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Effective antioxidant production through submerged fermentation of edible mushrooms

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ABSTRACT

Objective: The present work was designed to apply the submerged fermentation of five edible mushrooms including *Schizophyllum commune, Lentinus polychrous, Lentinula edodes, Ganoderma lucidum*, and *Lentinus squarrosulus* for antioxidant production. **Methods:** The antioxidant activity and total phenolic content (TPC) of 30 different broth and mycelium extracts acquired from the submerged mushroom fermentation in three liquid media, malt extract broth (MEB), potato dextrose broth (PDB), and yeast extract sucrose broth, were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and Folin-Ciocalteu colorimetric method, respectively. **Results:** All the extracts were found to possess antioxidant properties and contain phenolic compounds. The percentage of DPPH inhibition of the extracts was between 5.52% and 81.0% and TPC ranged of 6.46-154.47 mg gallic acid equivalent (GAE)/g extract. Species of mushroom and type of liquid medium demonstrated dominant effects on the antioxidant activity of the extracts correlated with TPC. The broth extracts obtained from the PDB and MEB submerged fermentation of *S. commune* showed the best antioxidant activity value of 81.0% and presented the highest level of phenolics at 154.47 mg GAE/g extract, respectively. There were three extracts having high total phenolics and exhibiting strong antioxidant capacities (78.85-81.0%) higher than ascorbic acid standard (75.25%). **Conclusion:** The results of this study introduced *S. commune* as a potential natural antioxidant producer and indicated that the submerged fermentation might serve as an effective alternative method for producing natural antioxidants which could probably be developed for applications in the pharmaceutical and cosmetic industries.

Keywords: Antioxidant activity, edible mushroom, submerged fermentation, total phenolic content

INTRODUCTION

In recent years, health and beauty have received increasing attention resulting in significant efforts to devote toward the exploration of new sources of biologically active compounds, especially antioxidants to be developed as potential and safe drugs, cosmetics, and nutritional products. Population growth as well as increase in pre-aging problems and health issues from antioxidant deficiency are driving the global demand for antioxidant products. Oxidation is a biological process which is essential to many organisms for the production of energy.[1] However, excess of oxygen-derived free radicals can induce cell damage causing the physiological process of aging and promotion of various diseases such as asthma, arthritis, cancer, cirrhosis, and diabetes.[2,3] Antioxidants are substances that can inhibit or delay onset of oxidation and can be classified as natural and synthetic. They, therefore, possess an ability to protect biological systems from the harmful effects of excessive oxidation. The use of synthetic antioxidants to prevent free radical damage has been reported to involve adverse effects due to their possible cytotoxic and carcinogenic potentials.[4,5] A rapidly increased attention has been toward natural antioxidants, which have generated considerable interest in preventive medicine and cosmetic company. Natural antioxidants such as phenolics, polysaccharides, glycosides, flavonoids, carotenoids, and ascorbic acid, which could prevent the free radical-induced damage and reduce the risk of chronic diseases, are found in food including fruits, mushrooms, and vegetables.[4,6]

Edible mushrooms have been recognized as nutritionally functional food due to their chemical composition and special fragrance. They provide a wealth of carbohydrates, proteins, vitamins, minerals, as well as dietary fiber and

are believed to be a harmless important source of natural antioxidants particularly phenolic compounds, which are of great significance recently as protective agents proved to help prevent and reduce the oxidative damage and thus protect the human body.[4] The antioxidant activity of edible mushrooms including *Agaricus bisporus*, *Lentinula edodes*, and *Volvariella volvacea* was reported to correlate with the phenolic content.[7] In addition, various phenolics present in edible mushrooms have been found to be excellent antioxidants and synergists, proved to exhibit a variety of beneficial biological properties including antiallergic, antiarthritic, anticarcinogenic, antihypertensive, antimicrobial, and anti-inflammatory activities and suggested to be the major bioactive compounds for nutraceutical and health benefits.[8] Furthermore, the rich diversity of edible mushrooms offers a promising source of novel natural antioxidants.

Edible mushrooms, namely, *Schizophyllum commune*, *Lentinus polychrous*, *L. edodes*, *Ganoderma lucidum*, and *Lentinus squarrosulus* have been largely consumed and commercialized particularly in Asia. There have been numerous reports on the antioxidant property of their fruiting bodies. Unlike many studies reported previously that used direct extraction of mushroom fruiting bodies, the antioxidant activity of the broth and mycelium extracts from the submerged fermentation of these edible mushrooms was evaluated in this work. The fruiting body and mycelium of some mushrooms were reported to contain different antioxidants exerting various antioxidant properties.[9,10] For the production of a diverse array of bioactive compounds, submerged fermentation technique has recently become attractive and has significant industrial potential because it gives rise to economic and environmental advantages of higher metabolite production in a compact space, easier controllability, shorter time, and lesser chance of contamination compared with the cultivation of fruiting bodies.[11] The previous reports indicated that the fermentation products of some edible mushrooms also had antioxidant, antitumor, antiviral, and immunomodulatory effects similar to and higher than the fruiting bodies.[9,12,13] Discovery of the bioactivities of several secondary metabolites has resulted in the further exploration of submerged fermentation as a production technique for these compounds. Moreover, an additional advantage of this technique is that various liquid media can be used to induce mushroom species to produce different secondary metabolites. In this study, malt extract broth (MEB), potato dextrose broth (PDB), and yeast extract sucrose (YES) broth recommended as suitable liquid media for the production of secondary metabolites by fungi were employed.[14,15] The total phenolic contents (TPCs) of the extracts both from fermentation broths and cultured mycelia of these edible mushrooms were also estimated.

MATERIALS AND METHODS

Preparation of Inocula

S. commune, *L. polychrous*, *L. edodes*, *G. lucidum*, and *L. squarrosulus* were procured from Biotechnology Research and Development Office, Department of Agriculture, Thailand. They were grown on potato dextrose agar plates at 25°C for 7 days.

Submerged Fermentation

Six pieces (6 × 6 mm^2) of each grown culture were transferred into the 1000 ml Erlenmeyer flask filled with 200 ml of each liquid medium and incubated at 25°C under the static condition for 21 days. All the liquid media used included MEB, PDB, and YES broth.

Preparation of Crude Extracts

The fermentation broth and mycelium were separated by filtration. The filtrate was extracted with equal volume of ethyl acetate for 3 times. The organic layers were combined and evaporated to obtain a crude broth extract. The mycelium was macerated in methanol (200 ml) at 25°C for 2 days. After maceration, the methanol layer was concentrated through evaporation, added with distilled water (200 ml), and partitioned twice with equal volume of ethyl acetate to yield a crude mycelium extract.

Determination of Antioxidant Activity

The *in vitro* antioxidant activity of crude extracts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to the procedure of Jiamworanunkul *et al*.[16] Each crude extract at a concentration of 0.5 mg/ml was mixed with equal volume of freshly prepared solution of 0.06 mM DPPH in ethanol. The mixture was shaken well and allowed to stand for 30 min in the dark at room temperature. The absorbance of the mixture was measured at 517 nm and ascorbic acid (0.5 mg/ml) was employed as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ inhibition} = (A_{control}\text{-}A_{sample})/(A_{control}) \times 100$$

where, $A_{control}$ and A_{sample} represent the absorbance of the control and sample respectively. The test was conducted in triplicate.

Determination of TPC

The TPC of crude extracts was estimated colorimetrically employing the method of Jiamworanunkul *et al*.[16] Briefly, each crude extract (800 μl) at a concentration of 5 mg/ml was mixed with Folin–Ciocalteu reagent (80 μl). Then, 7.5% sodium carbonate solution (1.6 ml) was added and the mixture was made up to the final volume of 4 ml. The mixture was shaken vigorously and allowed to stand for 60 min at room temperature. The absorbance was measured at 765 nm and gallic acid was used as standard. The concentration of total phenolic compounds was measured by potting the calibration curve of the standard and determined as mg of GAE/g of extract. The experiment was done in triplicate.

RESULTS AND DISCUSSION

Five species of edible mushrooms including *S. commune, L. polychrous*, *L. edodes*, *G. lucidum*, and *L. squarrosulus* were selected to be used for the antioxidant production. Interest in these species was due to their acknowledged medical importance, abundant nutritional components, edibility, and availability.[17-20] The fungal production of bioactive substances has utilized several different techniques such as submerged

and solid-state fermentations, together with direct extraction from fungi. Over the years, submerged fermentation has gained immense importance as a promising alternative for efficient production of biomass and valuable metabolites, especially for pharmaceutical and cosmetic applications. Instead of direct extraction from mushroom fruiting bodies, submerged fermentation was employed in the present study to produce fungal metabolites with antioxidant activity. MEB, PDB, as well as YES broth, recommended in the previous studies as suitable mycological media for inducing fungi to produce bioactive secondary metabolites, were used as liquid media.[14,15] The extraction of fungal metabolites both from fermentation broths and mycelia of the edible mushrooms was performed. According to the extraction results in Table 1, the obtained yields varied considerably, ranging from 3.65 to 635.20 mg and were found to be affected by differences in types of mushroom and liquid medium. The mushroom *L. edodes* cultured in YES broth and PDB gave the maximum and minimum yields, respectively.

DPPH radical has been widely accepted as a tool to assess antioxidant potency of various compounds.[18,21] It is a stable, nitrogen-centered free radical which produces violet color in ethanol solution and can accept a hydrogen atom to become a yellow-colored product, diphenylpicrylhydrazine. Hence, the capability of antioxidants to scavenge the DPPH radical is attributed to their hydrogen-donating abilities.[22] In this work, the antioxidant potentials of all the extracts were investigated using the DPPH radical scavenging assay. The assay is considered a most accurate method which is unaffected by certain side reactions such as metal chelation as well as enzyme inhibition and largely used to determine antioxidant activity over a relatively short time compared to other methods.[18,21] The DPPH scavenging activity of the broth and mycelium extracts is presented in Table 2. All the extracts showed antioxidant capacity with inhibition percentage of 5.52 ± 0.92–81.0 ± 0.81. The broth extracts of *S. commune* fermented in PDB, MEB, and YES exhibited the first three highest scavenging activities of 81.0%, 78.93%, and 78.85%, respectively, while the broth extract of *L. edodes* in YES gave the lowest value of 5.52%. Concerning the mycelium extracts, the highest scavenging power (70.37%) was found in the extract of *L. squarrosulus* in MEB followed by *L. edodes* (53.37%) in MEB, whereas the lowest (13.49%) was observed in the extract of *G. lucidum* in PDB. It should be noted that all the broth extracts of *S. commune* had more powerful antioxidant activities (78.85–81.0%) than the standard ascorbic acid (75.25%) and other examined extracts. The results also indicated that six crude extracts showed good scavenging effects of 69.73–81.0% on DPPH radicals compared to ascorbic acid: five extracts from broths (83.33%) and one extract from mycelia (16.67%). In the submerged fermentation, the mushrooms were induced to produce and secrete antioxidant metabolites into the culture media rather than accumulate in mycelia so the bioactive agents often were found to be extracellular. In addition, previously published reports revealed that DPPH scavenging activities were observed in the fruiting body extracts obtained through direct extraction of the mushrooms as followed: *S. commune* (70.52%), *L. polychrous* (71.09%), *L. edodes* (13.44%), *G. lucidum* (6.83%), and *L. squarrosulus* (40.54%).[17,18,23,24] Compared to the previous results, the extracts derived from the submerged fermentation using suitable liquid media in this research had greater DPPH radical scavenging powers in the same mushroom species including *S. commune* (81.0%), *L. polychrous* (72.57%), *L. edodes* (53.37%), *G. lucidum* (49.20%), and *L. squarrosulus* (70.37%), indicating that the submerged mushroom fermentation could be exploited as a promising alternative for the efficient production of secondary

Table 1: Yields of broth and mycelium extracts

Species of mushroom	Type of medium	Yield of extract (mg)/200 ml broth	
		Broth	Mycelium
S. commune	MEB	90.05	21.33
	PDB	76.51	12.00
	YES	44.13	38.32
L. polychrous	MEB	36.93	40.13
	PDB	57.68	43.55
	YES	155.09	13.52
L. edodes	MEB	248.43	15.44
	PDB	48.77	3.65
	YES	635.20	10.80
G. lucidum	MEB	11.39	33.97
	PDB	41.87	103.33
	YES	17.01	190.64
L. squarrosulus	MEB	67.73	17.55
	PDB	98.56	27.97
	YES	192.99	103.81

MEB: Malt extract broth, PDB: Potato dextrose broth, YES: Yeast extract sucrose, *S. commune: Schizophyllum commune, L. polychrous: Lentinus polychrous, L. edodes: Lentinula edodes, G. lucidum: Ganoderma lucidum, L. squarrosulus: Lentinus squarrosulus*

Table 2: DPPH radical scavenging activity of broth and mycelium extracts

Species of mushroom	Type of medium	% inhibition of DPPH±SD*	
		Broth	Mycelium
S. commune	MEB	78.93±0.81	34.29±0.54
	PDB	81.0±0.81	41.45±0.33
	YES	78.85±0.34	46.53±0.66
L. polychrous	MEB	72.57±0.35	28.30±0.66
	PDB	69.73±0.48	31.42±0.15
	YES	17.85±0.35	32.03±0.26
L. edodes	MEB	30.42±0.27	53.37±0.44
	PDB	41.30±0.35	31.42±0.40
	YES	5.52±0.92	46.34±0.59
G. lucidum	MEB	49.20±0.71	33.71±0.87
	PDB	34.12±0.26	13.49±0.44
	YES	26.90±0.60	27.26±0.54
L. squarrosulus	MEB	8.43±1.04	70.37±0.57
	PDB	14.33±0.27	23.36±0.75
	YES	13.41±0.70	22.32±0.59
Ascorbic acid		75.25±0.35	

*n=3. MEB: Malt extract broth, PDB: Potato dextrose broth, YES: Yeast extract sucrose, *S. commune: Schizophyllum commune, L. polychrous: Lentinus polychrous, L. edodes: Lentinula edodes, G. lucidum: Ganoderma lucidum, L. squarrosulus: Lentinus squarrosulus*, DPPH: 1,1-Diphenyl-2-picrylhydrazyl

metabolites with antioxidant activities. This study provides an introduction to more comprehensive work on the bioactive metabolites produced by the biotechnological approach. Further work is targeted at the isolation, purification, and structural elucidation of the bioactive compounds present in the strong active extracts that might have the potential to yield novel and potent natural antioxidants.

Phenolic compounds are important secondary metabolites which are vital in defense responses such as antioxidant, anti-aging, and anti-inflammatory activities and the major naturally occurring antioxidant components present in a large number of mushroom species.[25-29] They have been known as a great source of particular antioxidant substances having inhibitory effects on mutagenesis and carcinogenesis in humans by inhibiting free radical reactions.[30] Lately, they have obtained significant interest based on several reports of their conjectural part in holding back a variety of human illnesses.[31] The TPC of the broth and mycelium extracts was evaluated employing the Folin–Ciocalteu colorimetric method. As the results listed in Table 3, it was found that all the extracts showed a wide range of phenolic concentrations varying from 6.46 ± 0.27 to 154.47 ± 0.33 mg GAE/g extract. The broth extract of *S. commune* fermented in MEB ranked as highest phenolic extract (154.47 mg GAE/g extract) followed by the broth extracts of *L. polychrous* in MEB (124.18 mg GAE/g extract) and *S. commune* in PDB (98.09 mg GAE/g extract). The lowest phenolic content was observed in the mycelium extract from *G. lucidum* cultured in YES broth (6.46 mg GAE/g extract). The quantitative trend of TPC varied from one species to another, indicating that each species had its own unique approaches in phenolic synthesis and cell metabolism. High DPPH radical scavenging ability was shown in the extracts which also contained a good amount of total phenolics. Close association between the scavenging ability and TPC was found in accordance with earlier studies that indicated good correlation between both results.[23,32] Phenolics showed the scavenging activity due to their hydroxyl groups and might contribute directly to the antioxidative action.[33] In addition, the findings from this work revealed that the TPC values for the potential extracts of the five mushroom species grown in the submerged fermentation with appropriate liquid media ranged from 40.24 to 154.47 mg GAE/g extract which were higher than those varying from 0.30 to 45.69 mg GAE/g extract reported previously for the extracts obtained using direct extraction of fruiting bodies of the same mushrooms.[17,19,20,34,35] This suggested that the submerged mushroom fermentation could be potentially used for producing phenolic compounds, one of the most valuable metabolites.

From the submerged fermentation, the different quantities of phenolic compounds and, consequently, the different antioxidant activities were probably a consequence of the presence of different constituents with various antioxidant properties. This depended highly on mushroom species and liquid media used. The effects of the two factors on the antioxidant capacity were expressed in terms of DPPH radical scavenging activity corresponded to the total quantity of phenolics. With respect to target mushroom species, the influence of the liquid media was variable in consonance with the previous studies by Dulay *et al*.[36] and Bustillos *et al*.[37] The results in Table 2 disclosed that the liquid media MEB and PDB gave higher number of crude extracts with good DPPH radical scavenging activity (>69%) than YES broth as followed: MEB (three extracts), PDB (two extracts), and YES (one extract). Regarding TPC in Table 3, MEB and PDB also provided four extracts with high contents of phenolics

Table 3: Total phenolic contents of broth and mycelium extracts

Species of mushroom	Type of medium	TPC (mg GAE/g extract)±SD*	
		Broth	Mycelium
S. commune	MEB	154.47±0.33	28.36±0.50
	PDB	98.09±0.74	24.54±0.08
	YES	62.48±0.38	33.46±0.33
L. polychrous	MEB	124.18±0.57	16.12±0.98
	PDB	80.69±0.49	17.49±0.29
	YES	24.27±0.25	10.91±0.37
L. edodes	MEB	21.55±0.70	43.80±0.38
	PDB	41.53±0.10	12.09±0.69
	YES	7.97±0.34	19.0±0.13
G. lucidum	MEB	26.13±0.13	16.92±0.87
	PDB	24.10±0.24	11.27±1.36
	YES	40.24±1.26	6.46±0.27
L. squarrosulus	MEB	8.82±0.25	52.69±0.22
	PDB	29.18±1.26	18.92±0.37
	YES	6.68±0.38	18.01±0.34

*n=3. MEB: Malt extract broth, PDB: Potato dextrose broth, YES: Yeast extract sucrose, *S. commune: Schizophyllum commune, L. polychrous: Lentinus polychrous, L. edodes: Lentinula edodes, G. lucidum: Ganoderma lucidum, L. squarrosulus: Lentinus squarrosulus,* TPC: Total phenolic content, GAE: Gallic acid equivalent

(80.69–154.47 mg GAE/g extract) in comparison with other extracts (6.46–62.48 mg GAE/g extract). YES broth was found to give the crude extracts with the lowest TPC (6.46 mg GAE/g extract) and the least scavenging capacity (5.52%). As the results indicated, it was confirmed that the liquid media could affect the presence or absence of bioactive compounds and their level of production by the mushrooms. YES has previously been recommended as medium for the production of secondary metabolites by fungi.[15] In contrast to the previous report, YES was found to be inapplicable for the antioxidant production by the mushroom species in this study except *S. commune*. It was noteworthy that MEB and PDB were appropriate liquid media for the submerged fermentation process to achieve high quantity of secondary metabolites with good antioxidant properties synthesized by *S. commune* and *L. polychrous*. On the other hand, more selective or optimized liquid media might provide better expression for some phenotypes. Furthermore, the high differential among the liquid media suggested that at least two media comprising different carbon and nitrogen sources should be employed for screening of mushroom species for the production of bioactive metabolites.[15] In this work, it was apparent that the mushroom *S. commune* gave all the three broth extracts obtained from the submerged fermentation using the three different liquid media that were rich with phenolic compounds and presented outstanding antioxidant activities, indicating its great capacity for the antioxidant production.

CONCLUSION

The present study suggested that the submerged mushroom fermentation could potentially serve as an alternative method for the production of natural antioxidants, especially phenolic compounds. The results achieved in this work contribute to expand the knowledge of the submerged fermentation of the five edible mushrooms. Species of mushroom and type of liquid medium displayed notable effects on the antioxidant activity and TPC of the broth and mycelium extracts. The obtained results indicated the mushroom *S. commune* to be a potent natural antioxidant producer. Among the liquid media employed, MEB and PDB were more suitable in supporting the antioxidant production. To further highlight the importance of this research, the potential extracts derived from the submerged fermentation of all the mushrooms had higher antioxidant capacity and TPC than the fruiting body extracts obtained through direct extraction and some of them exhibited better antioxidant activity than ascorbic acid standard.

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