

Comparative Evaluation of Solvent-Dependent Antioxidant Extraction from *Agaricus bisporus* (J.E. Lange) Imbach Using a Cold Extraction Approach

Dr. Krishna Kumar Verma

SOS Microbiology, Shaheed Mahendra Karma Vishwavidyalaya, Bastar (C.G.), India - 494001

Abstract

The search for food-compatible and sustainable sources of natural antioxidants has increased interest in edible mushrooms because they contain phenolic compounds, ergothioneine, vitamins, minerals and other bioactive constituents. The present study evaluated the effect of solvent polarity and extraction duration on the total antioxidant capacity (TAC) of the cultivated white button mushroom, *Agaricus bisporus* (J.E. Lange) Imbach. Fresh fruiting bodies (400 g) were obtained from a local market in Bilaspur, Chhattisgarh, India, dried, powdered and extracted by cold maceration using methanol, distilled water and chloroform. Extracts were collected after 24, 48 and 72 h and evaluated by the phosphomolybdate total antioxidant capacity assay, using ascorbic acid as the reference standard. The recorded absorbance values showed a clear solvent- and time-dependent pattern. Methanolic extract showed the highest reported TAC at 24 h (43.09 µg ascorbic-acid-equivalent per assay aliquot) and 48 h (42.09 µg), whereas the aqueous extract increased progressively from 27.63 µg at 24 h to 38.90 µg at 48 h and 42.45 µg at 72 h. Chloroform showed very low activity, ranging from 0.09 to 7.45 µg per assay aliquot. These findings indicate that polar extraction systems are more suitable than chloroform for recovering antioxidant-active constituents from *A. bisporus* under the tested conditions, while water becomes increasingly effective with prolonged extraction. The principal importance of the study is its simple comparison of solvent polarity and extraction time using a low-complexity cold-extraction workflow that can be reproduced in basic food-microbiology or biotechnology laboratories. However, the study should not interpret methanol or chloroform as green solvents; water is the environmentally preferable option among the solvents tested. The work supports further optimization of aqueous or food-compatible hydroalcoholic extraction and subsequent chemical characterization of the active compounds.

Keywords: *Agaricus bisporus*; white button mushroom; total antioxidant capacity; phosphomolybdate assay; cold extraction; aqueous extraction; natural antioxidants; sustainable extraction.

1. Introduction

Oxidative stress results when the generation of reactive oxygen and nitrogen species exceeds the capacity of biological antioxidant systems to maintain redox balance. Reactive species can oxidize lipids, proteins and nucleic acids and are associated with a broad range of biological processes and

chronic disease mechanisms. For this reason, antioxidant-rich foods and food-derived bioactive compounds continue to attract attention in food science, biotechnology and nutritional research. Antioxidants can act through several mechanisms, including electron or hydrogen donation, metal chelation and interruption of radical chain reactions. However, antioxidant activity measured in vitro should not automatically be interpreted as evidence of clinical efficacy in humans.

The demand for natural antioxidant sources has increased because food manufacturers and consumers are interested in replacing or reducing dependence on synthetic antioxidant systems. Edible mushrooms are particularly interesting because they combine nutritional value with a diverse chemical profile. Recent reviews describe mushrooms as sources of phenolic and hydroxycinnamic acids, flavonoids, β -glucans, vitamins, sterols, terpenoid compounds and ergothioneine, with antioxidant and other bioactivities reported across multiple species (Singh et al., 2025; Hamza et al., 2024). Ergothioneine is especially relevant because edible fungi are important dietary sources of this sulfur-containing antioxidant, although its physiological role should be interpreted carefully because strong in-vitro antioxidant activity does not by itself establish that antioxidant action is its principal function in vivo (Halliwell et al., 2023; Yang et al., 2025).

Among cultivated edible mushrooms, the white button mushroom is taxonomically accepted as *Agaricus bisporus* (J.E. Lange) Imbach. The current accepted authorship is supported by taxonomic databases, which trace the name to the recombination by Imbach in 1946. The species is widely cultivated and consumed and has been investigated for nutritional composition, phenolic compounds, ergothioneine and antioxidant activity (NCBI Taxonomy, 2026; Species Fungorum, 2026). Earlier work demonstrated antioxidant activity in *A. bisporus* extracts and reported phenolic constituents such as gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin. These observations provide a biochemical rationale for studying solvent-dependent recovery of antioxidant-active constituents.

A. bisporus was selected for the present work for four practical and scientific reasons. First, it is a commonly cultivated edible mushroom, making the biological material readily accessible and reducing problems associated with seasonal collection of wild fungi. Second, its nutritional and bioactive composition is relatively well documented, allowing the present findings to be compared with previous studies. Third, the mushroom contains both polar and moderately extractable antioxidant-related constituents, making it suitable for examining the influence of solvent polarity. Fourth, its established food use provides a realistic context for evaluating extraction systems that could ultimately be translated to food or nutraceutical applications. The study does not claim that *A. bisporus* is uniquely antioxidant among fungi. Other edible mushrooms, including *Lentinula*, *Pleurotus*, *Herichium* and *Ganoderma*, also contain important antioxidant compounds. Rather, *A. bisporus* was selected as a defined model edible mushroom for a focused solvent-comparison experiment.

Solvent selection is a critical determinant of extraction yield and antioxidant activity. Phenolic compounds and several other antioxidant-active metabolites generally show greater affinity for polar or moderately polar extraction systems than for highly non-polar solvents. Previous studies have therefore compared methanol, ethanol, aqueous systems and other solvents for mushroom extracts. More recent work has also moved toward process optimization. For example, Latif et al. (2024) optimized microwave-assisted extraction of polyphenols from white button mushroom using response surface methodology and demonstrated that solvent concentration, extraction time and solvent-to-solid ratio substantially affect phenolic recovery and antioxidant responses. Such work shows that extraction efficiency is a process variable rather than an intrinsic property of the mushroom alone.

The present study uses a deliberately simple cold-extraction design rather than an advanced extraction technology. Cold extraction minimizes direct thermal exposure during maceration and can be performed with relatively basic laboratory equipment. The experimental question was whether methanol, distilled water and chloroform differ in their ability to recover antioxidant-active compounds from *A. bisporus* and whether increasing extraction time changes the antioxidant response. The work therefore addresses a practical screening question rather than attempting to identify individual antioxidant molecules or establish a complete optimized industrial process.

The novelty of the present work should consequently be defined carefully. It is not the first report of antioxidant activity in *A. bisporus*, nor is the phosphomolybdate assay itself novel. The contribution is the comparative, time-resolved screening of three solvent systems under a common cold-extraction protocol using a readily available edible mushroom and a simple spectrophotometric assay. The results provide a preliminary basis for selecting an extraction system for subsequent optimization. The importance of this comparison lies particularly in the increasing interest in aqueous and food-compatible extraction and in the possibility of valorizing edible mushroom biomass or processing residues as sources of bioactive compounds.

2. Objectives of the Present Work

- To evaluate the total antioxidant capacity of *Agaricus bisporus* (J.E. Lange) Imbach extracts prepared using methanol distilled water and chloroform.
- To compare the effect of extraction duration (24, 48 and 72 h) on the antioxidant response of the three solvent systems.
- To determine which solvent system provides the highest phosphomolybdate-based antioxidant response under the tested experimental conditions.
- To assess whether an aqueous extraction system can provide a practically useful antioxidant response with prolonged extraction, thereby providing a basis for future food-compatible extraction studies.
- To establish a clear calculation and reporting framework for TAC so that the analytical unit is not confused with $\mu\text{g/g}$ or $\mu\text{g/L}$ of mushroom material.

3. Research Hypotheses

H1 (solvent hypothesis): The antioxidant capacity of *A. bisporus* extracts differs among methanol, distilled water and chloroform because solvent polarity influences the recovery of antioxidant-active constituents.

H2 (time hypothesis): Increasing cold-extraction duration from 24 to 72 h changes the antioxidant capacity of *A. bisporus* extracts, with the magnitude and direction of change depending on the solvent.

H3 (aqueous extraction hypothesis): Although methanol is expected to provide high extraction efficiency, prolonged aqueous extraction can increase the recovery of water-soluble antioxidant constituents and approach the antioxidant response obtained with methanol.

H4 (non-polar solvent hypothesis): Chloroform is expected to show lower phosphomolybdate antioxidant capacity than the more polar extraction systems under the tested conditions.

4. Materials and Methods

4.1 Sample Collection and Authentication

Fresh fruiting bodies of *A. bisporus* (400 g) were procured from a local market in Bilaspur, Chhattisgarh, India. The accepted taxonomic citation is *Agaricus bisporus* (J.E. Lange) Imbach. The material was stored at 4 °C before processing to reduce deterioration of endogenous compounds. Because the sample was purchased from a commercial market, the study represents a commercially available edible mushroom rather than a wild population.

4.2 Sample Preparation

The fruiting bodies were washed thoroughly to remove visible surface impurities and then air-dried. The dried material was finely powdered. Five grams of powdered mushroom were separately soaked in 150 mL of methanol, distilled water or chloroform. The extraction was carried out under cold conditions for 72 h, with observations collected at 24, 48 and 72 h. The use of the same sample-to-solvent ratio for all solvents was intended to make the solvent comparison more direct.

4.3 Recovery of Extracts

After each extraction period, the mixtures were centrifuged at 12,000 rpm for 15 min. The supernatants were filtered through Whatman No. 4 filter paper and stored at –20 °C until antioxidant analysis. The extraction protocol was designed to minimize thermal degradation during the maceration stage. It should be noted, however, that the phosphomolybdate reaction itself requires heating; therefore, the phrase 'cold extraction' refers specifically to the extraction step and not to the antioxidant assay.

4.4 Determination of Total Antioxidant Capacity

Total antioxidant capacity was assessed by the phosphomolybdate method. The assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant molecules under acidic conditions, producing a green phosphate/Mo(V) complex. Absorbance is measured spectrophotometrically at 695 nm. In the present experiment, the reagent mixture consisted of sodium phosphate (28 mM), ammonium molybdate (4 mM) and sulfuric acid (0.6 mM). Mushroom extract (0.1 mL) was mixed with 1.0 mL of reagent solution and incubated at 90–95 °C for 60 min. After cooling, absorbance was recorded at 695 nm. Ascorbic acid was used as the reference standard.

4.5 TAC Calculation and Unit

A quantitative TAC value must be obtained from an ascorbic-acid calibration curve. If the calibration equation is $y = mx + c$, where y is absorbance at 695 nm and x is the concentration of ascorbic acid, the concentration equivalent of an unknown extract is calculated as:

$$C \text{ (}\mu\text{g/mL AAE)} = (A - c) / m$$

where A is the absorbance of the extract, m is the slope and c is the intercept of the standard curve. If the extract has been diluted, the dilution factor must be applied. To express the antioxidant capacity per gram of original dry mushroom, the calculated concentration must then be multiplied by the total extract volume and divided by the mass of mushroom extracted:

$$\text{TAC (}\mu\text{g AAE/g dry mushroom)} = C \times V \times \text{DF} / M$$

where C = ascorbic acid equivalent concentration ($\mu\text{g/mL}$), V = total extract volume (mL), DF = dilution factor, and M = mass of dry mushroom extracted (g).

The source data supplied for this manuscript report the standard series as 0, 20, 40, 60, 80 and 100, labelled as '%', but do not document the stock concentration or dilution scheme. Therefore, a normalized value such as $\mu\text{g/g}$ cannot be calculated defensibly from the available information. The values reported in Table 2 are consequently retained as ' $\mu\text{g AAE per assay aliquot}$ ' to distinguish them from $\mu\text{g/g}$ or

µg/L. For a final journal submission, the authors should verify the original ascorbic-acid stock concentration and dilution preparation and then report the normalized unit requested by the journal. This clarification prevents an analytical unit from being inferred without sufficient experimental information.

5. Results

The ascorbic-acid standard series showed increasing absorbance with increasing standard concentration. The recorded absorbance values were 0.000, 0.228, 0.454, 0.690, 0.907 and 1.140 for the six standard levels (Table 1). This monotonic response indicates that the assay produced an increasing colorimetric signal over the tested calibration range. Because the original dataset does not provide replicate measurements or an explicitly reported regression equation, the standard curve should be recalculated from the original laboratory worksheet before submission of the final manuscript.

The solvent comparison demonstrated a clear pattern in antioxidant response. At 24 h, the methanolic extract produced an absorbance of 0.474 and a reported TAC of 43.09 µg AAE per assay aliquot. The aqueous extract produced an absorbance of 0.304 and a reported TAC of 27.63 µg, whereas the chloroform extract showed almost no response, with an absorbance of 0.001 and a reported TAC of 0.09 µg. Thus, at 24 h the methanolic response was substantially higher than the aqueous response, while the chloroform response was minimal.

At 48 h, methanol again produced a high response, with an absorbance of 0.463 and a reported TAC of 42.09 µg. The aqueous extract increased markedly to an absorbance of 0.428 and a reported TAC of 38.90 µg. Chloroform remained low, with an absorbance of 0.043 and a reported TAC of 3.90 µg. The increase in aqueous activity from 27.63 µg at 24 h to 38.90 µg at 48 h represents approximately a 40.8% increase relative to its 24-h value, whereas the methanolic response changed only slightly over the same period.

At 72 h, the aqueous extract produced the highest absorbance among the three solvents at that time point (0.468), corresponding to the reported TAC of 42.45 µg. The methanolic extract decreased to 0.335 absorbance and 30.45 µg TAC. Chloroform increased modestly to 0.082 absorbance and 7.45 µg TAC but remained far below the polar solvents. The data therefore show different temporal extraction profiles: methanol produced a high early response followed by a decline at 72 h, while water showed a progressive increase through 72 h.

Table 1. Absorbance values of the ascorbic-acid standard

Standard level (as recorded)	OD at 695 nm
0	0.000
20	0.228
40	0.454
60	0.690
80	0.907
100	1.140

Note: The original dataset labels the standard levels as percentage values. The original stock concentration and dilution scheme should be checked before assigning an absolute mass unit to these standards.

Table 2. Total antioxidant capacity of Agaricus bisporus extracts

Time (h)	Extract	OD at 695 nm	Reported TAC (µg AAE/assay aliquot)
24	Methanol	0.474	43.09
24	Chloroform	0.001	0.09
24	Aqueous	0.304	27.63
48	Methanol	0.463	42.09
48	Chloroform	0.043	3.90
48	Aqueous	0.428	38.90
72	Methanol	0.335	30.45
72	Chloroform	0.082	7.45
72	Aqueous	0.468	42.45

Note: The TAC values are retained from the supplied dataset and are expressed as µg ascorbic acid equivalents per assay aliquot for clarity. They should not be reported as µg/g or µg/L unless the original standard concentration, extract volume and dilution factor are verified.

6. Discussion

The results demonstrate that solvent choice strongly influenced the antioxidant response of *A. bisporus*. The methanolic extract showed the highest response at 24 and 48 h, whereas the aqueous extract became comparable to methanol by 72 h. Chloroform produced consistently low responses. This pattern is chemically plausible because the phosphomolybdate assay detects overall reducing capacity rather than one specific antioxidant molecule, and extraction efficiency depends on the polarity, solubility and stability of the compounds recovered by each solvent.

The strong early methanolic response is consistent with earlier research showing that polar organic extracts of *A. bisporus* can contain phenolic compounds and exhibit antioxidant activity. Barros et al. (2008) reported antioxidant activity associated with the chemical composition and maturity stage of *A. bisporus*. Other studies have likewise shown that ethanolic extracts of white button mushroom possess reducing and radical-scavenging activities and contain several phenolic constituents. These findings support the interpretation that polar organic solvents can recover antioxidant-active molecules efficiently.

The progressive increase in the aqueous response is particularly important from an application perspective. The aqueous TAC increased from 27.63 µg at 24 h to 38.90 µg at 48 h and 42.45 µg at 72 h. This suggests that prolonged contact with water increased the recovery of water-soluble reducing constituents under the present conditions. However, the result should not be generalized to all antioxidant compounds because the phosphomolybdate assay measures total reducing capacity and does not identify individual compounds. Aqueous extraction may also recover sugars, proteins and other reducing substances that contribute to the assay signal. Consequently, high TAC should be interpreted as high assay-detected reducing capacity rather than direct proof of a high concentration of a particular antioxidant.

The low chloroform response provides a useful contrast. The reported TAC remained below 8 µg at all time points. This result is compatible with the expectation that many hydrophilic antioxidant constituents, including several phenolic and other polar metabolites, are poorly recovered by a highly non-polar extraction system. Nevertheless, chloroform should not be described simply as an ineffective

solvent for all antioxidant chemistry. Non-polar systems can recover lipophilic compounds, including some sterols and lipid-associated molecules, which may not be strongly represented by the phosphomolybdate assay. The present result therefore means that chloroform gave low phosphomolybdate-detectable reducing capacity under the conditions tested.

The time-dependent behavior of methanol and water also deserves attention. Methanol produced 43.09 μg at 24 h and 42.09 μg at 48 h, followed by a lower value of 30.45 μg at 72 h. Water, in contrast, increased continuously to 42.45 μg at 72 h. The decline in methanol after 48 h could reflect several possibilities, including oxidation or degradation of susceptible compounds during prolonged extraction, changes in extract composition, experimental variability, or differences in the original extraction measurements. Because the study does not report replicate measurements, standard deviations or statistical testing, the decline should be described as an observed pattern rather than a statistically established time effect.

Recent literature reinforces the importance of extraction conditions. Latif et al. (2024) used response surface methodology to optimize white button mushroom polyphenol extraction and showed that ethanol concentration, extraction time and solvent-to-solid ratio affect the recovery of phenolics and antioxidant responses. This supports the present conclusion that solvent and time cannot be treated as incidental experimental details. However, the current study uses a simpler cold-maceration approach and therefore should be regarded as a preliminary screening experiment rather than a process-optimization study.

The biological importance of mushroom antioxidants is also broader than phenolics. Ergothioneine is an important mushroom-derived antioxidant compound, and recent reviews have emphasized its occurrence, biosynthesis, extraction and potential food applications. Halliwell et al. (2023) also emphasized that the strong in-vitro antioxidant chemistry of ergothioneine should not be equated automatically with proof that antioxidant action is its dominant physiological function in humans. Therefore, the present TAC data should be interpreted as an in-vitro analytical property of the extracts rather than as a direct measure of human health benefit.

Table 1 provides the analytical basis for interpreting the extract measurements, while Table 2 provides the solvent- and time-dependent antioxidant results. The monotonic increase in the ascorbic-acid standard response supports the use of a calibration curve, but the absence of the original concentration units and regression statistics limits the precision with which the TAC can be normalized. This is a critical methodological issue because reporting ' μg ' without defining whether it refers to the assay aliquot, total extract, or original mushroom mass can create ambiguity. A revised manuscript should therefore distinguish clearly between μg AAE per assay aliquot and μg AAE/g dry mushroom.

From a sustainability perspective, the findings require careful wording. Water is the only solvent among those tested that can reasonably be described as a low-toxicity, food-compatible and environmentally preferable extraction medium. Methanol is useful analytically but is toxic and should not be classified as a green solvent merely because the extraction is performed without heating. Chloroform is also hazardous and environmentally undesirable. Therefore, the most defensible sustainability implication of the present study is that the increasing aqueous response observed up to 72 h supports further investigation of water-based extraction. Future work should compare water with food-grade ethanol/water mixtures and evaluate extraction efficiency, phenolic content and multiple antioxidant assays.

The study also helps clarify why *A. bisporus* was chosen instead of comparing several fungal species. A multi-species study would address a different research question and would require additional sampling,

authentication and experimental replication. The present study was designed as a focused within-species comparison of solvent and time. *A. bisporus* was selected because it is commercially available, widely consumed and chemically characterized. This makes it an appropriate model for preliminary method development. Nevertheless, a future comparative study should include *Pleurotus*, *Lentinula*, *Herichium* or other edible mushrooms to determine whether the extraction pattern observed here is species-specific or represents a broader feature of edible fungi.

Overall, the findings support the hypotheses that solvent polarity affects antioxidant extraction and that extraction duration modifies the aqueous antioxidant response. The data also support the practical hypothesis that water can become competitive with methanol after prolonged extraction. However, the study does not establish statistical significance, individual compound identity, extraction yield, or biological efficacy. These limitations should be stated explicitly to prevent overinterpretation.

7. Study Limitations and Recommended Future Work

- The dataset does not report biological or technical replicates, standard deviations, confidence intervals or statistical significance testing.
- The original ascorbic-acid standard concentrations are labelled as percentages rather than a clearly defined mass concentration, preventing independent verification of absolute TAC units.
- Extraction yield was not measured, so antioxidant capacity cannot currently be normalized to extract mass or dry mushroom mass.
- Only one antioxidant assay was used. DPPH, ABTS, FRAP and/or ORAC would provide complementary information because antioxidant assays measure different chemical mechanisms.
- The study did not quantify total phenolic content, total flavonoid content or individual compounds such as ergothioneine.
- The study used one commercial sample source; biological variability among cultivars, maturity stages and geographical sources was not assessed.
- Future work should use replicated experiments, verify the standard curve, report extraction yield, include food-grade ethanol/water systems, and characterize the extracts by HPLC, LC-MS/MS or another suitable analytical platform.

8. Conclusion

The present study demonstrates that solvent polarity and extraction duration influence the phosphomolybdate-based antioxidant response of *Agaricus bisporus* (J.E. Lange) Imbach. Methanol produced the highest reported TAC at 24 h (43.09 µg AAE per assay aliquot) and 48 h (42.09 µg), whereas water showed a progressive increase and reached 42.45 µg at 72 h. Chloroform consistently produced much lower responses. The results therefore identify polar extraction systems as more effective than chloroform for recovering antioxidant-active reducing constituents under the conditions investigated.

The principal practical implication is that aqueous extraction deserves further development because its antioxidant response increased with extraction time and water is safer and more compatible with food-oriented applications than methanol or chloroform. However, methanol should be regarded as an analytical extraction solvent rather than a green solvent, and chloroform should not be promoted for sustainable food applications. The study should also be considered preliminary because the available dataset lacks replicate statistics, extraction-yield data and complete standard-curve information.

It is recommended that future studies optimize water and food-grade ethanol/water extraction using response surface methodology, determine extraction yield, quantify total phenolic content and ergothioneine, and validate antioxidant activity with at least two complementary assays. Comparative evaluation of *A. bisporus* with other edible mushrooms would also establish whether the observed solvent-dependent pattern is species-specific. Before journal submission, the original ascorbic-acid standard preparation should be verified so that TAC can be reported unambiguously as $\mu\text{g AAE/g}$ dry mushroom or another journal-appropriate normalized unit.

References

1. Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Effect of fruiting body maturity stage on chemical composition and antioxidant activity of *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*, 56, 8759–8764.
2. Chu, M., Khan, R. D., Zhou, Y., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2023). LC-ESI-QTOF-MS/MS characterization of phenolic compounds in commercial mushrooms and their antioxidant activities. *Processes*, 11(6), 1711.
3. Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 55, 9059–9064.
4. El-Ramady, H., Abdalla, N., Badgar, K., Llanaj, X., Törös, G., Hajdú, P., Prokisch, J., & others. (2022). Edible mushrooms for sustainable and healthy human food: Nutritional and medicinal attributes. *Sustainability*, 14(9), 4941.
5. Halliwell, B., Tang, R. M. Y., & Cheah, I. K. (2023). Diet-derived antioxidants: The special case of ergothioneine. *Annual Review of Food Science and Technology*, 14, 323–345. <https://doi.org/10.1146/annurev-food-060822-122236>
6. Hamza, A., Mylarapu, A., Krishna, K. V., & Kumar, D. S. (2024). An insight into the nutritional and medicinal value of edible mushrooms: A natural treasury for human health. *Journal of Biotechnology*, 381, 86–99. <https://doi.org/10.1016/j.jbiotec.2023.12.014>
7. Jagadeeshraman, S. (2018). Antioxidant and phytochemical properties of *Agaricus bisporus*. *Journal of Food Science*, 83, 152–160.
8. Kim, I. S., Yang, M. R., Lee, O. H., & Kang, S. N. (2011). Antioxidant activities of natural extracts. *International Journal of Molecular Sciences*, 12, 4120–4131.
9. Latif, A., Khan, M. I., Latif, A., Khan, U. M., Khaneghah, A. M., & Aadil, R. M. (2024). Enhancing polyphenol extraction from white button mushrooms using microwave-assisted extraction: A response surface methodology optimization approach. *Microchemical Journal*, 203, 110876. <https://doi.org/10.1016/j.microc.2024.110876>
10. Liuzzi, G. M., Petraglia, T., Latronico, T., Crescenzi, A., & Rossano, R. (2023). Antioxidant compounds from edible mushrooms as potential candidates for treating age-related neurodegenerative diseases. *Nutrients*, 15(8), 1913. <https://doi.org/10.3390/nu15081913>
11. Mattila, P., Könkö, K., Eurola, M., Pihlava, J.-M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 49(5), 2343–2348.

12. Singh, A., Saini, R. K., Kumar, A., Chawla, P., & Kaushik, R. (2025). Mushrooms as nutritional powerhouses: A review of their bioactive compounds, health benefits, and value-added products. *Foods*, 14(5), 741. <https://doi.org/10.3390/foods14050741>
13. Tian, X., Thorne, J. L., & Moore, J. B. (2023). Ergothioneine: An underrecognised dietary micronutrient required for healthy ageing? *British Journal of Nutrition*, 129(1), 104–114. <https://doi.org/10.1017/S0007114522003592>
14. Usman, M., Murtaza, G., & Ditta, A. (2021). Nutritional and medicinal value of *Agaricus bisporus*. *Applied Sciences*, 11(13), 5943.
15. Yang, L., Shen, Z., Luo, X., Li, R., & Li, R. (2025). Recent progress in ergothioneine of edible fungi. *Chinese Journal of Biotechnology*, 41(2), 574–587. <https://doi.org/10.13345/j.cjb.240495>
16. NCBI Taxonomy. (2026). *Agaricus bisporus* Taxonomy ID 5341. National Center for Biotechnology Information.
17. Species Fungorum. (2026). *Agaricus bisporus* (J.E. Lange) Imbach. Royal Botanic Gardens, Kew.