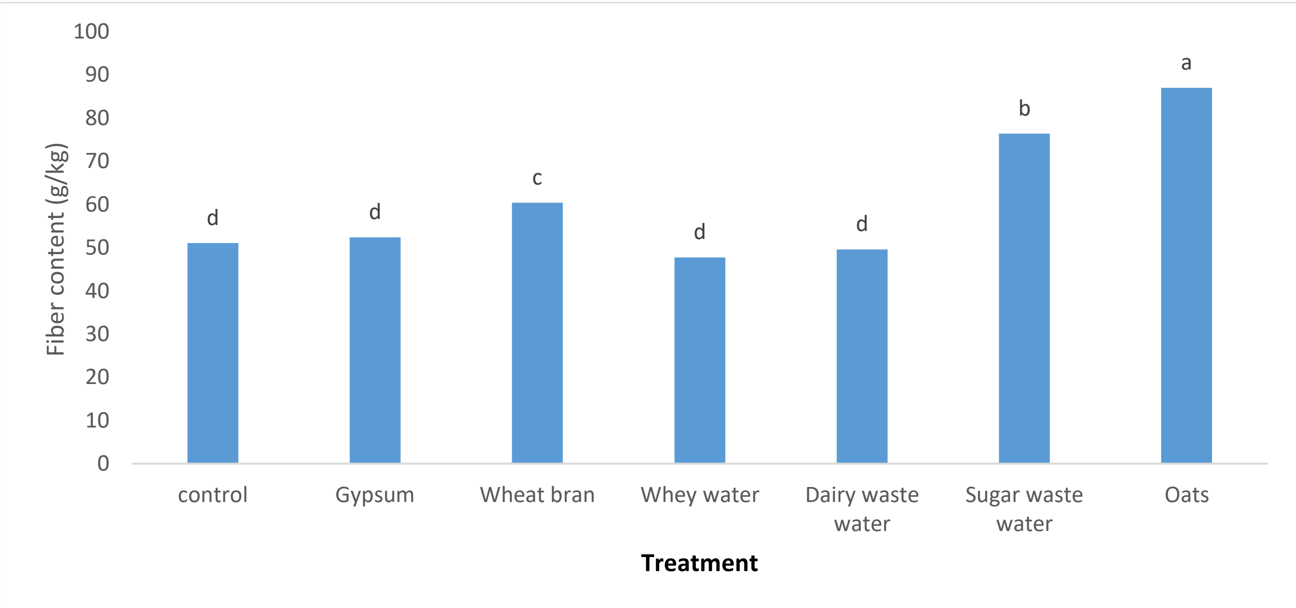

Figure 1. Mean comparison among the treatments for Fiber content.
Means having the same letter(s) don't show significant difference based on LSD test at 5% probability level

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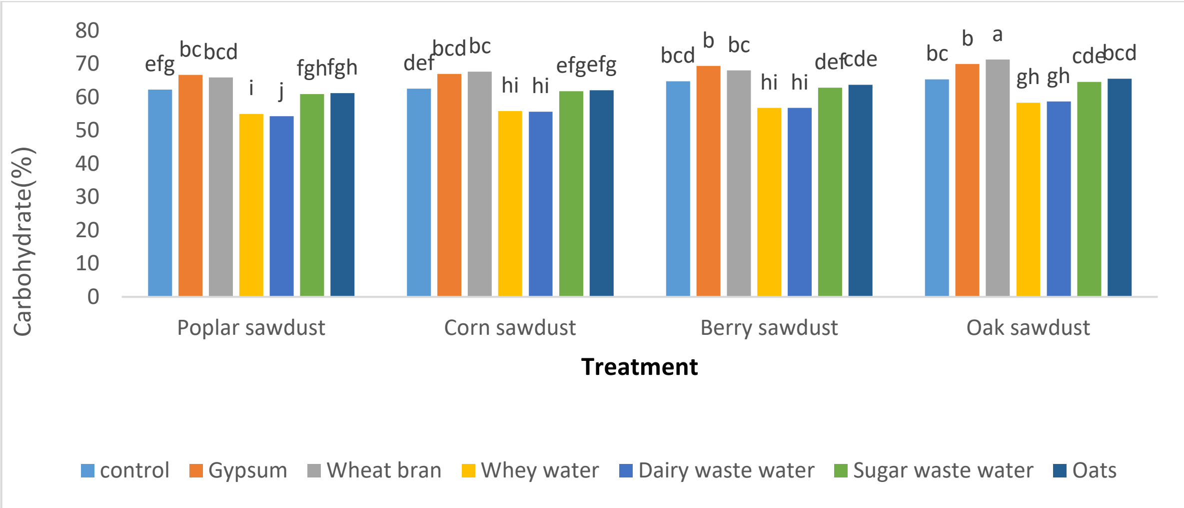

Figure 2. Mean comparison of interaction between the treatments and substrate for Fiber content.
Means having the same letter(s) don't show significant difference based on LSD test at 5% probability level

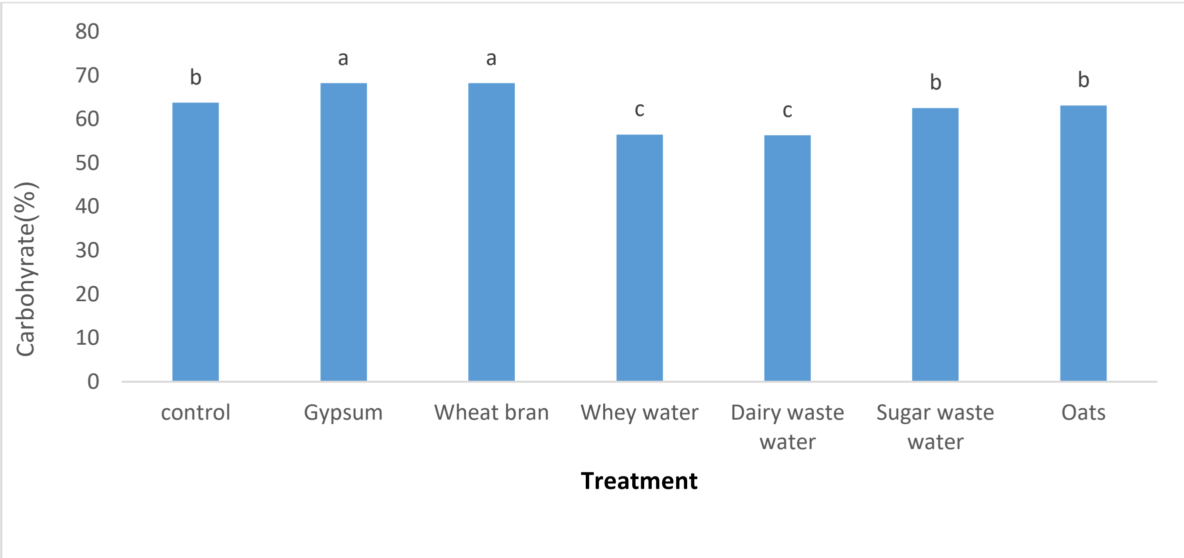

Figure 3. Mean comparison among the treatments for carbohydrate content.
Means having the same letter(s) don't show significant difference based on LSD test at 5% probability level

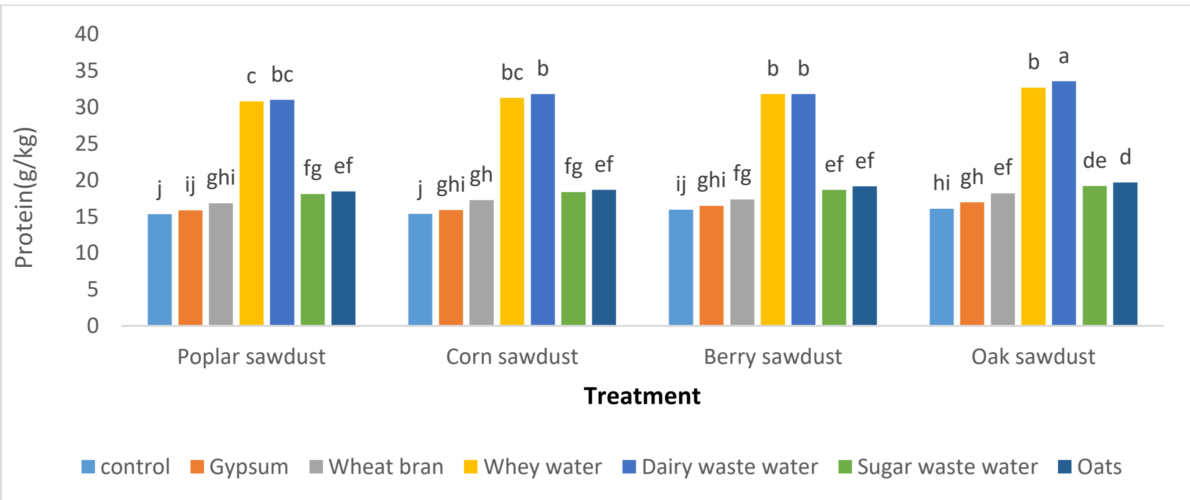

Figure 4. Mean comparison of interaction between the treatments and substrate for protein content.
Means having the same letter(s) don't show significant difference based on LSD test at 5% probability level

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The results of the analysis of variance indicated that the effect of the treatment and the interaction between substrate × treatment were significant on the protein content of Shiitake mushroom fruiting bodies at the 1% probability level. However, the effect of the substrate was not significant at the 5% probability level for this trait (Table 1). The results of the mean comparison showed that the interaction between substrate × treatment was significant at the 5% level in LSD test for the protein content of Shiitake mushrooms. According to the results, the highest protein content of Shiitake mushrooms (33.54 g per kg of dry matter) was found in the oak sawdust treatment with dairy wastewater supplement. The lowest protein content (15.34 and 15.37 g per kg of dry matter) was observed in the poplar sawdust and corn treatments without any supplements, respectively (Fig. 4). No significant statistical differences were observed between some treatments at the 5% level of LSD test (Fig. 5).

The results of this study showed that the highest amount of Shiitake mushroom fiber was obtained in the oat treatment, and the lowest amount was observed in the whey, dairy wastewater, control, and gypsum treatments. Considering the influential role of the culture medium in increasing the quality of the produced mushrooms, the high contents of fiber in Shiitake mushrooms produced in the oat-containing medium can be related to the nature and physical and chemical properties of oats. In addition to the nature of greater degradability, the use of oat supplements increases the rate of decomposition of the lignocellulosic components (lignin, cellulose, and hemicellulose) of the culture medium, which leads to the degradability and humification of organic matter increase, and the carbon to nitrogen ratio is adjusted, making nutrients more available to the mushroom mycelium, which in turn transfers more nutrients to the fruiting body the mycelium transfers more nutrients to the fruiting body (Sarhadi et al., 2017), which can be an important factor in increasing the fiber composition of the fruiting body of Shiitake mushrooms. Previous research on other Pleurotus species supports the use of lignocellulosic waste. A study by Kazemi Jeznabadi et al. (2024) found that locally available wheat straw and sugarcane bagasse were optimal substrates for Pleurotus eryngii, reducing the colonization period and maximizing yield.

It has been shown that gypsum ($CaSO_4 \cdot 2H_2O$) in sawdust culture medium increases mushroom yield. Acidification of the medium to pH = 3.5-4 is essential for the growth of Shiitake mushrooms. In this study, it was found that oxalic acid excreted by Shiitake mushrooms is responsible for the acidification of the medium. Oxalic acid biosynthesis was regulated by the pH of the medium and the buffer capacity of the medium. To acidify the sawdust medium, the total and dissolved oxalate concentrations were 1.51 and 10.8 mmol/kg, respectively. When the dissolved oxalate concentration was 8 mmol/kg, the mycelium growth rate decreased by 29% compared to the control. When the amount of gypsum in the sawdust culture medium was more than 25 mmol/kg, the dissolved oxalate decreased to less than 1 mmol/kg and the mycelium growth rate increased by 32% compared to the control. As a result, gypsum improved the growth and performance of Shiitake mushrooms by reducing oxalate toxicity and facilitating the acidification of the sawdust environment (Li et al., 2022).

The results of this study showed that the highest carbohydrate content of Shiitake mushrooms was obtained in the oak sawdust treatment containing wheat bran supplements. Mushrooms contain relatively high amounts

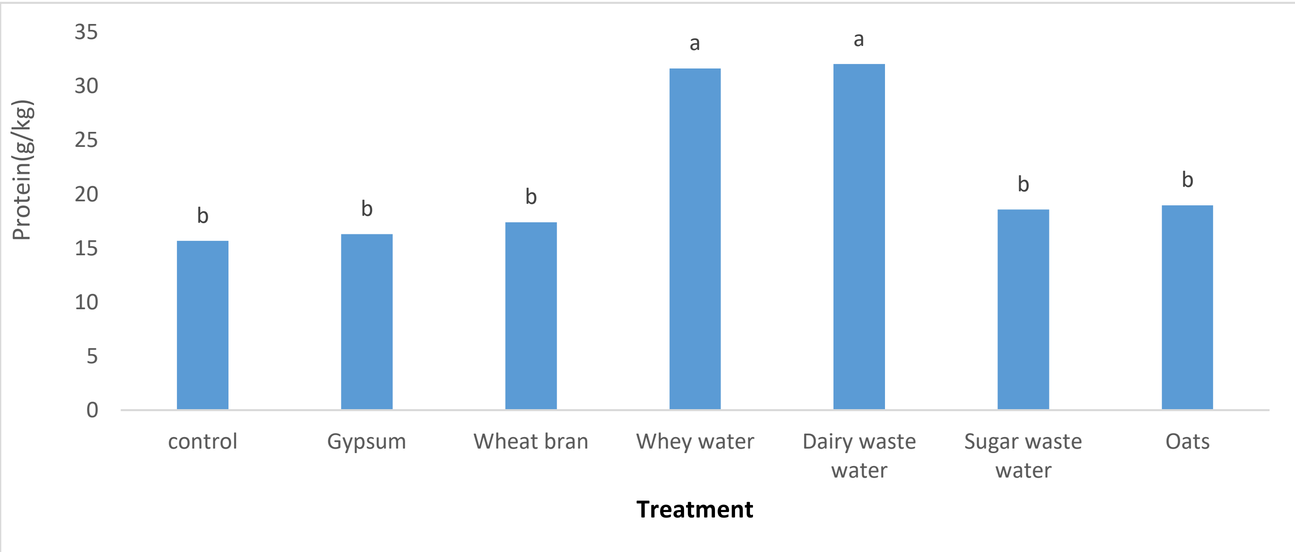

Figure 5. Mean comparison among the treatments for protein content.
Means having the same letter(s) don’t show significant difference based on LSD test at 5% probability level

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of carbohydrates, which have an antioxidant role. Carbohydrates also play an important role in regulating cell osmosis under stress conditions and, as a result, protect the structure of macromolecules and cell membranes (Yao et al., 2012). Cellulose and hemicellulose are the main sources of carbohydrates for Shiitake mushrooms, which, together with lignin surrounding cellulose and hemicellulose and the different ratio of these compounds and substrate nitrogen, can affect Shiitake mushroom growth, yield and quality (compounds constituting the fruiting body of Shiitake mushrooms). The recalcitrant structure of lignocellulosic biomass, primarily due to lignin content, is a key factor influencing its conversion efficiency, whether through biological cultivation methods or thermochemical processes like supercritical water gasification (Safari et al., 2024).

Considering the nature of the mentioned substrates, it seems that oak sawdust substrate enriched with wheat bran supplement has increased the carbohydrate of Shiitake mushroom by increasing the transfer of nutrients from the culture substrate to the fruiting body of the mushroom (Sarhadi et al., 2017). The high level of carbohydrates, amino acids and minerals in rice bran is an important factor in increasing its effect on mushroom efficiency and high carbohydrate content. According to the results of chemical analysis of the culture substrates studied in this study, oak sawdust was one of the effective components in increasing the amount of element zinc in the culture substrate. Mineral elements, including zinc, are an important micronutrient for the metabolism of various cellular macromolecules such as protein and carbohydrates and play an important role in the biosynthesis of carbohydrates (Soliman et al., 2015; Kozarski et al., 2015).

The results of this study showed that the highest protein content of Shiitake mushrooms was obtained in the oak sawdust treatment containing dairy wastewater supplement. Mushrooms have high levels of high-quality protein, the amount of which is also affected by the type of culture medium. The levels of protein in the fruiting bodies of edible-medicinal mushrooms have a direct relationship with the physical and chemical properties and also the carbon to nitrogen ratio of the culture medium. It has been reported that the protein content of mushrooms is strongly affected by the nature of the medium, the amount of nutrients in the culture medium, the mushroom isolate, the stage of development and its age after harvest (Marlina et al., 2015). Also, the presence of high levels of nitrogen in the culture medium will not be a reason for high nitrogen in the mushroom fruiting bodies (Atila et al., 2018). Considering the nitrogen measured in the media, the reason for this is probably the difference in the amount of usable nitrogen, which can affect the quantity and quality of edible mushrooms. The results indicated that nitrogen plays a very important role in the development and growth of Shiitake mushrooms. According to the results obtained regarding the quality of Shiitake mushroom fruiting bodies, the relationship between the presence of nitrogen in the composition of the culture medium and the mentioned characteristics is evident (Gaitán-Hernández et al., 2011; Curvetto et al., 2005).

The results of the analysis of the chemical composition of the culture mediums indicated that the highest amount of nitrogen in the culture medium was obtained in the oak sawdust treatment containing dairy wastewater and whey supplements. Therefore, it seems that the use of dairy wastewater supplement in combination with oak sawdust substrate due to its high nitrogen and protein compounds has increased the protein content of Shiitake mushroom fruiting bodies (Royse et al., 2017). Also, the increase in the protein content of Shiitake mushrooms in the oak sawdust treatment containing dairy wastewater supplement may be due to the provision of a significant number of prerequisite elements for protein production. In fact, the enrichment of the substrate treatment with organic nitrogen compounds increased the vegetative growth of the mushroom mycelium, so that the lignocellulosic components of the substrate treatment were broken down into simpler components and the mushroom mycelium transferred more nutrients from the substrate treatment to the fruiting body (Ramazan et al., 2019; Bellettini et al., 2019).

4. Conclusion

The amounts of nitrogen, protein, and other chemical properties of the mushroom basidiocarp are directly related to the amounts of these compounds in the culture medium. Therefore, in order to achieve maximum growth and yield, it is very important to accurately match the type of mushroom spices with the culture medium. Due to the high carbon to nitrogen ratio of the culture medium (agricultural and forest waste), high lignin content and the lack of complete degradability of cellulosic compounds, the mushroom hyphae cannot fully absorb the nutrients in the culture medium. Hence, mixed substrates and organic supplements (wheat bran, rice bran, barley grain, etc.) are used to adjust the carbon to nitrogen ratio and increase the activity of enzymes that degrade cellulose, hemicellulose and lignin and increase the efficiency of bioconversion. In fact, by choosing the right culture medium and examining the balanced ratio of carbon to nitrogen in the substrate, the desired quantitative and qualitative traits in Shiitake mushrooms can be improved. Based on the present research, the maximum carbohydrate and protein content were obtained in the oak sawdust treatment with wheat bran and dairy wastewater supplementations, respectively.

Authors Contribution

All authors contributed equally to this study. All authors prepared the manuscript.

Availability of data and materials:

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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