Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

Yuen SIM, Kheng; Young LIEW, Jeng; DING, Xin Yi; CHOONG, Wan Shin; INTAN, Seri
Effect of vacuum and oven drying on the radical scavenging activity and nutritional contents of submerged fermented Maitake (Grifola frondosa) mycelia
Ciência e Tecnologia de Alimentos, vol. 37, núm. 1, diciembre, 2017, pp. 131-135
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

DOI: http://dx.doi.org/10.1590/1678-457X.28816

Effect of vacuum and oven drying on the radical scavenging activity and nutritional contents of submerged fermented *Maitake* (*Grifola frondosa*) mycelia

Kheng Yuen SIM[1]*, Jeng Young LIEW[2], Xin Yi DING[2], Wan Shin CHOONG[2], Seri INTAN[2]

Abstract

Maitake (*Grifola frondosa*) mycelia contain high dietary and medicinal values that have gained great attentions from consumers. Proper drying can preserve their bio-availabilities prior to subsequent processing or consumption. Pure *Maitake* (*Grifola frondosa*) strain was cultivated in Mushroom Complete Medium (MCM) using submerged fermentation for 14 days. *Maitake* mycelia were harvested and treated respectively by vacuum, oven drying treatments while the fresh mycelia were assigned as control. All the samples were tested for Total Phenolic Content, DPPH radical scavenging assays and nutritional contents. Submerged fermentation produced mycelia biomass (1.3 ± 0.1 g/L) within 14 days of fermentation. Both the pH and reduced sugar content had decreased ($P < 0.05$) throughout fermentation. Vacuum dried mycelia had shown higher ($P < 0.05$) total phenolic (20.0 ± 0.4 mg GAE/g), DPPH radical scavenging activity (84.7 ± 0.1%), and soluble protein content (283.6 ± 7.1 μg/mL) as compared to other treated samples. The crude protein (39.1 ± 0.2), fat (5.7 ± 0.7), ash (11.1 ± 0.3%) of mycelia were well preserved using vacuum drying as compared to oven dried samples. The study has suggested that vacuum drying at 70 °C, 1000mBar has the advantage to preserve the nutritional and radical scavenging activity of high value *maitake* mycelia effectively at lower cost.

Keywords: DPPH radical scavenging; *maitake* mycelia; submerged fermentation; total soluble protein; vacuum drying.

Practical Application: Cultivation of high value *maitake* biomass via submerged fermentation within shorter time at desirable yield. The use of vacuum drying at 70 °C, 1000 mBar to preserve its bioactive and nutritional compositions of *maitake* mycelia which can be considered as cost efficient dehydration unit for subsequent post-harvest or downstream process.

1 Introduction

Edible mushrooms are popular macro fungi that have been consumed for millennia as part of our dietary source. They are high in nutritional compositions especially protein, essential amino acids, carbohydrate, fatty acid, vitamins and minerals. Some of them even possess bioactive components namely polysaccharides, glycoproteins, antioxidants and polyphenolic compounds that exert great medicinal effects which include anticancer, antibiotic, antiviral activities, immunity blood lipid lowering effect (Toledo et al., 2013; Bhattacharya et al., 2014). Therefore, large varieties of mushrooms have gained great attentions from consumers in different cultures who wish to maintain their health as well as to boost up their immuno- modulatory systems.

Maitake or *Grifola frondosa* is a Basidiomycete fungus that grows in small and overlapping-tongue or fan shaped caps with stalks. *Maitake* is considered as a newly cultivated species with accelerating market consumption and demand similar to other cultivated ones, especially the *Pleurotus*, *Agaricus*, *Lentinus*, *Volvariella* and *Shiitake* sp (Zhang et al., 2014). In fact, maitake has been used as medicinal source in Asia for a very long time to treat high blood pressure, elevated serum cholesterol, improving immunity and anti-ageing (Deng at al., 2009; Yeh et al., 2011). The *Maitake*-D fraction or specifically the β-glucans group is the important active compounds available in *maitake* which can enhance one's immune system, limits or reverse tumour growth, delaying cell ageing and cancer development (Gregori et al., 2011). Various downstream of *maitake* products are available ranging from the *maitake* mushroom tea, mycelia biomass and capsule of *maitake* extract with potent bioactivities (Wu et al., 2016).

Commercial mushroom cultivation relies on the complete fruiting body development which is time consuming (approximately 2 months) and labour intensive. The quality of the mushroom is inconsistent as it relies on the successful rate for each cultivation batch. An extra cost will be incurred if the solid cultivation substrate is contaminated. Hence, the submerged fermentation has been used as alternative to accelerate the production of mushroom biomass and valuable metabolites. A liquid complete medium will be used to cultivate respective mushroom species under controlled environment and condition. The method has the potential to obtain higher mushroom biomass or mycelia within shorter time of consistent quality (Kim et al., 2007). Some researchers discovered that the nutritional value of mushroom biomass cultivated via submerged fermentation is comparable to those found in fruiting body (Ulziijargal & Mau, 2011). Besides, the texture is also unique as compared to fruiting body. This actually encourages more mushroom growers to opt for submerged cultivation on mushroom biomass especially growing mycelia to meet the market demand.

Received 07 Dec, 2016
Accepted 13 July, 2017
[1] *Faculty of Science, Universiti Tunku Abdul Rahman, Kampar Campus, Kampar, Perak, Malaysia*
[2] *Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli Kelantan, Malaysia*
*Corresponding author: simky@utar.edu.my

Mushrooms are highly perishable upon harvest, as they need appropriate postharvest treatments to prolong their storage time and to preserve the nutritional and chemical compositions. Generally, dehydration using heat is the most common practice to preserve the mushroom from getting spoil. For instance, the use of hot air, sun and microwave oven that aims to reduce the excessive moisture within mushrooms prior other processes (Manaa et al., 2013). As far as the nutritional and chemical compositions are concerned, the use of direct thermal processing is detrimental to the valuable properties as well as the sensory attributes of mushrooms. However, little information is available on the suitability of drying approaches in preserving the chemical and the proximate compositions of the high commercial value of *maitake* mycelia that was derived from submerged fermentation. Therefore, the present study aims to compare the influence of vacuum and oven drying methods on the antioxidant capacity and nutritional compositions of fermented *maitake* mycelia.

2 Materials and methods

2.1 Maitake stock culture preparation

The stock cultures of *maitake* (*Grifola frondosa*) were obtained from Mushroom Research Centre (MRC), University of Malaya and maintained on malt extract agar (MEA). The stock culture was prepared by cutting 10 plugs (approximately 5mm each) aseptically prior to transferring into a 250mL Erlenmeyer flask containing Mushroom Complete Medium (MCM) as basal medium and incubated at 25 °C using orbital shaker at 150 rpm for 7 days.

2.2 Submerged fermentation and harvest of mycelia biomass

A total of 10% (v/v) of the seed culture was transferred aseptically into a new 100 mL conical flask containing 100mL MCM solution. The flask was then sealed with cotton plug and allowed to incubate at 25 °C using orbital shaker at 150 rpm for 7 to 14 days. The biomass obtained was rinsed with distilled water and filtered by Whatman no. 1 filter paper. The filtrate was recovered from the liquid medium and centrifuged twice at 6000 rpm, 15 minutes. The mycelia pellet was collected and left to dry to remove excessive moisture before stored in air-tide container for subsequent analysis.

2.3 Drying treatments

Mycelia biomass was separated into three portions; the first portion of mycelium biomass was vacuum and dried under the temperature of 70 °C, 1000 mbar for 24 hours (Memmert VO, Germany) and the sample was then stored in sterilized falcon tube. The second portion of sample was dried using oven dryer (Memmert, Germany) at 105 °C for 24 hours. The fresh sample without underwent any drying treatment was assigned as control. All treated samples were milled into powder form using mortar and pestle and stored in a freezer at –20 °C until further analysis.

2.4 Determination of chemical analysis of maitake mycelia

Maitake mycelia were extracted overnight with methanol solvent at room temperature on a shaker at 150 rpm and filtered via Whatman no. 4 filter paper. The filtrate was further extracted by rotary evaporator (Buchi R210, US). The dried extract was used for analysis and re-dissolved in methanol to a concentration of 10 mg / mL. The total phenolic content of the *maitake* mycelia extract was determined using Folin-Ciocalteu method as described by Wong & Chye (2009). A total of 0.1mL extract prepared earlier was added with 0.75 mL of Folin-Ciocalteu reagent (Sigma). The resulting mixture was then measured at 725 nm after incubated for 90 minutes and the data was calculated by comparing to the gallic acid standard curve. The results were expressed as mg of Gallic Acid Equivalents (GAE) per gram of *maitake* mycelia extract.

Then, the DPPH radical scavenging activity of the *maitake* mycelia was tested for DPPH radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method as reported by Wong & Chye (2009). The procedure involved the measurement of test solution at 515 nm and the percentage of DPPH radical scavenging activity was calculated according to Formula 1 given:

$$\text{Radical Scavenging Activity } (\%) = \left[\left(A_{control} - A_{sample}\right) / A_{control}\right] \times 100\% \ (\text{A}: absorbance) \quad (1)$$

The determination of total soluble protein content was done using Bradford method and the Bovine Serum Albumin (BSA) as a standard procedure. The standard procedure was done with reference to Tripathy et al. (2014) procedure. The amount of soluble protein in *maitake* mycelia was calculated and expressed as μg protein / mL.

2.5 Determination of nutritional compositions of maitake mycelia

The nutritional composition analyses were done according to the Association of Officials Analytical Association (Association of Official Analytical Chemists, 1995). The moisture content of all mycelia samples were determined by drying at 105 °C for 6 hours. The protein and crude fat content were determined using Kjeldahl digestion and Soxhlet extraction method respectively. The ash content was determined by burning the samples in furnace at 550 °C for 3hours while the total carbohydrate content was calculated by subtracting the percentage of protein, crude fat, and ash content of tested samples (% Dry Weight).

2.6 Statistical analysis

All analyses were performed in triplicate and data were expressed as mean ± SEM. Statistical analyses were subjected to one-way ANOVA and Duncan's Multiple Range Test using SPSS (version 17) at 0.05 confident level.

3 Results and discussion

3.1 Yield of biomass and reducing sugar contents of submerged fermented maitake mycelia

The initial pH of the culture broth with *maitake* inoculums (10% v/v) was recorded at pH 5 and reduced to 4.2 ± 0.2 after 14 days of fermentation. The pH is always considered as one of the critical indicators for mycelia growth during fermentation. The decrease of pH indicated that the mycelia underwent submerged fermentation and the synthesis of metabolites was in place. Besides, the initial reducing sugar content of *maitake* mycelia was 74.8 ± 0.2 before reduced to 25.2 ± 0.3 µg / mL at the end of fermentation. As far as the fermentation was concerned, *maitake* culture utilized the nutrients in MCM and transformed them into desired polysaccharides, ketones and aldehydes that attributed to the formation of exopolysaccharides (EPS). According to Ding et al. (2012), the glucose level of the cultivation broth tends to reduce as a positive effort to promote formation of pallets and mycelium of mushroom.

The yield of biomass was 1.3 ± 0.1 g/L upon completion of submerged fermentation (14 days). However, the result obtained from this study was relatively lower than what was reported by Shih et al. (2008) who obtained 2.7 ± 0.1 g/L of biomass yielded after 9 days of cultivation. This might due to the use of different cultivation ingredients in their optimized growing medium. In fact, the growing medium used by Shih et al. (2008) had higher amount of yeast extract and corn steep powder as compared the one used in this study.

3.2 Total phenolic content of maitake mycelia treated by various drying methods

Phenolic are naturally heat sensitive phytonutrients that is available in plants including mushroom. Current result revealed that vacuum drying best preserve the phenolic content in *maitake* mycelia as compared to other treatments (Table 1). On contrary, the oven dried mycelia showed slightly lost ($P > 0.05$) of total phenolic content as compared to the fresh one. This clearly indicated that choosing a suitable drying approach is vital to maintain the valuable bioactivities of *maitake* mycelia during post-harvest stage. The vacuum drying preserved most of the phenolic components in mycelia as the dehydration on excessive moisture was done under vacuum condition. The lowering of atmospheric pressure enables the water loss efficiently without affecting its quality as compared to hot air drying (Pedro et al., 2010). Besides, the above scenario also related to the presence of additional phenolic compounds after the breakdown of plant's cellular constituents by vacuum heat (Chang et al., 2006)

Table 1. Effect of different treatments on the total phenolic content of *maitake* mycelia.

Samples / Treatments	Total Phenolic Content (mg GAE/g)
Vacuum	20.0 ± 0.4^{a}
Oven	16.5 ± 0.6^{b}
Fresh (Control)	17.0 ± 0.8^{b}

Data shown was means ± standard deviation with three replicates. Value with different superscripts within a column indicated significant different ($P < 0.05$) among samples.

3.3 The DPPH radical scavenging activity of maitake mycelia

The DPPH radical scavenging activity of *maitake* mycelia treated with various different treatments was marked with variation as shown in Figure 1. For example, the scavenging activity of *maitake* mycelia was in the order of: vacuum dried > fresh > oven dried samples. This indicated that vacuum drying enhanced the scavenging ability of *maitake* mycelia by 24% compared to the fresh sample (control), while oven drying showed the adverse effect. This can be attributed by the effectiveness of vacuum drying to preserve the heat-sensitive phenolic compounds within mycelia fibre from oxidation and denaturation. On contrary, the continuous direct heating on maitake mycelia has interrupted its hydrogen donating ability.

3.4 The nutritional compositions and soluble protein contents of maitake mycelia

The nutritional value of some edible mushroom mycelia was comparable or even higher than those found in their fruiting body (Cohen et al., 2014). This clearly indicated that the mushroom mycelia have the potential to be harvested and utilized at an early stage instead of until the full fruiting body development.

The use of proper dehydration approaches in post-harvest stage is critical to preserve and extend the shelf life of mycelia prior to further processing or consumption. Both vacuum and oven dried mycelia did not show significant ($p > 0.05$) difference for moisture content (Table 2) after treatments. However, the crude protein of oven dried mycelia was slightly higher ($p < 0.05$) than those found in vacuum dried mycelia and the controlled specimen. The use of oven drying promotes speedy removal of water molecule from mycelia and caused the breaking down of hydrogen bonds bonded with *maitake*'s protein backbone. Thus, it enhanced the hydrolysis of nitrogen-based components in mycelia matrices that eventually contributed to the crude protein content (Agoreyo et al., 2011). The crude protein content in this study was in accordance to Ulziijargal & Mau (2011) who reported that the protein content in *maitake* mycelia was relatively higher than those in fruiting body.

The drying treatments that was applied in this study were significantly ($p < 0.05$) affecting the crude fat content of *maitake* mycelia. The crude fat content for all treated samples was in

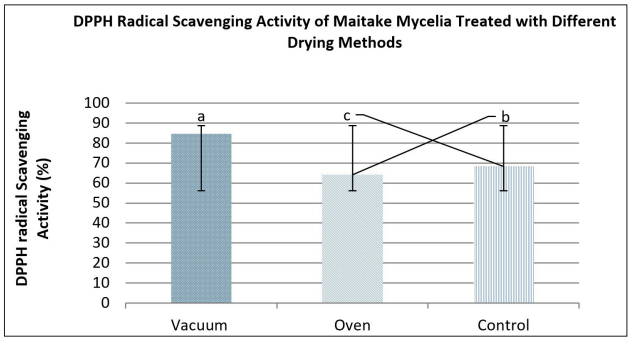

Figure 1. DPPH radical scavenging activity (%) of *maitake* mycelia treated with different drying methods. The data is indicated as the mean ± SEM; (n = 3), bars that display different letters are shown as significantly different ($P < 0.05$).

Table 2. Nutritional and chemical compositions of *maitake* mycelia treated with different drying approaches.

Samples	Moisture Content (%)	Nutritional Compositions (%DW)				Chemical Composition (% DW)
		Crude Protein	Crude Fat	Ash	Total Carbohydrate	Soluble Protein (μg/mL)
Vacuum	92.9 ± 0.9[a]	39.1 ± 0.2[a]	5.7 ± 0.7[a]	11.1 ± 0.3[a]	44.2 ± 0.2[c]	283.6 ± 7.1[a]
Oven	92.1 ± 0.3[a]	34.8 ± 0.1[b]	3.6 ± 1.2[b]	9.8 ± 0.2[b]	51.8 ± 0.9[b]	274.1 ± 9.5[c]
Fresh (Control)	N.A	3.1 ± 0.1[bc]	0.7 ± 0.2[c]	0.9 ± 0.1[c]	95.4 ± 0.3[a]	279.8 ± 4.8[b]

Data shown was means ± standard deviation with three replicates. Value with different superscripts within a column indicated significant different ($P < 0.05$) among samples.

the order of: vacuum dried > oven dried and control. Vacuum drying preserved the crude fat of mycelia as it reduces the boiling point of water at lower pressure. In fact, this enables the water molecules inside mycelia to evaporate easier and thus more non-polar molecules (especially fatty acids) were presented. This result was in line with those reported by Kalmis et al. (2011) that mushroom mycelia usually contains higher crude fat level at the initial development stages.

The ash content for vacuum dried mycelia was relatively ($p < 0.05$) higher than the oven dried mycelia. The high ash content could be attributed by the abundant of minerals such as sodium, calcium, iron, potassium and zinc within mycelia after incineration. Generally, the ash content for fruiting body and mycelia of edible mushroom species especially *maitake* were approximately 10% and 7% respectively (Ulziijargal & Mau, 2011). However, the total ash content of *maitake* mycelia found in this study was higher than those reported. On the other hand, the total carbohydrate content of fresh sample was the highest among all treated samples. Nevertheless, these carbohydrates tend to lose its function just similar to most heat sensitive nutrients of food. This explained why the total carbohydrate content for both vacuum and oven dried samples were relatively lower than the free ones.

Mushrooms have high protein content that is comparable to animal-based protein sources. According to Kalmis et al. (2011), the protein content of mushroom mycelia was comparatively higher than those available in fruiting body. Nevertheless, the protein content of mushroom can be affected by several factors such as their development stage, type of mushroom, the location of cultivation and post-harvest treatments. The trend of total soluble protein was in the order of vacuum dried mycelia > oven dried mycelia > fresh sample. Vacuum drying promotes better dehydration and releases of unbound peptides and amino acids of *maitake* mycelia. Vacuum drying encourages dehydration of moisture at lower pressure which might improve the release of the soluble peptide within *maitake* biomass. On contrary, oven drying manage to remove the moisture efficiently; however, it has caused the losses of volatile organic substances and low molecular compounds such as peptides, amino acids, organic acids and sugars.

4 Conclusion

The current study revealed that submerged fermentation was able to cultivate the high value *maitake* mycelia with desirable biomass. The *maitake* mycelia exhibited higher phenolic contents after underwent vacuum drying. Vacuum drying at 70 °C, 1000 mBar was found to be effective in preserving the radical scavenging activity and major nutritional contents of *maitake* mycelia as compared to direct oven drying. This study concluded that vacuum drying can be considered as one of the low cost post-harvest approach in improving *maitake* quality.

Acknowledgements

The authors are grateful for the financial support of this research from the Ministry of Higher Education of Malaysia by awarding the RAGS grant (R/RAGS/A07.00/01153A/001/2015/000213). A special thanks to Professor Dr Vikineswary Sabaratnam and research staffs from the Mushroom Research Centre, University of Malaya for the technical support throughout the project.

References

Agoreyo, B. O., Akpiroroh, O., Orukpe, O. A., Osaweren, O. R., & Owabor, C. N. (2011). The effects of various drying methods on the nutritional composition of Musa paradisiaa, Dioscorea rotundata and Colocasia. *Asian Journal of Biochemistry*, 1, 1-7. http://dx.doi.org/10.3923/ajb.2011.458.464.

Association of Official Analytical Chemists – AOAC. (1995). *Official methods of analysis of the Association of Official Analytical Chemists* (16th ed). Arlington: AOAC.

Bhattacharya, M., Srivastav, P. P., & Mishra, H. N. (2014). Optimization of microwave-convective drying of Oyster mushrooms (*Pleurotus ostreatus*) using response-surface methodology. *International Food Research Journal*, 21(4), 1575-1581.

Chang, C. H., Lin, H. Y., Chang, C. Y., & Liu, Y. C. (2006). Comparisons on the antioxidant properties of fresh freeze-dried and hot-air-dried tomatoes. *Journal of Food Engineering*, 77(3), 475-485. http://dx.doi.org/10.1016/j.jfoodeng.2005.06.061.

Cohen, N., Cohen, J., Asatiani, M. D., Varshney, V. K., Yu, H. T., Yang, Y. C., Li, Y. H., Mau, J. L., & Wasser, S. P. (2014). Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary-medicinal higher basidiomycetes mushrooms. *International Journal of Medicinal Mushrooms*, 16(3), 273-291. PMid:24941169. http://dx.doi.org/10.1615/IntJMedMushr.v16.i3.80.

Deng, G., Lin, H., Seidman, A., Fornier, M., D'Andrea, G., Wesa, K., Yeung, S., Cunningham-Rundles, S., Vickers, A. J., & Cassileth, B. (2009). A phase I/II trial of a polysaccharide extract from *Grifola frondosa* (Maitake mushroom) in breast cancer patients: Immunological effects. *Journal of Cancer Research and Clinical Oncology*, 135(9), 1215-1221. PMid:19253021. http://dx.doi.org/10.1007/s00432-009-0562-z.

Ding, Z. Y., Wang, Q. Q., Peng, L., Zhang, L., Gu, Z. H., Shi, G. Y., & Zhang, K. C. (2012). Relationship between mycelium morphology and extracellular polysaccharide production of medicinal mushroom *Ganoderma lucidum* in submerged culture. *Journal of Medicinal Plants Research*, 6(14), 2868-2874. http://dx.doi.org/10.1016/j.jhazmat.2010.12.097.

Gregori, A., Svagelj, M., Berovic, M., Wraber, B., Simcic, S., & Boh, B. (2011). Immunomodulating activities of cultivated Maitake medicinal mushroom Grifola frondosa (Dicks.: Fr) Gray (Higher basidiomycetes) on peripheral blood mononuclear cells. *International Journal of Medicinal Mushrooms*, 14(4), 377-383. http://dx.doi.org/10.1615/IntJMedMushr.v14.i4.50. PMid:22164768.

Kalmis, E., Yildiz, H., Ergonul, B., Kalyoncu, F., & Solak, M. H. (2011). Chemical composition and nutritional value of a wild edible ectomycorrhizal mushroom, *Tricholoma anatolicum. Turkish Journal of Biology*, 35, 627-633. http://dx.doi.org/10.3906/biy-0909-100.

Kim, S. W., Hwang, H. J., Lee, B. C., & Yun, J. W. (2007). Submerged production and characterization of *Grifola frondosa* polysaccharides: a new application to cosmeceuticals. *Food Technology and Biotechnology*, 45(3), 295-305.

Manaa, S., Younsi, M., & Moummi, N. (2013). Study of methods for drying dates: review the traditional drying methods in the Region of Touat Wilaya of *Adrar-Algeria. Energy Procedia*, 36, 521-524. http://dx.doi.org/10.1016/j.egypro.2013.07.060.

Pedro, M. A. M., Telis-Romero, J., & Telis, V. R. N. (2010). Effect of drying method on the adsoption isotherms and isosteric heat of passion fruit pulp powder. *Ciencia Technologia Alimentos*, 30(4), 993-1000. http://dx.doi.org/10.1590/S0101-20612010000400024.

Shih, I. L., Chou, B. W., Chen, C. C., Wu, J. Y., & Hsieh, C. (2008). Study of mycelia growth and bioactive polysaccharide production in batch and fed-batch culture of Grifola frondosa. *Bioresource Technology*, 99(4), 785-793. PMid:17363244. http://dx.doi.org/10.1016/j.biortech.2007.01.030.

Toledo, R. C. C., Carvalho, M. A., Carlos, L., Lima, O., Vilas-boas, E. V. D. B., & Dias, E. S. (2013). Measurement of β-glucan and other nutritional characteristics in distinct strains of *Agaricus subrufescens* mushrooms. *African Journal of Biotechnology*, 12(43), 6203-6209. http://dx.doi.org/10.5897/AJB2013.13024.

Tripathy, S. S., Rajoriya, A., & Gupta, N. (2014). Nutritive properties and antioxidative activity of *Amanita caesarea* and *A. loosii* wild edible mushrooms from Odisha. *Innovative Drug Discovery*, 4(3), 124-129.

Ulziijargal, E., & Mau, J. L. (2011). Nutrient compositions of culinary-medicinal mushroom fruiting bodies and mycelia. *International Journal of Medicinal Mushrooms*, 13(4), 343-349. PMid:22164764. http://dx.doi.org/10.1615/IntJMedMushr.v13.i4.40.

Wong, J. Y., & Chye, F. Y. (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis*, 22(4), 269-277. http://dx.doi.org/10.1016/j.jfca.2008.11.021.

Wu, T. X., Wang, N., Zhang, Y., & Xu, X. B. (2016). Research progress in submerged mycelia culture of *Grifola frondosa*, a culinary-medicinal mushroom. *African Journal of Microbiological Research*, 10(6), 138-146. http://dx.doi.org/10.5897/AJMR2013.6453.

Yeh, J. Y., Hsieh, L. H., Wu, K. T., & Tsai, C. F. (2011). Antioxidant properties and antioxidant compounds of various extracts from the edible Basidiomycete *Grifola frondosa* (maitake). *Molecules (Basel, Switzerland)*, 16(4), 3197-3211. PMid:21499220. http://dx.doi.org/10.3390/molecules16043197.

Zhang, Y. Q., Geng, W., Shen, V. Q., Wang, Y. L., & Dai, Y. C. (2014). Edible mushrooms cultivation for food security and rural development in China: bio-innovation, technological dissemination and marketing. *Sustainability*, 6(5), 2961-2973. http://dx.doi.org/10.3390/su6052961.