Journal of Tropical Biodiversity and Biotechnology
Volume 11, Issue 03 (2026): jtbb19888
DOI: 10.22146/jtbb.19888

Short Communication

Assessment of Phenolic Content and Antioxidative Properties of Wheat Fermented with S*chizophyllum commune* Mushroom

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Keywords:
Antioxidant
Fermentation
Mushroom
Phenolic
Schizophyllum commune
Wheat
Submitted:
12 February 2025
Accepted:
13 October 2025
Published:
13 July 2026
Editors:
Miftahul Ilmi
Annisaa Widyasari

ABSTRACT
Schizophyllum commune, a tropical mushroom with health-promoting properties, has limited exploitation for high-value products. Realising the potential of fermentation to transform wheat into a value-added product, this study optimised wheat fermentation with *S. commune* to boost phenolic production and antioxidative activity. Results showed that 30-day fermentation produced the highest phenolic content and antioxidative properties, significantly increasing the FRAP value from 0.181 mg AAE g^{-1} in non-fermented wheat to 2.207 mg AAE g^{-1} and boosting the DPPH value from 6.28 % to 81.63 %. *S. commune*-fermented wheat shows potential to be explored as a sustainable health-promoting food product.

How to cite:
Abd Razak, D.L., Khulidin, K.A. & Ismail, A., 2026. Assessment of Phenolic Content and Antioxidative Properties of Wheat Fermented with *Schizophyllum commune* Mushroom. *Journal of Tropical Biodiversity and Biotechnology*, 11 (3), jtbb19888. doi: 10.22146/jtbb.19888

Mushrooms have long been harvested and utilised in nutraceuticals and dietary supplements because of their bioactive components. Traditional methods for producing the fruitbodies of the mushroom may take several months and involve extensive labour. On the other hand, mushroom mycelia production takes less time, and mushroom mycelia are easier for humans to digest due to their thin and permeable cell walls that permit the passage of enzymes and the absorption of nutrients. Recently, there has been a lot of interest in mushroom-based functional foods, which presents a clear scientific impression of the rising demand for mushroom fruitbodies and mycelia. Additionally, the potential for industrial-scale implementation and the substantial health benefits of mushrooms mycelia make them an auspicious and sustainable alternative to traditional protein sources (Guo et al. 2025). *Schizophyllum commune*, known as split gill mushroom (Figure 1a), is a worldwide distributed wood-degrading basidiomycete regarded as an important edible and medicinal mushroom especially in tropical Asia such as Indonesia, Thailand and Malaysia as well as in East Asia countries such as Korea. Due to its edible and therapeutic characteristics, as well as its widespread dissemination, *S. commune* is deemed a global source of food and medicine. Despite its expanding popularity, research on *S. commune* is still limited, especially regarding its utilisation as a potential source for the development of health-promoting products. One of the important and sought-after health-promoting properties in food products is their antioxidative capacity, as antioxidants, including the phenolic and/or flavonoid compounds found in plants and mushrooms are molecules with radical scavenging capacity intended to counteract free radical damage (Rashed & Butnariu 2014). This property is crucial for maintaining overall health.

The fermentation process has been used to enhance the nutritional quality, bioavailability as well as biological properties of many types of grains including wheat (Nkhata et al. 2018). Solid-state fermentation (SSF) of wheat by many mushroom species is currently used for spawn production in mushroom cultivation. At present, other potential uses of fermented wheat are scarce. Due to its enhanced nutritional and biological properties, fermented wheat, including the encompassing mushroom mycelia, has a significant advantage for development as a new health-promoting food product. Consuming mycelial biomass delivers all the benefits of the mycelium, its extracellular chemicals that are diffused into the substrate, and the fermented substrate itself. Studies have been conducted on the solid-state fermentation of grains with mushrooms (Subramaniam et al. 2014; Benson et al. 2019; Clark et al. 2022) which exhibits promising potential and warrants more research, especially on the underutilised mushroom species. Considering these factors, the exploration of *S. commune*-fermented wheat as a potential sustainable food product is currently being undertaken. In conjunction with this effort, the current study was carried out to determine the optimal length of fermentation time to obtain optimum phenolic content and antioxidative activities of fermented wheat. To the best of our knowledge, this is the first report on the phenolic content and antioxidative activities of wheat fermented with *S. commune* for up to 30 days of fermentation.

Schizophyllum commune was obtained from the Spawn Production Laboratory, MARDI Headquarters, Malaysia. The wheat grains were washed several times, soaked in hot boiling water for 1 hour, and left to cool before being packed into a polypropylene plastic bag (30 cm x 10 cm x 10 cm). The wheat grain bags were weighed at about 200 grams each and then autoclaved for 21 mins at 121 °C (Hirayama, Japan). The sterilized wheat grain was inoculated with 5 mL of 7-day-old *S. commune* mycelial liquid culture using a sterile pipettor under aseptic conditions and incubated for 30 days at 28 ± 2 °C (Memmert, Germany) in the dark. Non-fermented and fermented samples

(Figure 1b) were dried to constant weight in an oven at 40 °C and then finely ground into powder form. Extraction was carried out using distilled water (1:10 w v^{-1}) at 37 ± 2 °C for 1 hour in a water bath, followed by centrifugation to obtain a clear solution of crude extract. Determination of total phenolic content (TPC), expressed as mg gallic acid equivalent (mg GAE g^{-1} sample), total flavonoid content (TFC), expressed as μg quercetin equivalent (μg QE g^{-1} sample), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (expressed as %) and ferric-reducing antioxidant power (FRAP), expressed as mg ascorbic acid equivalent (mg AAE g^{-1} sample) were performed with reference to a previously described methods (Abd Razak et al. 2019). Data obtained from triplicate samples were used to calculate mean values and standard deviations. One-way analysis of variance (ANOVA) was conducted with Tukey's test at a significance level of $p < 0.05$ using Minitab Statistical Software (version 14). Correlation and regression tests were conducted using Microsoft Excel. Data are represented as mean value ± standard deviation.

As shown in Table 1 and Figure 2, the extracts of fermented wheat showed no significant difference in TPC, TFC, FRAP, and DPPH compared to non-fermented wheat for the first two days of fermentation, but a gradual increment with some fluctuations were observed from day 6 onwards. Overall, the highest TPC, TFC, and antioxidative activities were observed towards the end of fermentation period, notably from day 26 to day 30. During fermentation, microbial proteolytic enzymes hydrolise phenolic complexes, converting them into simpler, more soluble, and biologically active forms that are easily absorbed (Haile & Kang 2019). This process, coupled with the structural breakdown of plant cell walls, enhances the extraction of bioactive compounds. Furthermore, enzymes like β-glucosidase, α-amylase, and lactase improve the overall phenolic profile (Salar et al. 2017).

(a)

(b)

Figure 1. (a) *Schizophyllum commune* mushroom; (b) cross-section of *S. commune*-fermented wheat.

In this study, wheat fermented for 30 days produced an almost 15-fold increment of total phenolic content, with a value of 5.694 ± 0.188 mg GAE g^{-1} sample. For TFC, the highest amount (255.98 ± 3.07 µg QE g^{-1} sample) was observed on day 26. However, similar to TPC, no significant difference was observed between TPC and TFC from day 26 to day 30.

Wheat fermentation significantly improves its antioxidative properties, as demonstrated by studies using *Ganoderma* spp. (Subramaniam et al. 2014), *Taiwanofungus salmoneus* (Chiang et al. 2015), *Agaricus blazei* (Zhai et al. 2015), and *S. commune* (Boonthatui et al. 2021). This enhancement is caused by biochemical changes during the fermentation process, which facilitate the release or modification of antioxidant compounds, such as phenolics, making them more bioavailable. Compared to the start of fermentation, the enhancement in DPPH scavenging activity of *S. commune*-fermented wheat was up to 13-fold, with the highest activity (81.63 ± 0.72 %) obtained on the 30th day of fermentation. The highest ferric-reducing activity was also observed on day 30, with a value of 2.207 ± 0.194 mg AAE g^{-1} sample, which significantly differed from the amount observed on earlier fermentation times. This study demonstrated a 12-fold increase in the FRAP value of wheat after 30 days of *S. commune* fermentation. These results are consistent with (Boonthatui et al. 2021), whose research noted an improvement of up to 19-fold in the ferric-reducing capacity of wheat following *S. commune* fermentation.

Contrary to the findings of this present study, a previous study (Boonthatui et al. 2021) observed a steady increase in TPC, TFC, FRAP and DPPH of the *S. commune*-fermented wheat water extract. It is important to note the difference in mushroom strain, form, and quantity of the starter culture, fermentation time, drying method, and extraction procedures used in these two studies. This study provides a more detailed time-course analysis (up to 30 days) that captures the full profile, including the initial lag phase, which might be missed in shorter or less frequent sampling. This offers more refined insights into the optimal fermentation duration for *S. commune* on wheat.

Wheat fermented with *Agaricus blazei*, also known as almond mushroom, showed the highest TPC, protein content, reducing sugar content, and antioxidative properties on the 30th day of fermentation (Zhai et al. 2015). Similar observations were reported by Xu et al. (2018) for wheat fermented with three types of mushrooms: *Agaricus bisporus*, *Helvella lacunose*, and *Fomitiporia yanbeiensis*. A previous study on the fermentation of wheat with *Aspergillus oryzae* (Joginder et al. 2016) showed no significant changes in TPC and antioxidant activities of the methanol extract from day 3 to day 6 of fermentation. On the contrary, results from the same study showed increased TPC in the ethanol extract up to day 5, but decreased on day 6 of fermentation. Differences in TPC between wheat cultivars and fluctuations in TPC were also observed during the fermentation of wheat (Sandhu et al. 2017). Other studies found that phenolic content and antioxidative properties may vary noticeably between species and even between strains of the same species. Moreover, it may vary according to the variety of wheat grains and extracting solvent (Bhanja Dey & Kuhad 2014). Throughout fermentation, the modification of phenolic content can be attributed to many factors such as breakdown of the cereal cell wall, activities of enzymes, synthesis and liberation of new compounds, as well as binding of compounds with other constituents (Adebo & Medina-Meza 2020).

As predicted, the phenolic content was significantly correlated with FRAP ($r = 0.93$, $p < 0.05$) and DPPH ($r = 0.95$, $p < 0.05$). Total flavonoid content was also significantly correlated with FRAP ($r = 0.92$, $p < 0.05$) and DPPH ($r = 0.95$, $p < 0.05$) (data not shown). Most phenolic compounds in cereals are bound to cell wall polysaccharides and are more readily extractable

Table 1. Total phenolic and total flavonoid content of water extracts from non-fermented and *S. commune*-fermented wheat.

Sample[1]	Total Phenolic Content (TPC) (mg GAE g^{-1} sample)[2]	Total Flavonoid Content (TFC) (µg QE g^{-1} sample)[2]
NFW	0.388 ± 0.051^{i}	57.19 ± 6.2^{j}
FW Day 0	0.456 ± 0.001^{hi}	62.22 ± 1.42^{ij}
FW Day 2	0.460 ± 0.001^{hi}	63.83 ± 11.18^{ij}
FW Day 4	1.045 ± 0.023^{gh}	86.12 ± 3.33^{hij}
FW Day 6	1.429 ± 0.322^{g}	90.7 ± 24.03^{hi}
FW Day 8	2.185 ± 0.082^{f}	105.83 ± 13.97^{h}
FW Day 10	2.506 ± 0.196^{ef}	117.61 ± 19.51^{fgh}
FW Day 12	2.702 ± 0.308^{ef}	115.8 ± 30.4^{gh}
FW Day 14	3.091 ± 0.003^{e}	142.29 ± 8.61^{efg}
FW Day 16	4.211 ± 0.448^{d}	150.2 ± 33.6^{ef}
FW Day 18	4.292 ± 0.461^{d}	167.04 ± 12.68^{de}
FW Day 20	4.614 ± 0.355^{cd}	201.68 ± 8.68^{bc}
FW Day 22	5.039 ± 0.142^{bc}	208.93 ± 6.25^{bc}
FW Day 24	4.716 ± 0.712^{cd}	191.4 ± 25.2^{cd}
FW Day 26	5.651 ± 0.217^{ab}	255.98 ± 3.07^{a}
FW Day 28	5.678 ± 0.349^{a}	255.21 ± 5.72^{a}
FW Day 30	5.694 ± 0.188^{a}	231.72 ± 6.59^{ab}

[1]NFW: non-fermented wheat; FW: *S. commune*-fermented wheat. [2]Results expressed as mean ± standard deviation. Means that do not share a letter are significantly different ($p < 0.05$).

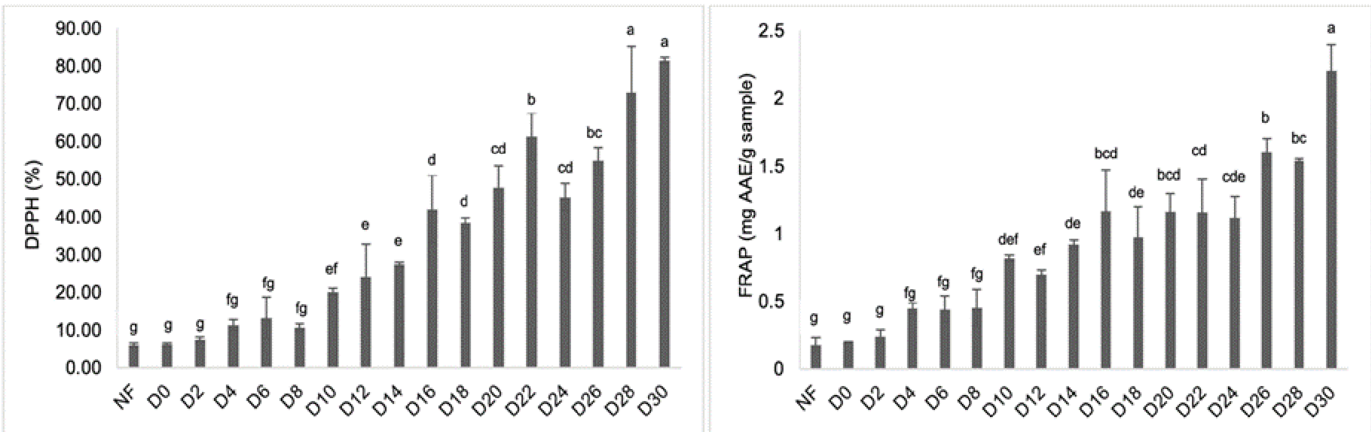

Figure 2. Antioxidative properties of wheat fermented with *S. commune*. DPPH radical scavenging activity (left, expressed as a scavenging percentage) and ferric-reducing antioxidant activity (right, expressed as milligrams ascorbic acid equivalent per gram sample) of non-fermented wheat (NF) and wheat fermented with *S. commune* from day 0 (D0) until day 30 (D30). Results are expressed as mean ± standard deviation. Means that do not share a letter are significantly different ($p < 0.05$).

through acid or alkaline hydrolysis (Sani et al. 2012). This may explain the results of the present study, where TPC was lower in the earlier stages of fermentation. The higher TPC was due to a higher content of free phenolics which were extractable by water.

The findings of this current study are based on the tested parameters where a more comprehensive optimisation across a wider range of fermentation conditions was not explicitly explored. Further optimization of the fermentation conditions, such as moisture content, concentration of the starter culture, and substrate particle size, could potentially yield better results. Additionally, while increased phenolic content and antioxidative properties were observed in this study, the specific types and profiles of these phenolic compounds were not characterized. Also, the antioxidative properties reported may be due to other antioxidant compounds besides phenolics, such as polysaccharides, tocopherols, and vitamins. The content of these compounds was not investigated in the current study.

In conclusion, through SSF, *S. commune* successfully transformed the biological properties of wheat, resulting in a substantial increase in antioxidative compounds. A fermentation period of 30 days was identified as optimal, yielding the highest phenolic content and antioxidative properties in the fermented wheat. These findings underscored the health-promoting value of *S. commune*-fermented wheat and highlight its considerable potential as a sustainable, readily available, and economical health-promoting food product. Future research should focus on elucidating its complete nutritional and bioactive profiles, as well as the mechanisms underlying its health benefits. Aligning with the global push for sustainable food production, this study demonstrates that SSF using mushroom mycelia, is an environmentally conscious approach for converting raw agricultural materials such as wheat into functional foods.

AUTHOR CONTRIBUTION

D.L.A.R designed the study, collected and analysed the data and wrote the manuscript, K.A.K prepared the research material and wrote the manuscript, A.I. supervised all the processes and edited the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors wish to thank Nur Kartinee Kassim from Universiti Putra Malaysia (UPM) and Norliza Abu Bakar from the Malaysian Agricultural Research and Development Institute (MARDI) for their support.

CONFLICT OF INTEREST

The authors declare that they have no known conflict of interest relating to the study depicted in this manuscript.

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