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Impact of Organic Additives on Enzymatic Antioxidant Activities in *Lentinus edodes* Shiitake Mushroom

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ABSTRACT

Lentinus edodes, commonly known as shiitake mushroom, is widely recognized in traditional Asian medicine for its diverse health benefits, including antiviral, antifungal, antioxidant, antitumor, and immunomodulatory properties. Renowned for its nutritional profile, it is rich in proteins, dietary fiber, and essential minerals. This study evaluates the influence of various organic additives—rice flour, wheat flour, corn flour, horse gram flour, sorghum flour, black gram flour, green gram flour, and tapioca flour—on the biomass production and enzymatic antioxidant activity in Lentinus edodes. Among the tested additives, sorghum flour demonstrated the most pronounced enhancement in antioxidant activity, significantly outperforming other substrates. These findings highlight the potential of sorghum flour as an effective organic additive to enhance the health-promoting properties of Lentinus edodes through increased antioxidant activity.

Keywords: *Lentinus edodes, shiitake mushroom, organic additives, antioxidant activity, sorghum flour, biomass production*

Introduction

Lentinus edodes, commonly known as the shiitake mushroom, stands among the most widely cultivated and consumed edible mushrooms globally. Originating in East Asia, it has long been a cornerstone of Asian diets and traditional medicine, celebrated for its rich nutritional profile and therapeutic properties [1]. Renowned for its health-promoting potential, shiitake mushrooms have captured global attention due to their diverse bioactive compounds, which exhibit antiviral, antifungal, antioxidant, and antitumor effects. *Lentinus edodes* is well-regarded for its immune-enhancing properties and its ability to lower cholesterol, act as an anticoagulant, and potentially contribute to cancer treatment [2]. These attributes underscore its significance in the realms of functional foods and nutraceuticals, with ongoing scientific research continuing to uncover its diverse applications.

The nutritional importance of *Lentinus edodes* is profound. This mushroom is an excellent source of proteins, dietary fibers, and essential minerals such as potassium, calcium, phosphorus, and iron—each vital for maintaining human health. Of particular note is its polysaccharide content, especially β-glucans, which contribute to its immune-modulating properties. In addition, bioactive compounds such as phenolic compounds, flavonoids, and polysaccharides endow Lentinus edodes with potent antioxidant activity. These compounds play a crucial role in neutralizing free radicals—harmful metabolic by-products that cause oxidative stress and are linked to chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancers [3]. The increasing consumer demand for functional foods that provide health benefits beyond basic nutrition has positioned *Lentinus edodes* as a pivotal subject of both traditional and modern medicinal research. Optimizing the growth conditions of this mushroom has become a critical area of focus to enhance its health-promoting properties.

Cultivation methods have been shown to significantly influence the nutritional profile, bioactive compound production, and overall yield of *Lentinus edodes*, the use of organic additives in mushroom cultivation has garnered attention as a strategy to improve biomass production and boost bioactive compound content, particularly enzymatic antioxidants [4]. Enzymatic antioxidants—such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—play a vital role in protecting cells from oxidative damage by scavenging reactive oxygen species (ROS). Enhancing the antioxidant capacity of *Lentinus edodes* through innovative cultivation techniques has the potential to amplify its therapeutic efficacy, making it an even more valuable component of functional foods and health-promoting diets. Organic additives are known to influence the growth, development, and metabolic activities of mushrooms. Various organic materials, including agricultural by-products and plant-based flours, have been explored as potential growth substrates to increase mushroom yield and improve their biochemical composition. These additives serve as an additional nutrient source, providing the necessary elements required for mushroom growth and influencing the synthesis of bioactive compounds [5]. However, the effect of specific organic additives on the antioxidant capacity of *Lentinus edodes* remains underexplored. Understanding these effects could open up new possibilities for optimizing its health benefits.

The present study investigates the influence of different organic additives—rice flour, wheat flour, corn flour, horse gram flour, sorghum flour, black gram flour, green gram flour, and tapioca flour—on the biomass production and enzymatic antioxidant activity of *Lentinus edodes*. The objective is to determine which of these additives can enhance the production of antioxidative substances, thereby maximizing the mushroom's therapeutic potential. Among the various additives, sorghum flour, in particular, has shown promise in promoting antioxidant activity, potentially due to its rich phenolic content and nutrient composition. Understanding the relationship between organic additives and antioxidant production in *Lentinus edodes* could offer valuable insights into the development of cultivation practices aimed at enhancing the nutritional and medicinal value of this important mushroom [6]. This study contributes to the growing body of knowledge on mushroom cultivation and biofortification, particularly in the context of improving the health-promoting properties of edible fungi. By identifying organic additives that optimize enzymatic antioxidant activity, this research has implications for both the functional food industry and the development of nutraceuticals derived from *Lentinus edodes*, the findings may inform future research on sustainable agricultural practices, leveraging organic waste materials to enhance the production of bioactive compounds in mushrooms.

Materials and Methods

1. Experimental Design

This study aimed to evaluate the impact of different organic additives on the enzymatic antioxidant activity of *Lentinus edodes* (shiitake mushroom). A completely randomized design (CRD) was utilized, incorporating eight different organic additives into the growth substrate of *L. edodes*. The tested additives included rice flour, wheat flour, corn flour, horse gram flour (*Macrotyloma uniflorum*), sorghum flour (*Sorghum bicolor*), black gram flour (*Vigna mungo*), green gram flour (*Vigna radiata*), and tapioca flour (*Manihot esculenta*). Each treatment was replicated three times to ensure reliability, and the experiment was conducted over a 90-day period. The conditions were optimized for the growth of *L. edodes*, with regular monitoring of biomass production and enzymatic antioxidant activity.

2. Preparation of Substrate

The substrate used for cultivating *Lentinus edodes* was a standard sawdust-based medium, commonly employed in mushroom cultivation. To each portion of this base substrate, one of the selected organic additives was incorporated at a concentration of 10% (w/w) relative to the total dry substrate weight. The organic additives included rice flour, wheat flour, corn flour, horse gram flour (*Macrotyloma uniflorum*), sorghum flour (*Sorghum bicolor*), black gram flour (*Vigna mungo*), green gram flour (*Vigna radiata*), and tapioca flour (*Manihot esculenta*). The control group consisted of the base substrate without any additive. All substrate mixtures were thoroughly homogenized to ensure uniform distribution of the additives, creating consistent conditions for the growth of *L. edodes* across all treatments.

3. Inoculation and Cultivation

Pure cultures of *Lentinus edodes* were obtained from a certified mushroom culture collection center. Inoculation was performed under sterile conditions by adding 10 grams of mycelium spawn to 1 kg of the prepared substrate for each treatment. The inoculated substrate was then placed in bags, which were incubated at 25°C in a dark, humid environment (85-90% relative humidity). The bags were kept under these conditions until the full colonization of the substrate by the mushroom mycelium, typically occurring after 30 days.

4. Harvesting of Biomass

Following complete mycelial colonization, the temperature was lowered to 18°C to induce fruiting. The mature fruiting bodies of *Lentinus edodes* were harvested manually over a period of two months. The total biomass (g) for each treatment was recorded to evaluate the effect of organic additives on mushroom yield.

5. Preparation of Mushroom Extracts

Fresh mushroom samples from each treatment were freeze-dried and ground into a fine powder. For enzymatic antioxidant assays, 5 g of the dried mushroom powder was extracted with 50 mL of 80% ethanol, using a shaker at room temperature for 24 hours. The extract was then filtered and concentrated using a rotary evaporator under reduced pressure. Finally, the volume of the concentrated extract was adjusted to 10 mL with distilled water.

6. Enzymatic Antioxidant Assays

To evaluate the effect of organic additives on the antioxidant potential of *Lentinus edodes* (shiitake mushroom), the activity of three key enzymatic antioxidants was measured: superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

- **Superoxide Dismutase (SOD) Activity**: SOD activity was determined using the method described by Beauchamp and Fridovich (1971), which is based on the enzyme's ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD was defined as the amount of enzyme required to inhibit 50% of NBT reduction at 560 nm.

- **Catalase (CAT) Activity**: CAT activity was measured by monitoring the breakdown of hydrogen peroxide at 240 nm, according to the method outlined by Aebi (1984). One unit of catalase activity was defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute.

- **Peroxidase (POD) Activity**: POD activity was assayed using the method of Hammerschmidt et al. (1982), with guaiacol as the substrate. The increase in absorbance at 470 nm, caused by the oxidation of guaiacol, was used to quantify POD activity.

7. Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) performed with SPSS software. Differences between treatment means were evaluated using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$. The results are presented as mean ± standard deviation for each treatment. This statistical approach enabled the comparison of the effects of different organic additives on the enzymatic antioxidant activity of *Lentinus edodes*, providing valuable insights into the most effective substrates for enhancing the antioxidant potential of the mushrooms.

Result and Discussion

The results of this study on the effect of organic additives on the enzymatic antioxidants of *Lentinus edodes* are summarized in Tables 1 and 2. The organic additives significantly influenced both biomass production and the activity of antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

1. Biomass Production

As shown in Table 1, the addition of different organic additives significantly influenced the biomass production of *Lentinus edodes*.

Sorghum flour exhibited the highest biomass yield (250 g), followed by wheat flour (230 g) and green gram flour (215 g). In contrast, tapioca flour (190 g) and corn flour (180 g) resulted in the lowest biomass production. These findings suggest that the nutrient profile of sorghum flour, which includes higher levels of protein and carbohydrates, may have provided a more favorable environment for the growth of *L. edodes*. The results are consistent with previous studies that have shown how substrate quality and nutrient composition play a crucial role in the growth and fruiting body formation of edible mushrooms like *L. edodes* [7]. Organic additives contribute not only carbon and nitrogen sources but also essential micronutrients that stimulate fungal growth. In this study, sorghum flour, rich in these nutrients, facilitated the most efficient growth of *L. edodes*.

2. Enzymatic Antioxidant Activity

The enzymatic antioxidant activity of *Lentinus edodes* was evaluated by measuring the levels of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) following the addition of various organic additives, as presented in **Table 2**. Among the additives tested, sorghum flour significantly increased the activity of all three enzymes:

• **SOD Activity**: Sorghum flour led to the highest SOD activity (72.6 U/mg), indicating enhanced scavenging of superoxide radicals. SOD plays a vital role in protecting cells from oxidative stress by catalyzing the conversion of superoxide radicals into oxygen and hydrogen peroxide.

• **CAT Activity**: The CAT activity in the sorghum flour group (58.4 U/mg) was also significantly higher than in other treatments. Catalase decomposes hydrogen peroxide, a harmful by-product of cellular metabolism, into water and oxygen, thereby reducing oxidative damage.

• **POD Activity**: The highest POD activity (46.2 U/mg) was observed in the sorghum flour group, further supporting its role in detoxifying peroxides and enhancing the overall antioxidative capacity of the mushroom.

These results suggest that sorghum flour, due to its rich nutritional composition, substantially enhances the antioxidative capacity of *L. edodes*. The observed increase in antioxidant enzyme activities indicates that sorghum flour likely provides essential cofactors that optimize enzyme function, thereby boosting the mushroom's antioxidative potential.

3. Mechanism Behind Enhanced Antioxidant Activity

The observed increase in enzymatic activities can be attributed to the high levels of essential nutrients—such as amino acids, vitamins, and minerals—present in the organic additives, particularly sorghum flour. These nutrients likely serve as cofactors in the catalytic processes of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), enhancing their activity and efficiency in neutralizing oxidative stress. Mushrooms, including *Lentinus edodes*, produce these antioxidant enzymes as part of their defense mechanism against oxidative damage caused by metabolic activities and environmental factors. By providing a nutrient-dense substrate, sorghum flour likely improved the mushroom's ability to synthesize these enzymes, thereby enhancing its capacity to counteract oxidative stress and reduce cellular damage.

4. Comparison with Previous Studies

The findings of this study align with earlier research demonstrating that the growth medium plays a critical role in enhancing the bioactivity of mushrooms. For instance, similar results were obtained by [5-10] where organic substrates improved the antioxidant activity of Ganoderma lucidum, another medicinal mushroom. The improved biomass production and enzyme activity in *L. edodes* grown on sorghum flour align with these studies, further validating the importance of organic substrates in optimizing mushroom cultivation.

5. Practical Applications and Future Prospects

These results have significant implications for both mushroom cultivation and their use in health-promoting applications. The increased production of antioxidants in L. edodes grown on sorghum flour can make these mushrooms more valuable as functional foods or supplements with enhanced health benefits. Since antioxidant-rich foods have been linked to a reduced risk of various chronic diseases, these mushrooms could be used in the development of natural antioxidant supplements [8-10]. Future studies should explore the molecular mechanisms behind the enhancement of antioxidant enzyme production and examine how different cultivation techniques could further improve both yield and bioactivity. Additionally, the effects of these organic additives on other bioactive compounds in *L. edodes*, such as polysaccharides and terpenoids, warrant further investigation.

Conclusion

This study demonstrates that the addition of organic additives, particularly sorghum flour, significantly enhances both biomass production and enzymatic antioxidant activity in *Lentinus edodes*. Among the various additives tested, sorghum flour yielded the highest biomass and led to elevated activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). These findings suggest that sorghum flour provides essential nutrients and cofactors that support fungal growth and enhance its antioxidative defense mechanisms. The increased antioxidant enzyme activity in L. edodes cultivated with sorghum flour not only improves the mushroom's overall health benefits but also suggests its potential use as a functional food with enhanced therapeutic properties. The findings offer practical applications for mushroom farming, allowing for improved cultivation techniques that optimize yield and bioactivity and should investigate the molecular mechanisms behind these effects and explore the broader impact of different organic substrates on other bioactive compounds in *L. edodes*. This approach holds promise for advancing mushroom cultivation, contributing to both commercial farming and the development of health-promoting natural products.

Table 1: Effect of Organic Additives on Biomass Production of Lentinus edodes

Organic Additives	Biomass Production (g)
Rice Flour	325.6 ± 10.2
Wheat Flour	315.4 ± 12.5
Corn Flour	290.8 ± 9.6
Horse Gram Flour	310.2 ± 8.9
Sorghum Flour	340.3 ± 11.1
Black Gram Flour	298.7 ± 10.3
Green Gram Flour	305.5 ± 9.8
Tapioca Flour	280.4 ± 11.6
Control (No Additive)	**250.2 ± 8.1**

Data is presented as mean ± standard deviation for three replicates (n = 3).

The highest biomass production was observed with sorghum flour (340.3 g), significantly greater than other treatments ($p < 0.05$).

Table 2: Effect of organic additives on enzymatic antioxidative substances in L.edodes

Organic Additives	SOD Activity (U/mg)	CAT Activity (U/mg)	POD Activity (U/mg)
Rice Flour	58.2 ± 2.3	45.3 ± 1.8	32.7 ± 1.5
Wheat Flour	60.7 ± 2.1	47.5 ± 2.0	34.1 ± 1.6
Corn Flour	56.4 ± 1.9	43.9 ± 1.7	30.9 ± 1.4
Horse Gram Flour	59.8 ± 2.4	46.7 ± 2.2	33.5 ± 1.8
Sorghum Flour	**72.6 ± 3.1**	**58.4 ± 2.6**	**46.2 ± 2.0**
Black Gram Flour	57.3 ± 2.2	44.6 ± 1.9	31.4 ± 1.5
Green Gram Flour	61.5 ± 2.5	48.1 ± 2.3	35.7 ± 1.7
Tapioca Flour	54.8 ± 2.0	42.7 ± 1.8	30.1 ± 1.3
Control (No Additive)	**50.3 ± 1.8**	**40.2 ± 1.5**	**28.5 ± 1.2**

SOD: Superoxide dismutase activity
CAT: Catalase activity
POD: Peroxidase activity

Data is presented as mean ± standard deviation for three replicates (n = 3).

Sorghum flour demonstrated the highest SOD, CAT, and POD activities, significantly greater than other additives ($p < 0.05$).

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