Special Publication of TARI No. 148

International Training Course on Edible Mushroom Production for Asian Farmers and Entrepreneurs

November 22-26, 2010

Venue: Taiwan Agricultural Research Institute, COA, Taiwan, ROC



Organizers

Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC/ASPAC)

Taiwan Agricultural Research Institute (TARI), Council of Agriculture (COA), Taiwan, ROC

Asia-Pacific Association of Agricultural Research Institutions (APAARI)

Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA)

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Practical Handbook for Edible Mushroom

Edited by
M. H. Chen, T. Y. Ku, and H. D. Shih

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International Training Course on Edible Mushroom Production for Asian Farmers and Entrepreneurs

Nov 22-Nov 26, 2010 TARI/COA, Taichung, Taiwan, R.O.C

Program of Activities

***Nov 21* (Sun)**	Arrival of overseas participants Stay at Enterprise Hotel, Taichung City No.160, Ho Ping St., Taichung City, Taiwan Tel: +886-4-2220-7733 Fax: +886-4-2221-7540
***Nov 22* (Mon)**	Field trip to observe edible mushroom cultivation and management including on-farm demonstration, technical orientation and sharing of practical experiences with mushroom growers vis-à-vis, and Q&D at mushroom farms
08:10	Hotel pick-up
09:00-12:10	Visit button mushroom farm in Changhua County and straw mushroom farm in Nantou County as a household industry Dr. Hsin-Der Shih
12:10-13:30	Lunch break
13:30-17:00	Visit Richyear mushroom farm and other farms en route at Puli area, Nantou County
18:00-19:20	En route to hotel, informal dinner to be arranged at TARI
19:20-	Back to Hotel Stay at Enterprise Hotel, Taichung City
***Nov 23* (Tue)**	
08:10	Hotel pick-up
09:00-09:40	Visit and briefing of Taiwan Agricultural Research Institute
09:40-10:00	Registration
10:00-10:25	Opening session
10:25-10:40	Tea/coffee break

10:40-12:00	Overview and prospects of edible and medicinal mushrooms: Production, consumption, and marketing in Taiwan	Dr. Jin-Torng Peng
12:00-13:30	Lunch break	
13:30-15:20	Practicum: Preparation of potato-dextrose agar (PDA) and grain spawn	Mr. Kaun-Tzer Wu Dr. Yun-Sheng Leu
15:20-15:30	Tea/coffee break	
15:30-16:20	Practicum: Tissue isolation and sub-culture	
16:30-17:20	Practicum: Preservation of mushroom cultures	
18:30-20:30	Welcome dinner hosted by TARI/COA Stay at Enterprise Hotel, Taichung City	

***Nov 24* (Wed)**

08:10	Hotel pick-up	
09:00-10:50	Using agro-wastes as basic raw materials for cultivation of edible mushrooms in Taiwan	Dr. Jin-Torng Peng
10:50-11:10	Tea/coffee break	
11:10-12:00	Practicum: Cultivation technologies for button mushroom	Dr. Jin-Torng Peng Mr. Jin-Tong Chen
12:00-13:30	Lunch break	
13:30-17:20	Practicum: Substrate preparation techniques for mushrooms Stay at Enterprise Hotel, Taichung City	Mr. Jin-Tong Chen Mr. Wei-Sung Li

***Nov 25* (Thu)**

08:10	Hotel pick-up	
09:00-10:00	Nutritional and medicinal value of mushrooms	Dr. Jeng-Leun Mau
10:00-10:10	Tea/coffee break	
10:10-12:00	Sawdust-bag cultivation technologies for mushroom production	Dr. Mei-Hsing Chen
12:00-13:30	Lunch break	

13:30-15:20	Practicum: Inoculation of logs and bagged substrates for mushroom cultivation	Mr. Wei-Sung Li Dr. Yun-Sheng Leu
15:20-15:40	Tea/coffee break	
15:40-17:30	Discussion and final wrap-up, distribution of certificate and closing	
18:30-20:30	Appreciation dinner hosted by FFTC Stay at Enterprise Hotel, Taichung City	

***Nov 26* (Fri)**	Field trip	
08:00	Hotel pick-up	
09:00-10:00	Visit Tai Mushroom Farm to observe mushroom as a full-blown business and rural enterprise development	Dr. Hsin-Der Shih
10:00-12:10	En route to visit Q-Yo Bio-Technology Farm in Changhua County	
12:10-13:30	Lunch break	
13:30-16:30	Depart for Taipei City Free in the rest of the day Stay at Howard International House Taipei No.30, Xin Sheng South Rd., Sec 3, Taipei City Tel: + 886-2-8369-1155 Fax: + 886-2-8369-1177	

***Nov 27* (Sat)** Departure of overseas participant to respective country

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Preface

Mushrooms are used as food as well as medicine since time immemorial. Their production can be fitted in as a prime model for the 21st century's Green Production System – characterized by resource conservation and utilization – in which various agricultural and forestry wastes/leftovers are converted into high value food/mushroom products, and where zero-emission of wastes can be established in the process of mushroom production where complete recycling of natural materials is attainable. Thus, mushroom production using agricultural and forestry wastes/ leftovers could contribute greatly to the development of a self-sustaining rural community, particularly in countries of the Asian and Pacific (ASPAC) region.

Cultivation techniques on mushroom are well developed in Taiwan. There are more than twenty kinds of mushroom available all year-round and are used in many kinds of table dishes. Mushrooms are valuable health food which is low in calories, and high in vegetable proteins, fiber, vitamins and minerals. They have a long history of use in traditional Chinese/oriental medicine to promote good health and vitality and in increasing the body's adaptive abilities. Consequently, the production of edible mushrooms is increasing rapidly in Taiwan and throughout some parts of the world.

The Taiwan Agricultural Research Institute (TARI) has long been being the only government-supported mushroom research station in Taiwan. In its mushroom laboratory, research and extension cover many aspects of edible and medicinal mushrooms, including breeding and collecting mushroom varieties, improving culture techniques, and developing mushroom products. More than fifteen mushroom species have been studied in this laboratory in the past sixty years.

Therefore, it is my pleasure to present this report to the attention of participants to the first International Training Course on Edible Mushroom Production for Asian Farmers and Entrepreneurs, to be held on November 21-27, 2010 at our institute, TARI/COA in Taichung, Taiwan ROC. I hope this handbook will be useful in providing participants – researchers, government officers, and extension

workers in the ASPAC region with a working knowledge on improved modern technologies on household-scale or industrial mushroom production. We take the liberty to conduct this first international training program on mushroom at TARI with a strong belief that this activity will enhance the sustainable commercial production of edible mushroom by Asian farmers and entrepreneurs who want to incorporate mushrooms into their systems either as additional enterprise or as a full-blown business.

I would like to thank the authors and lecturers from TARI and from other institutions for their support in completing this report. Furthermore, I would like to thank those who jointly organized this training course – the Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC/ASPAC), the Asia-Pacific Association of Agricultural Research Institutions (APAARI), and the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA). Finally, special thanks go to the Council of Agriculture (COA) of Taiwan ROC for funding this training course.

Junne-Jih Chen, Ph. D.
Director General
Taiwan Agricultural Research Institute

Overview and prospects of edible and medicinal mushrooms: Production, consumption, and marketing in Taiwan

Dr. Jin-Torng Peng

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Introduction

The cultivation of edible mushroom has been starting quite earlier in the midst of 20th century in Taiwan and resulted quickly in developing its canned mushroom (common white mushroom) exporting market led the world for more than a decade in 1970s. During the high time of maintaining its exporting market, the farmer grown common white mushroom mainly in a rather simple plastic house which was constructed with local materials of bamboo or wood and covered with a sheet of red mud plastics to keep away from raining and sun lights. The mushroom house was installed with a simple ventilator and a plastic duct for keeping ventilation and re-circulation of air. To maintain steady supply and demand for the exporting market, more than ten thousands of farmers were keeping on line to fully utilize these simple constructed mushroom houses to cultivate the common white mushroom during cooler months of the year from October to March.

Thus, incentive measures were established to encourage mushroom growers/farmers such as they could get subsidiary payments from government to build their own mushroom house or to buy pesticides or other agricultural chemicals to control mushroom diseases and insects. In addition, to improve their technology to grow the mushroom, farmers could also get needed information and training from local Farmers' Association. The latter obtained financial supports

from the government. In order to maintain the consistent production of the common white mushroom, the farmers were obligated to buy mushroom spawn from domestic registered spawn markers who got the mother culture of the common white mushroom from the governmental research institute (TARI). The institute conducted the research on the improvement and maintenance of living mushroom cultures and supplied new hybrids to the spawn makers for making and selling mushroom spawn to mushroom farmers.

Mushrooms thus produced were brought by mushroom farmers themselves to collection farm gate/station for weighing, recording and pre-washing were done by the staffs from canneries prior shipping to mushroom canneries/factories. Furthermore, all mushrooms were sold at a guarantee price which was settled down by negotiation by both parties of the government and canneries.

Since mid-1980s, however, due to increased production costs and the appreciation of New Taiwan dollar against US dollar, these have resulted in great impact on its competitiveness edge of Taiwan's canned mushrooms in international markets. The government authority decided to pull out its production system for common white mushroom for exporting market during the crop year of 1993/1994. Since then the production of common white mushroom along with other kinds of edible mushrooms was directed toward their marketing mainly for domestic consumption. To meet demand for local consumption, more and more mushroom farmers joined in to expand to add in growing a variety of edible or medicinal mushrooms, to name a few such as *Volvariella volvacea, Auricularia* spp., *Lentinula edodes*, *Pleurotus* spp., *Ganoderma* spp. etc. mainly for domestic markets and limited for exportation. Along with the demand in mushroom industry in Taiwan, earlier for exporting market and in recent years to meet the demand for domestic consumption, greater efforts have been driving toward research and professional enhancement in harnessing technology for sustainable development of mushroom production. For example, in addition to grow a variety of conventional edible mushrooms, several new mushroom species were also developed for large-scale commercial cultivation in environmentally controlled

facilities/rooms or traditional plastic mushroom houses during the past 20 to 30 years, such as *Flammulina velutipes*, *Agrocybe cylindracea*, *Pleurotus eryngii*, *Hypsizigus marmoreus*, *Hericium erinaceum*, *Pleurotus sajor-caju*, *Pleurotus citriopileatus*, *Agaricus braziliensis*, *Clitocybe nuda* etc. All these mushrooms are produced mainly to meet an increase in demand for domestic market and consumption and a small portion of them for export.

Major edible and medicinal mushrooms grown in Taiwan

My report will confine to the most recent situation of the development of major edible and medicinal mushrooms commonly grown in Taiwan. There is no official statistic data available yet for the production and consumption of various major commonly grown edible and medicinal mushrooms for the crop year of 2009/2010 in this country. I'll use the estimated figures based on data supplied by local mushroom traders and mushroom spawn suppliers or their inoculated substrate used in the mushroom production areas. Therefore the production data of these mushrooms reported here is an estimated figure as shown in Table 1. The major commonly grown and consumed edible and medicinal mushrooms in Taiwan are described below.

• **Golden mushroom—*Flammulina velutipes***

Nowadays, golden mushroom is grown exclusively on a sawdust substrate filled in bottles in an environmentally controlled room and the sawdust used is derived mainly from the tree of *Cunninghamia lanceolata* var. *lanceolata* or broad leaf trees. Rice bran is commonly used as the major nutritional supplement with a small portion of wheat bran and soybean meal. Operation of most growing processes, including substrate filling, sterilization, inoculation and removing out of old inoculants and spent substrates are conducted with automatic machines. It takes 55 days to 60 days from inoculation up to the end of crop harvest.

Due to the increasing costs of energy and raw materials, including sawdust and nutritional supplements, and the long standing low price of golden mushrooms in recent years, some small

mushroom farms were forced to reduce or even abandon their production, while some other farms have switched to growing other profitable mushroom species, such as *Pleurotus eryngii* and *Hypsizigus marmoreus*. Currently, there are 25 golden mushroom growers with their production amounts to an estimated 24,000 MT in the crop year of 2009/2010 and were about 30,000 MT in the last crop year 2008/2009. Farm gate price for golden mushroom was about US$ 0.8～1.3/kg varying depending on the season of the year. This mushroom is consumed mostly in cool months or cold winter season and festivities by serving with cooking in a hot pot with assorted meats, vegetables and other food ingredients. Again, due to its low price and the introduction of some newly emerged mushroom species into markets instead, it is foreseeable that the production and consumption of golden mushroom shall be declining in the coming years.

• **Shiitake**—***Lentinula edodes***

Shiitake has been one of the oldest mushroom favorite consumed in Taiwan and used to be mostly cultivated on wood logs or decayed tree cuts. In recent decades, however, the production techniques have been gradually changing to use sawdust substrate filled in a PP bag instead in replacement of wood logs. Nowadays, the majority of mushroom growers are using sawdust substrate to cultivate the mushroom. The sawdust used for Shiitake cultivation is mostly derived from the hard wood trees such as *Acacia confusa* and *Liquidamber formosana* and/or some others. Rice bran is the main nutritional supplement with addition of a small portion of wheat bran and corn meal. Filling of substrate into bags is conducted by a filling machine. The substrate is pasteurized at ca 98～100℃ for at least 4 hours. Wheat grain spawn is used for inoculation of the pasteurized substrate. After incubation it usually takes 3 to 4 months before stimulation for fruiting.

In the crop year of 2009/2010 seasons, total production of shiitake was an estimated 28,000 MT, comparing to 36,000 MT of the last crop year 2008/2009 up to current. This reduction in total production was due partly to the drastic change in weather extreme

conditions, either too cold or too hot, to decreasing quality of sawdust and to a possible change in genetic make-up of shiitake spawn supplied by a few spawn makers. The current farm gate price of fresh shiitake rose to about US$3.1～3.5/kg in comparing to US$2.6～3.1/kg of the last crop year. Difference in the consumption pattern of Shiitake mushroom in a year may be not significant as that of the declining of golden mushroom because of their different cooking ways and prices of the two mushroom species which both determine the amount of consumption. Shiitake has been extensively used in Chinese cuisine and not easily replaced by other edible mushrooms species. The price of this mushroom is quite stable, either fresh or dried products in the marketplaces, comparing to other mushroom species. As long as the genetic stability of the spawn strain and the reliability of sawdust sources can be maintained and solved, shiitake is still a profitable crop in the future.

• **King oyster mushroom**—***Pleurotus eryngii***

This mushroom was firstly developed by Taiwan Agricultural Research Institute for growing on the substrate filled in a bottle because such way of cultivation can be highly automatic with consistent production in an environmentally controlled mushroom house. It can be grown with the same machineries and facilities as those for growing golden mushroom during the period of low mushroom price of the latter, i.e. in summer. King oyster mushroom can be grown on rice straw or sawdust substrate, however it is more convenient to use sawdust rather than rice straw due to the easiness of operation during cultivation when using sawdust as basic raw material. Rice bran is the main nutrient supplement with a small proportion of wheat bran. The sawdust substrate can also be filled in a PP bag for sterilization or pasteurization. After such heat treatment, the substrate is ready for inoculation when it cools down to 20℃. Incubation for spawn running at 21℃ usually takes 35 to 40 days prior to reducing temperature to initiate fruiting. Mature fruiting bodies can be harvested 15 to 20 days after stimulation depending on the spawn strain used.

The technology for growing king oyster mushroom using bottle system has been developed for almost 20 years. Due to its excellent culinary characteristics and unique texture of its cap and stalk it has been accepted and become the favorite food of many people. It can be cooked in many ways enhancing the consumption of this mushroom. There are more than 50 growers to cultivate this new king oyster mushroom species with an estimated total yearly production of about 12,000 MT for the crop year of 2009/2010. The fresh products of king oyster mushroom are classified into four grades, i.e. A, B, C and D. The farm gate prices for the above grades are US$2.6～3.3, 2.2～2.6, 1.6～1.9 and 1.0～1.3/kg respectively.

King oyster mushroom is a weak fungus and vulnerable to the competition of nutritional requirements from other harmful microorganisms and thus, preventive management measures need to be taken to keep away of contamination. In addition, the growth and development of this mushroom is also very sensitive to the changes in environmental factors during cultivation, such as temperature, humidity, concentration of carbon dioxide, movement of air ventilation and light intensity. These results lead to difficult to maintain its stability for production and thus prone to risk to lose of profitability. Due to the difficulties in the management to grow this mushroom species, many growers shifted to growing a newly introduced mushroom species called Bunashimeji – *Hypsizigus marmoreus,* from Japan, *thereby,* resulting in shortage of the supply of king oyster mushroom and also in the rise of its farm gate price during the crop year of 2009/2010. Since this mushroom species has become very popular in the market and can be cooked in many ways in Chinese cuisine, the demand for this mushroom in the market will still be strong in the coming years. However its farm gate price might go down if the stability of the technology for this mushroom species can be solved or its import from Korea or other countries is greatly increased.

• **Phoenix tail mushroom**—***Pleurotus sajor-caju***

This mushroom is grown exclusively on sawdust substrate filled in a PP plastic bag. The substrate is pasteurized or sterilized before

inoculation. It takes about three months for incubation prior to subjecting them in reducing temperature for the induction of fruiting. This mushroom is usually grown in a traditional plastic mushroom house without a climate control and is able to grow above 20℃. In order to have a uniform fruiting it is suggested that the fully colonized substrate be subjected to low temperature treatment (chilling). Four to six flushes or even more can be harvested with a yield of 300 to 400g per bag (with1.2 kg of fresh substrate). Fruiting bodies of the spawn strain cultivated in Taiwan are small ones with a thin context of cap. Besides, the fruiting bodies grow very fast, so that the management of humidity in a mushroom house is extremely important to this mushroom strain. It is usually necessary to harvest the mushroom three times a day to keep the high quality level of the products. There were more than 100 mushroom growers to cultivate this mushroom species with a yearly production of an estimated 12,000 MT in the crop year of 2009/2010. Farm gate price for this mushroom was US$1.8～2.7/kg, however it was US$1.4～1.7/kg in the last crop year.

Due to the inefficient use of mushroom house and the vulnerability to green molds (*Trichoderma spp.* and *Penicillium spp.*) after the first flush, many growers who used to cultivate this mushroom with air-conditioning facilities switched to growing Bunashimeji. This resulted in the reduction of production and the rise of farm gate price of this mushroom in the crop year of 2009/2010. The price of this mushroom species may still keep high for a period of time, however the demand for this mushroom and its production as well may gradually reduce if the consumers are getting used to other new mushroom species.

• **Common white mushroom—*Agaricus bisporus***

There are still a few mushroom growers who cultivate the common white mushroom in a traditional plastic mushroom house without a climate control. These mushroom growers cultivate this mushroom mainly in cooler weathers during fall and winter seasons. After cropping/harvesting, most of the mushroom farmers use the spent composts again to grow other mushroom species, such as straw

mushroom (*Volvariella volvacea*) or hot mushroom (*Agaricus bitorquis*), so that maximize the utilization efficiency of spent compost and mushroom house facilities to cut down production cost.

In Taiwan, nowadays most mushroom farmers cultivate common white mushroom in a well insulated mushroom house with a climate control. However, the scale of their white mushroom production is not as big as those of developed countries and most often Taiwan's farmers own only 6 to 12 or a little more mushroom growing rooms. They also have one Phase I tunnel, two Phase II tunnels and two Phase III tunnels for making their own compost and spawn run compost. Some mushroom growers do not have Phase I tunnel; they prepare Phase I compost outdoors instead.

Most mushroom growers cultivate only one mushroom species, however, very few of them manage to grow other species, i.e. *Clitocybe nuda*. There were about 20 mushroom growers with a year production of an estimated 3,000 MT in the crop year of 2009/2010, while farm gate prices fluctuated for domestic fresh markets for this mushroom kept at the level of US$3.2~4.5/kg.

The price of the common white mushroom appears quite stable in most parts of the year but the price may drop to some extend in the winter due to competition from the cheap mushrooms grown in a traditional mushroom house. Growing common white mushroom is labor intensive and hard working, fewer farmers want to involve in its cultivation, so that there is a tendency in the number of the common white mushroom growers to reduce in the recent years. However, there is no competition from imported mushrooms, the above mushroom price and the consumption of this mushroom species anticipate stable for the years to come.

• **Bunashimeji—*Hypsizigus marmoreus***

This new mushroom species was introduced into Taiwan markets from Japan by one of our mushroom traders several years ago. It appears good market potential and acceptable by local consumers. Some mushroom growers start growing this mushroom and the Japanese supplier of this mushroom has already set up production premises in

southern Taiwan for commercial cultivation and has already started supplying the mushroom to marketplaces. Bunashimeji mushroom can be grown with a bottle or bag system in good control modern facilities. When using bottle system the operation of growing processes can be very automatic.

Now there are two strains of this Bunashimeji mushroom species on the markets, one is brown and another is white. The brown one is exclusively grown in a bottle, while the white one can be grown in a bottle or in a bag. The fruiting bodies of the Bunashimeji grown in a bottle have a small cap and a short stalk with a solid texture, while those grown in a bag usually appear to have a larger cap and a longer stalk with a softer texture than the one grown in a bottle. When using bottle system, incubation of the above two bunashimeji strains takes nearly three months before they can be stimulated to initiate fruiting by reducing temperature and increase ventilation in giving supply of more fresh air. When using bag system, the white strain also needs three months for incubation of the inoculated substrate. It takes about 30 days from stimulation to harvest no matter what cultivating system is used. When using bag system, 300 to 400 grams of mushroom can be harvested from one bag which has 1.2kg of fresh substrate. The growers usually harvest one flush only.

Due to the easiness to get initiation of fruiting, the stability and profitability of production as well as the less labor cost, many mushroom growers who used to cultivate king oyster mushroom or phoenix tail mushroom, shifted to growing Bunashimeji in the last crop year 2008/2009. This resulted in lowering of the farm gate price of this mushroom. The total production of Bunashimeji in the crop year of 2009/2010 was an estimated 2,000 MT and the farm gate price for the one grown/harvest in a bottle was in the range of US$ 4.8～9.7 /kg, while the one grown in a bag was at the level of US$2.3～3.2/kg.

The difference in farm gate prices between golden mushroom and Bunashimeji is so evident that it is anticipated the growers of the former will have a strong tendency to shift to growing the latter for profitability for the years ahead. This may result in gradually lowering of the price of Bunashimeji mushroom. Besides, the golden mushroom

growers may have the advantage to fully utilize their existing facilities and bottle system to cultivate Bunashimeji mushroom than growers whom are cultivating king oyster mushroom or phoenix tail mushroom.

• **Jew's ear-- *Auricularia* spp.**

This fungus is grown on a sawdust substrate filled in a bag and the substrate is pasteurized before inoculation with sawdust spawn. Incubation takes about one month before stimulating it to fructify. Most of the fungal growers do not prepare their own bags of substrate -- they just buy them from the professional substrate bag makers. The fungus is cultivated under a mushroom shed covered with plastic sheet and black plastic net. It takes about 21 days from initiation of fruiting bodies to harvest. Four to six flushes can be harvested before termination of the crop. The total production of Jew's ear fungus in the crop year of 2009/2010 in Taiwan estimated about 3,000 MT and the farm gate price for this fungus ranges at the level of US$ 1.3～1.9/kg. The price of Jew's ear mushroom is comparatively low in comparing with the prices of most of other mushroom species. Nevertheless, since consumers recognize its nutrient value and medicinal benefits to health, the consumption of this fungus remains steady increases in the recent years.

• **Straw mushroom—*Volvariella volvacea***

This mushroom is usually to be grown on a pasteurized cotton waste which is covered on the top of spent mushroom compost in a traditional plastic mushroom house. The same spent substrates can be repeatedly used for cultivation of straw mushroom four to five times in the same mushroom house without removing the old spent substrates prior to again starting a new crop of the common white mushroom. Two to three flushes can be harvested for each crop of straw mushroom with a yield of 900 to 1,200 kg in a mushroom house of which bed surfaces are 165 square meters. The total production of straw mushroom in the crop year of 2009/2010 estimated about 1,000 MT and the farm gate price for this mushroom ranges at the level of

US$ 1.3～1.7/kg, in Taiwan. All fresh straw mushrooms produced were sold to the markets for domestic consumption.

• **Summer oyster mushroom—*Pleurotus cystidiosus***

This mushroom species has been growing for more than 40 years in Taiwan. Even with easiness in the cultivation of summer oyster mushroom whole year round in Taiwan, nevertheless, due to the emerging development or introduction of some new edible mushroom species in recent years, such as phoenix tail mushroom, king oyster mushroom and Bunashimeji, the summer oyster mushroom is facing increased competition in its market share from those new mushroom species. The total production of this mushroom in the crop year of 2009/2010 estimated about 600 MT and the farm gate price for this fungus ranges at the level of US$ 0.4～0.6/kg.

• **Yuhuanmo—*Pleurotus citriopileatus***

This mushroom is usually grown on a pasteurized sawdust substrate filled in a bag. The growers buy inoculated substrate bags and incubate them in their own mushroom house or shed. It takes about one month for colonization of spawn mycelium prior to initiating/stimulation for fruiting. In Taiwan, the total production of this mushroom in the crop year of 2009/2010 estimated about 300 MT and the farm gate price for this fungus ranges at the level of US$ 0.6～1.1/kg.

• **Brown swordbelt—*Agrocybe* spp.**

This mushroom can be cultivated either on sterilized or pasteurized sawdust substrates filled in a bottle or in a plastic bag depending on the mushroom strain used. High temperature strain of brown swordbelt is usually grown in bags without a climate control, while the low temperature one is grown in bottles in a well insulated mushroom house with an environmental control. In Taiwan, the total production of *Agrocybe* spp. in the crop year of 2009/2010 estimated about 300 MT and the farm gate price for the one grown in a bottle ranges at the

level of US$ 3.2～4.8/kg, while the one grown in a bag ranges at the level of US$2.9～3.9/kg.

• **Sunny mushroom—*Agaricus braziliensis***

Sunny mushroom is mostly grown on a pasteurized sawdust substrate filled in a bag. After the substrate has been fully colonized by the fungal mycelium, it is necessary to cover the substrate surfaces with a layer of casing soil for fruiting. Some farmers cultivate this mushroom on rice straw compost. The total production of *A. braziliensis* in the crop year of 2009/2010 was an estimated 350 MT and the farm gate price for fresh mushroom ranges at the level of US$ 7.1～9.7/kg, while for the dried products was in the range of US$96.8～112.9/kg.

• **Oyster mushroom—*Pleurotus ostreatus***

The oyster mushroom is usually grown on a pasteurized sawdust substrate filled in a bag. When the pasteurized sawdust substrate has been fully colonized by the fungal mycelium grown out from wheat grain spawn, the substrate bags are moved to a well insulated mushroom house of which environmental factors, such as temperature, relative humidity and carbon dioxide concentration and light, are strictly monitored and well controlled. About 200 to 250 grams of fresh mushrooms can be harvested in the first flush from each bag with 900 grams of fresh substrate. In Taiwan, the total production of oyster mushroom in the crop year of 2009/2010 estimated to reach 100 MT and the farm gate price for fresh mushroom was in the range of US$ 2.6～3.2/kg.

• **Shaggy cap—*Coprinus comatus***

This mushroom is grown on a pasteurized sawdust substrate filled in a bag. When the Shaggy mushroom mycelium has fully grown through the substrate, the plastic bags are completely removed then a layer of casing soil is laid on the surfaces of exposed substrate for inducing fructification. In Taiwan, the total production of shaggy cap in the crop year of 2009/2010 estimated to reach 50 MT and the farm gate price for fresh mushroom was in the range of US$4.0～5.2/kg.

• **Lingzi—*Ganoderma* spp.**

Lingzi is a medicinal mushroom which is usually grown on a sterilized sawdust substrate filled in a bag under a plastic mushroom shed. It takes about one month for incubation prior to it can be stimulated for fruiting and then takes a duration three weeks to one month for the development of fruiting bodies to the stage to be harvestable. The Lingzi products of harvested fruiting bodies are usually dried for direct sale or to be processed for extraction of useful components either to make capsules or as liquid drinks. The total production of dry Lingzi products in the crop year of 2009/2010 estimated about 15 MT and the farm gate price for dry Lingzi mushroom ranges at the level of US$15.4~21.5/kg.

Marketing and distribution of mushroom products

There has been well established for stakeholders in dealing with the marketing and distribution of fresh or dried mushroom products. Small volume growers generally market their fresh mushrooms to jobbers (secondary wholesalers) or shippers, while large volume growers usually market their mushrooms to shippers and wholesalers. Jobbers sell their mushrooms to traditional markets (retailers), restaurants, groceries and institutions. Shippers collect and buy fresh mushrooms from both small and large volume growers and sell their mushrooms to jobbers, traditional markets, supermarkets, hypermarkets and wholesalers as well as restaurants and Institutions. Wholesalers sell their mushrooms to supermarkets or hypermarkets (Fig. 1).

Fresh mushrooms are packed loose in bulk bags or in various sizes of boxes, or in food-service and retail packages supplied by both small and large growers. To keep fresh mushrooms in good quality and wholesomeness, products packed loose in bulk bags or boxes are usually re-packed by shippers and wholesalers, and kept in refrigerated conditions. Then shippers and wholesalers transport the packed fresh mushrooms to marketplaces by refrigerated truck during distribution.

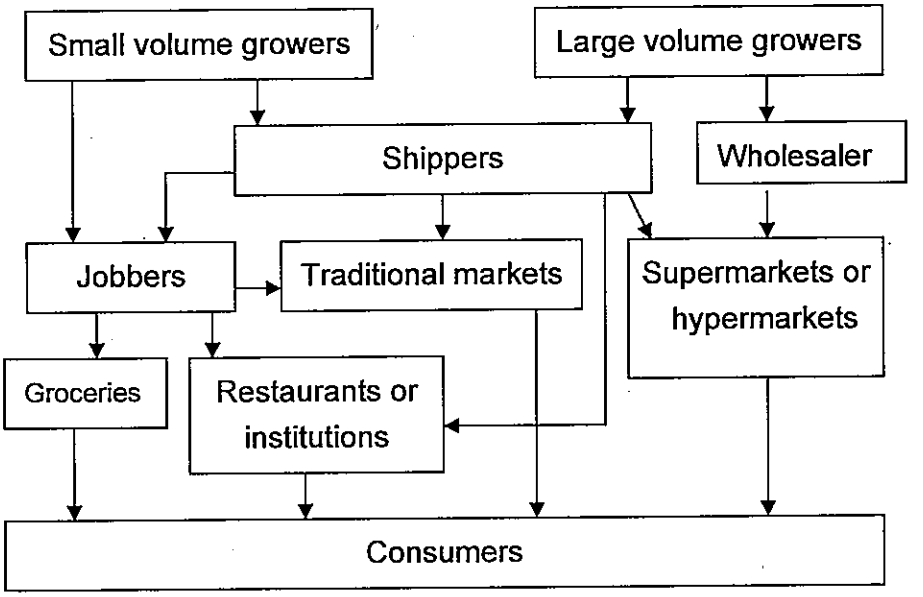


Fig. 1. Major distribution channels for Fresh mushroom sales in Taiwan.

Table 1. Estimated production of edible and medicinal mushrooms in the crop year of 2009/2010 in Taiwan

Common name	Scientific name	Estimated production (MT)	Unit price (US$/kg)
Golden mushroom	*Flammulina velutipes*	24,000	0.8-1.3
Shiitake	*Lentinula edodes*	28,000	3.1-3.5
King oyster mushroom	*Pleurotus eryngii*	12,000	2.6-3.2
Bunashimeji	*Hypsizigus marmoreus*	2,000	Bottle:4.8-9.7 Bag:2.3-3.2
Phoenix tail mushroom	*Pleurotus sajor-caju*	10,000	1.8-2.7
Jew's ear	*Auricularia spp.*	3,000	1.3-1.9
Common white mushroom	*Agaricus bisporus*	2,000	3.2-4.5
Straw mushroom	*Volvariella volvacea*	1,000	1.3-1.7
Summer oyster mushroom	*Pleurotus cystidiosus*	600	0.4-0.6
Yuhuanmo	*Pleurotus citriopileatus*	300	0.6-1.1
Brown swordbelt	*Agrocybe spp.*	300	Bottle:3.2-4.8 Bag:2.9-3.9
Sunny mushroom	*Agaricus braziliensis*	350	7.1-9.7
Oyster mushroom	*Pleurotus ostreatus*	100	2.6-3.2
Shaggy cap	*Coprinus comatus*	50	4.0-5.2
Lingzi	*Ganoderma spp.*	15 (dry)	15.4-21.5

Using agro-wastes as basic raw materials for cultivation of edible mushrooms in Taiwan

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The output of agro-wastes in Taiwan

According to the estimation made by the Council of Agriculture, Executive Yuan, Taiwan ROC, the total output of agricultural wastes estimated amount to 4.6 million MT in the year of 2007.

The major parts of agro-wastes are from rice straws, livestock and poultry manure, and the yearly output for rice straws alone amounts to 1,363,458 MT, while from livestock and poultry manure reaches to 2,437,242 MT (Table 1). Thus, the output of these two agro-wastes combined reached 3,800,700 MT which account for of 86.05% of the total agro-waste production in Taiwan. The agro-wastes from animal sources livestock and poultry have been properly treated and from rice straws for making composts prior to use as basic raw materials for mushroom cultivation. Nowadays, agro-wastes from rice straws if not use for mushroom raw cultivation materials, most of the rice straws are ended up directly plowed under in situ or burned. It deems important to recycling use of rice straws in paddy rice production areas either for use in growing edible mushrooms or regeneration resource of bio-energy, thus to avoid wasteful of lignocellulosic resource indeed.

Estimated over 300,000 MT of food could be generated from the use of rice straws alone as raw materials for the production of edible mushrooms in Taiwan

According to the method of estimation reported by Professor S. T. Chang(2007), If we could use one third of the output of rice straws, we could produce 306,778 MT of edible mushrooms based on the calculation of an average of 67.5% of biological efficiency (fresh weight of mushroom divided by the dry weight of substrate). However, at present we are only able to produce about 2,000 MT of fresh mushroom products to use rice straws as basis raw materials for cultivation of mushrooms. This shows the potential of rice straws alone remains profoundly huge, optimistic challenges and opportunities to be explored in the transformation of recycling use of agro-wastes in the production of highly valued mushroom products as food sources in the future.

According to Chiu et al.(2000) the biological efficiency of a cultivated mushroom strain ranges from 17 to 250%, thus mushroom cultivation can well be viewed as an effective mean to extract resources left behind in agro-waste.

This waste bioconversion process is simultaneously also a sound environmental protection strategy too. Besides, most edible mushrooms are regarded as a nutrient and healthy food due to their nutriceutical value and their pharmacological potential (eCAM 2005:2(3)285-299). More than ever, there are ample opportunity and challenge ahead of to tackle and to study how to convert rice straw efficiently into highly valued products by growing edible or medicinal mushrooms, among others.

The most extensively used agro-wastes for the production of edible mushrooms in the world

- Wheat and rice straw
- Cotton seed hull and cotton waste
- Sawdust or wood chips
- Wheat and rice bran
- Chicken and horse manure

- Corn cob
- Sugarcane bagasse

Table 1. Output of major agro-wastes in Taiwan in 2007

Kind of waste	Production		Properly treated	
	Amount (ton)	%	Amount (ton)	%
Rice hull	272,691	6.17	272,691	100
Rice straw	1,363,458	30.87	1,363,458	100
Spent sawdust	152,000	3.44	152,000	100
Oyster shell	169,194	3.67	162,000	95.75
Livestock and poultry manure	2,437,242	55.18	2,225,000	91.29
Food processed waste	22,400	0.51	22,400	100

Other agro-wastes have also been used or tried to be used, such as

- Cotton stalk
- Coffee pulp
- Soybean straw
- Banana pseudo stem
- Waste paper
- Grasses
- Groundnut haulms
- Jute stick and caddis
- Water hyacinth
- Powdered oil seed cakes (sunflower, cotton and soybean)
- Sorghum stover

The most popular agro-wastes for mushroom production in Taiwan

- Rice straw
- Sawdust or wood chips
- Cotton waste

- Chicken manure
- Rice and wheat bran

Estimate value generated from recycling use of agro-wastes for mushroom production in Taiwan

These agro-wastes have been converted into a great number of edible and medicinal mushrooms creating a total value of more than US$0.24 billion per year contributing to the prosperous economics of rural areas in Taiwan.

Basic raw materials for modern production of mushrooms in Taiwan

- Cotton waste from a spinning mill > *Volvariella volvacea*
- Rice straw > *Agaricus bisporus*, *A. bitorquis*, *A. braziliensis*, *Clitocybe nuda*, *Coprinus comatus*
- Sawdust or wood chips > *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus spp.*, *Auricularia spp.*, *Agrocybe cylindracea*, *Hypysizigus marmoreus*, *Ganoderma spp.*

Advantages of using cotton waste as basic material for growing straw mushroom

- This mushroom can be grown in a plastic mushroom house without a sophisticated climatic control
- Basic raw material needs no fermentation and nutrient supplement
- It can be grown successively 3 to 4 times without necessity to remove spent substrate after each crop.
- Short production cycle
- Cost effective—a. efficiency of the use of mushroom house, b. cheap raw material, c. low labor cost, d. low energy cost.

Ps: Prices of cotton waste--US$0.03/kg
Prices of heavy oil—US$0.45/kg
Labor cost—US$48.48/day/person

Successive growing of 3 mushroom species in the same mushroom house

Straw mushroom (Oct.) →Common white mushroom (Nov. to Feb.) → Straw mushroom (Mar.) → Hot mushroom (April to July) → Straw mushroom (Aug.) → Straw mushroom (Sep.) → Cooking out and removing spent substrate for a new crop of straw mushroom.

Procedures for growing straw mushroom including substrate preparation

- 1st day—loosening, spreading, wetting and pressing of cotton waste.
- 2nd and 3rd day—percolation of wetted cotton waste
- 4th day—filling the wetted cotton waste onto mushroom bed with a thickness of ca.10cm on the top of spent rice straw compost, then closing doors and windows
- 5th day—bed temp. going up to 30-40℃
- 6th day—pasteurizing at 70℃ for 12 hrs or 62-65℃ for 24 hrs.
- 8th day—spawning (open corner windows one night before)> air temp. 30-32℃, bed temp. 37-38℃, closing doors, central and corner windows after spawning.
- 13th or 14th day—spraying water onto mushroom bed and opening doors for 1 day.
- 14th or 15th day to 16th or 17th day—opening corner windows
- 17th or 18th day—appearance of pinheads
- 22th or 23th day—harvesting 1st flush
- 28th or 29th day—harvesting 2nd flush

Ps: Total production—1.2 to 1.5 ton/165 square meters

Rice straw as basic material for growing mushrooms in Taiwan

- There are three types of mushroom production using rice straw as basic material according to the combinations of making compost (Phase I and Phase II), spawn running (Phase III) and cropping.
- Phase I—stacking and composting
- Phase II—pasteurization and conditioning

- Phase III—spawning and spawn running

Table 2. Three types of mushroom production using rice straw compost

Type	Phase I done	Phase II done	Phase III done	Cropping done
1	outdoors	in plastic mushroom house	in plastic mushroom house	in plastic mushroom house
2	outdoors	in tunnel	in tunnel	in climatically controlled room
3	in tunnel	in tunnel	in tunnel	in climatically controlled room

Table 3. Suggested formula for rice straw compost in Taiwan

Ingredient	%
Air dried rice straw	100
Chicken manure	20
Rice bran	4
Soybean meal	1
Ammonium sulfate	2
Calcium super-phosphate	3
Calcium carbonate	2
Gypsum	3

Preparation of rice straw compost and the outdoor processes to be carried out for Phase I in Taiwan

- 1st day—Cutting (2.5-5.0 cm) and wetting of rice straw
- 2nd to 5th day—Wetting of rice straw
- 6th day—Adding all ingredients except soybean meal and gypsum, stacking of mixture into a pile
- 9th day—First turning, adding soybean meal and gypsum, making a pile as above
- 11th day— Second turning, making a pile as above

- 13th day—Turning and mixing with rice bran, then filling

Preparation of rice straw compost and the outdoor processes to be carried out for Phase II in a plastic mushroom house and in tunnel in Taiwan

- 15th day —Pasteurizing at 58 - 60℃ for 6 to 8 hrs
- 16 to 17th day —Reduce compost temp. to 52℃ in two days
- 18 to 21th day —Control compost temp. at 48 - 52℃
- 22 to 24th day — Control compost temp. at 45 - 48℃
- 25 to 26th day — Reduce compost temp. to 25 - 27℃ for spawning

Procedures for growing *Agaricus bisporus* from spawning to harvesting in a traditional plastic mushroom house in Taiwan

- 26th day — Mixing spawn grains with compost as thoroughly as possible, closing the doors and windows, wetting the ground and walls of mushroom house, re-circulating room air.
- 27 to 39th day — Spawn running, keeping compost temperature not over 27℃, maintaining RH at 90-95%, ventilating once in the morning if compost temperature rising not over 27℃.
- 40th day — Casing with wetted disinfected casing soil with a layer of 2.5cm, closing the doors and windows, wetting the ground and walls of mushroom house, re-circulating room ai
- 41 to 47th day — Casing running, keeping compost temperature not over 27℃, maintaining RH at 90-95%, ventilating once (about 30 min) in the morning if compost temperature rising not over 27℃, gradually increasing the water content of casing soil, when 70-80% of the surfaces of casing layer covered with mushroom mycelium, it is ready for scratching.
- 48th day — Wetting heavily in the morning, scratching the casing surfaces down to the interfaces between compost and casing layer in the afternoon or next morning.
- 49 to 50th day — Re-growing of mycelial fragments,

maintaining high RH and CO_2 in the mushroom house, re-circulating the air inside the house if necessary, not introducing fresh air, not spreading water onto casing surfaces.

- 51th day — Wetting heavily, introducing fresh air and re-circulating room air using ventilator and extract fan to lower both temperature and CO_2 as well as RH to promote pinning, keeping surfaces of casing layer moisten by spraying water lightly if it showing a little bit too dry.
- 52 to 58th day — Keeping aerating until appropriate amount of pins and small fruit-bodies formed.
- 59 to 65th day — When pins reaching the size of a pea, spraying water as much as they need, maintaining proper concentration of CO_2(900-1200ppm) and RH at 85-90%, the 1st flush mushrooms will be ready for harvest 20 to 25 days after casing depending on the mushroom strains to be used.

Preparation of rice straw composts and the suggested processes to be carried out for Phase I, II and III, in a tunnel in Taiwan

- 1st day — Cutting and wetting of rice straw
- 2nd to 5th day — Wetting of rice straw
- 6th day — Mixing all ingredients of compost and filling the mixture into a tunnel for conducting phase I
- 7 to 9th day — Compost temp. going up to 75-80℃, controlling oxygen level not below 10%
- 10th day — Moving out Phase I compost, mixing with 5% of Phase II compost (previously made) and re-filling in a tunnel
- 11 to 12th day — Controlling compost temp. at 48 - 52℃
- 13th day — Pasteurizing at 58-60℃for 6 to 8 hrs
- 14 to 15th day — Reducing compost temp. to 52℃ in two days
- 16 to19th day — Controlling compost temp. at 48 - 52℃
- 20 to 22th day — Pasteurizing at 58-60℃for 6 to 8 hrs
- 23 to 24th day — Reducing compost temp. to 52℃ in two days

Growing procedures for *Agaricus bisporus* from spawning to harvesting in an environmentally controlled room

- 25th day — Moving out Phase II compost for spawning and refilling of the spawned compost for spawn running in a tunnel (Phase III).
- 26 to 37 day — Spawn running, maintaining compost temp. at ca 25℃ by air conditioning unit and re-circulating the air in the tunnel, supplying fresh air if it needs.
- 38th day — Moving out of Phase III compost for refilling into iron boxes, then for casing with disinfected soil, stacking compost boxes and moving them in an environmentally controlled room. Maintaining the compost temp. not over 27℃.
- 39 to 40th day — Re-growing of fragmented mycelia, maintaining compost temp. at 27℃, introducing fresh air if necessary, keeping RH at 90-95%, re-circulating the room air.
- 41 to 45th day — Spraying water onto casing layer in consecutive three days, keeping compost temp. not over 27℃, keeping RH at 90-95%, re-circulating the room air.
- 46th day — Spraying water heavily in the late afternoon, maintaining high CO_2 and high RH of the room.
- 47th day — Scratching the casing layer down to the interfaces between compost and casing layer.
- 48 to 49th day — Re-growing of fragmented mycelia in the casing layer, maintaining high CO_2 and high RH of the room, maintaining compost temp. at 27℃.
- 50 to 54th day — Reducing CO_2 to 900-1000ppm by introducing cooled fresh air, lowering air temp. to 15℃, in 3 days, reducing RH to 85-90% for pinning.
- 55 to 57th day — Appearing of pinheads and gradually developing into small fruit-bodies reaching the size of peas, raising air temp. to 16℃.
- 58 to 63th day — Spraying water heavily as necessary, maintaining air temp. at 16℃ until harvesting the 1st flush.

Characteristics of mushroom growing in a traditional plastic mushroom house with rice straw composts and cotton wastes in Taiwan

- Growing different mushroom species in different seasons to adapt different climatic conditions increasing the efficiency of using mushroom house and reducing pests and diseases.
- Supplementing organic nutrients to increase microbial activity to give off heat saving energy cost.
- Growing the second mushroom species without removing the spent substrate of the previous crop saving both raw material and labor cost.
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- Growing the second mushroom species without removing the spent substrate of the previous crop saving both raw material and labor cost.
- All mushroom houses are family owned, hiring outside employee as few as possible.
- Most mushroom growers are relatively old.

Characteristics of mushroom growing in an environmentally controlled mushroom house with rice straw composts in Taiwan

- Most mushroom houses are family owned, hiring outside employee as few as possible.
- Most mushroom growers are relatively young.
- Most growers cultivate single mushroom species whole year round.
- All mushroom farms are small, not more than 10 cropping rooms, each room with not more than 200 square meters of mushroom bed surfaces.
- No big machine for the operation of various works in the farm.
- It is not necessary to supply live steam during Phase I and Phase II to keep the optimum air or compost temperature except for cooking out of the spent substrate at the end of cropping.

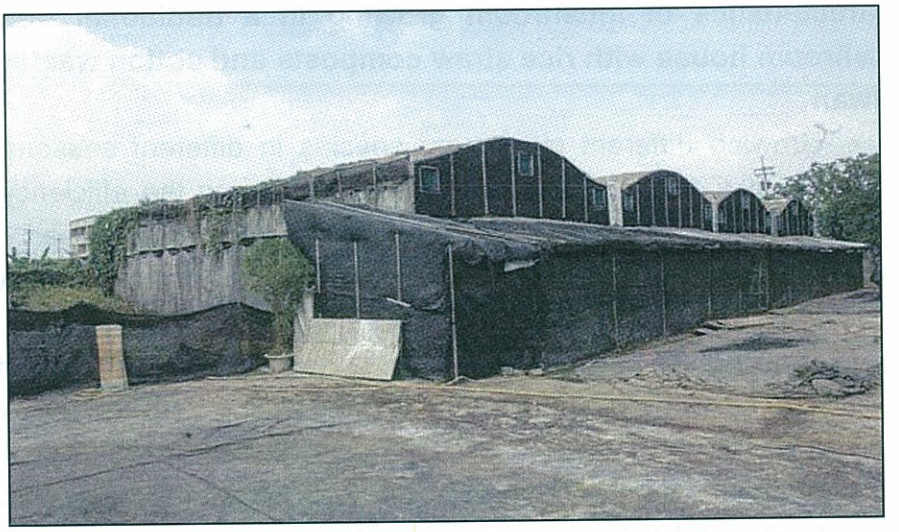


Fig. 1. Traditional plastic mushroom houses for growing straw mushroom or the common white mushroom.

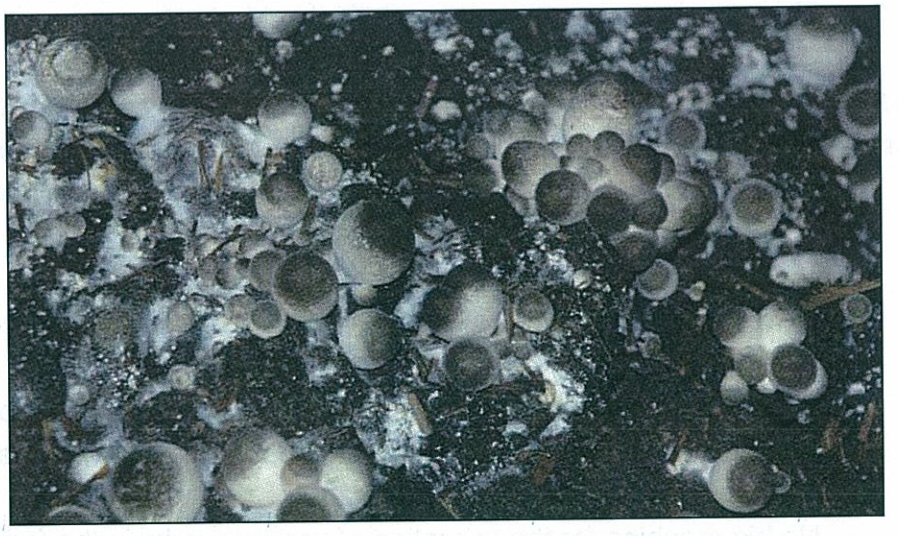


Fig.2.Straw mushroom grown on pasteurized cotton wastes in a traditional plastic mushroom house.

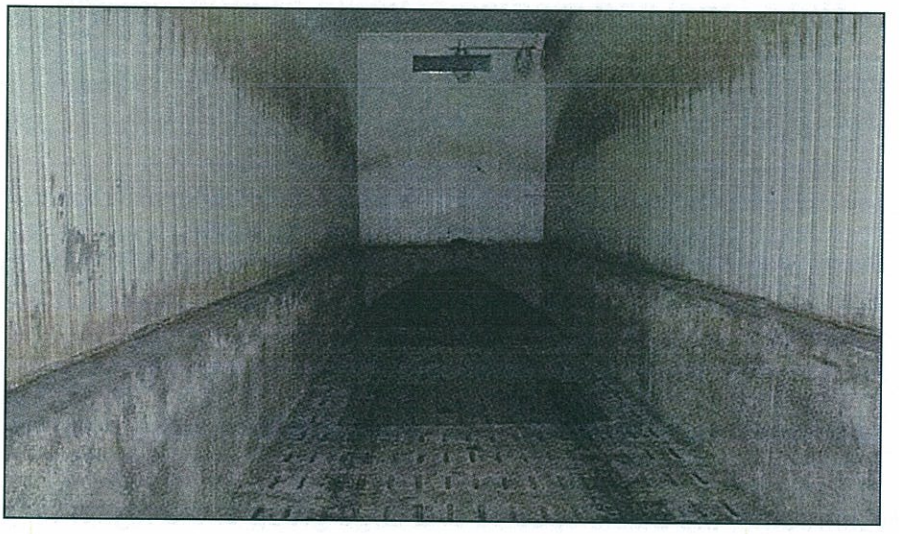


Fig.3. Phase II tunnel for pasteurization and conditioning of phase I compost.

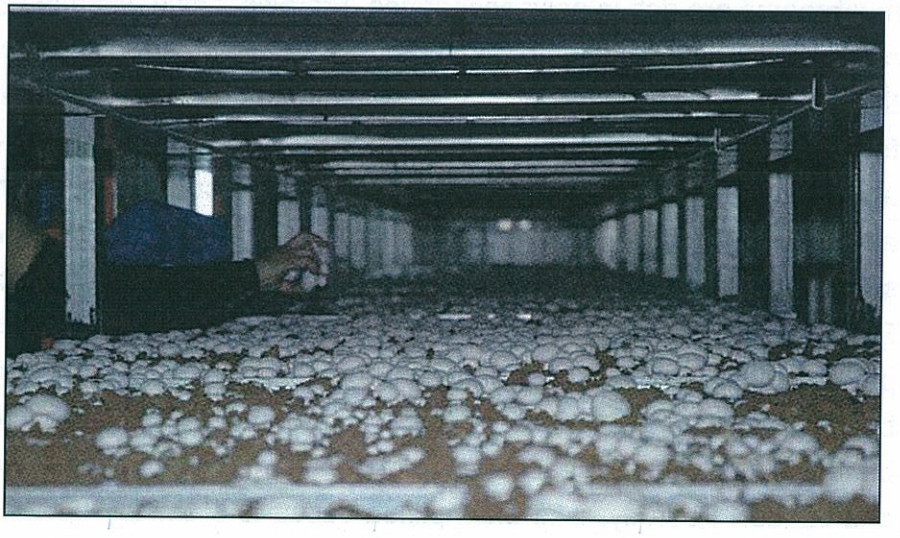


Fig. 4. First flush mushrooms on a mushroom bed.

Nutritional and medicinal value of mushrooms

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Introduction

Mushrooms, the edible species of a large group of the higher fungi, are produced with high quality and high economic value. Mushrooms have been used as foods and food flavoring materials in soups and sauces for centuries, due to their unique and subtle flavor. Generally, mushrooms possess all four functionalities of food -- nutritional, tasty, physiological and cultural. For the first functionality of nutritional values, mushrooms are rich in polysaccharides, proteins, chitin, vitamin D_2 and minerals and low in fat, calories and contain no cholesterols. Secondly, the functionality of tasty properties features mushrooms as delicious food and contain food flavoring substances due to their unique flavor. In addition to the volatile eight-carbon compounds such as 1-octen-3-ol and 1-octen-3-one, the typical mushroom flavor consists of water soluble taste components including free amino acids and 5'-nucleotides. For the third functionality of physiological effects, as several physiologically active substances contained therein, mushrooms become a valuable health food. Mushrooms are thought to be beneficial for such diseases as hypertension, hypercholesterolemia, and cancer. Fourthly, take exemplified cases to show the functionality of mushrooms in cultural aspect; truffle is related to French cuisine and matsutake is famous in Japan, while Chang-chih (*Antrodia camphorata*) is only found in Taiwan.

Edible mushroom products are always available in fruiting body forms as food sources or as food additives for cooking whereas medicinal mushrooms are always available in mycelia due to their rare and expensive nature. Nevertheless, some are consumed in the form of sclerotia such as truffle while some species of mushroom spores are made into broken products for use in pharmaceutical formulation such as Lingzi (*Ganoderma lucium*). Due to increased health awareness for human life, it appears mushroom production and consumption is increasing in recent years.

Currently, there are many mushroom products/fruiting bodies available in market either produced domestically or imported from other countries and some mycelia produced via submerged fermentation mainly used as an ingredient for pharmaceutical formulations in Taiwan. These mushrooms are common mushroom [*Agaricus bisporus* (Lange) Imbach], hot mushroom [*Agaricus bitorquis* (Quelet) Saccardo], shiitake [*Lentinula edodes* (Berkeley) Pegler], paddy straw mushroom [*Volvariella volvacea* (Bulliard: Fries) Singer], winter mushroom [*Flammulina velutipes* (Curtis: Fries) Singer], willow-pine mushroom [*Agrocybe cylindracea* (de Candolle: Fries) Maire], summer oyster (*Pleurotus cystidiosus* Miller), mini oyster [*Pleurotus ostreatus* (Jacquin: Fries) Kummer], king oyster [*Pleurotus eryngii* (de Candolle: Fries) Quelet], golden oyster (*Pleurotus citrinopileatus* Singer), ferulae mushroom [*Pleurotus ferulae* (DC.: Fr) Quel.], big cup mushroom (*Clitocybe maxima* Gaertn. et G. Mey.: Fr.), black ear [*Auricularia mesenterica* (Dickson) Persoon], red ear [*Auricularia polytricha* (Montagne) Sacchardo], jin ear[*Auricularia fuscosuccinea* (Montagne) Farlow], silver ear (*Tremella fuciformis* Berkeley), hon-shimeji [*Hypsizigus marmoreus* (Peck.) Bigelow], lion's mane mushroom [*Hericium erinaceus* (Bulliard: Fries) Persoon], basket stinkhorn (*Dictyophora indusiata* Fischer), king bolete (*Boletus edulis* Bull.: Fr.), maitake [*Grifola frondosa* (Dickson: Fries) Gray], white matsutake (*Tricholoma giganteum* Massee), wood blewit (*Lepista nuda* Bulliard ex Fries), Chaga [*Inonotus obliquus* (Fr.) Pilat],

Ling-chih [*Ganoderma lucidum* (Curtis: Fries) Karsten], Tsuga Ling-chih (*Ganoderma tsugae* Murrill), Yun-chih [*Coriolus versicolor* (Fries) Quelet], Brazilian mushroom (*Agaricus blazei* Murrill), Chang-chih [*Antrodia camphorata* (Chang & Chou) Wu, Ryvarden & Chang], shiang-shan-chih (*Antrodia salmonea* Chang & Chou), caterpillar fungus [*Cordyceps sinensis* (Berk.) Sacc.], Northern caterpillar fungus [*Cordyceps militaris* (L.) Link], san-huang [*Phellinus linteus* (Berkeley & Curtis) Teng], termite fungus [*Termitomyces albuminosus* (Berkeley & Broome) Heim], and chicken-leg mushroom [*Coprinus comatus* (Muller: Fries) S.F. Gray].

This paper focuses on nutrition and medicinal values of edible and medicinal mushrooms including proximate composition, taste components and bioactive components.

- **Proximate composition**

Table 1 shows the proximate composition of some mushroom fruiting bodies available in Taiwan. In order to show the consistent comparison on the same basis, the data were calculated based on the dry matter. The composition of mushrooms can vary considerably from species to species and strains to strains. In addition, the cultivation techniques including use of different substrates, maturity at harvest and methods of analysis also result in different composition. However, the results can provide basic information about several important mushrooms.

Most fresh mushrooms contained 85% to 93% of moisture and generally around 90%. Therefore, upon the conversion of the dry basis to the fresh basis, the moisture content of mushrooms was arbitrarily assigned to be 90%. The ash, carbohydrate, fat, fiber and protein contents shown in Table 1 can be converted into fresh basis by a factor of 0.1, i.e., one log reduction or one decimal reduction.

Generally, mushrooms are a good source of protein. Mushroom protein is of good quality in vegetable in addition to legume protein. However, in Table 1, protein contents range from 4.2% to 44.94%.

Agrocybe and *Agaricus* provide high amount of protein (30-40%), *Lentinula* and *Pleurotus* provide middle amount (10-30%) and *Auricularia*, *Tremella*, *Ganoderma* and *Antrodia* are poor source of protein.

Usually, mushrooms are low in fat. However, the fat content in *Antrodia* is high (32.23-37.23%). Besides, the fat contents in other mushrooms are lower than 10%. The fat contents in *Auricularia* are even lower than 1%. *Agaricus* contained high amount of ash (12.6%). The ash contents in other mushrooms are lower than 10%. The fat contents in *Antrodia* and *Ganoderma* are around 1-2%.

The carbohydrate content (%) was calculated by subtracting the contents of crude ash, fat, fiber and protein from 100% of dry matter. Carbohydrate contents in Table 1 widely range from 10.41% to 88.14%. However, carbohydrate content can be subdivided into total reducing sugar (TRS) and non-reducing sugar (NRS) contents. TRS is the energy of mushrooms whereas NRS is so called soluble polysaccharide, which is another kind of dietary fiber in addition to insoluble polysaccharide. In Table 1, *Tremella* and *Auricularia* are rich in soluble polysaccharides. Nevertheless, *Ganoderma* is not rich in TRS or NRS.

The fiber contents in *Ganoderma* and *Lentinula edodes* stipe are high (59-73% and 32-47%). The fiber contents in *Pleurotus*, *Auricularia* and *Tremella* are lower than 10% and those in other mushrooms are between 10-30%. The fiber is acid-, alkali- and alcohol-insoluble polysaccharide and is ineffective in taste. The major component of crude fiber in mushrooms is chitin, which is an important structural polysaccharide found in the cell wall. The fiber and NRS contents together are called dietary fiber. Generally, mushrooms are rich in dietary fiber and some mushrooms are rich in soluble polysaccharides and other mushrooms are rich in insoluble polysaccharides. Both dietary fiber and polysaccharides are good for health-care.

- **Vitamins**

Mushrooms are known to be a good dietary source of B-complex vitamins. Besides, mushrooms are a good source of vitamin D_2, ergocalciferol and the only non-animal fresh food source of vitamin D. Also, mushrooms contain high amount of ergosterol, provitamin D_2, which can be converted to vitamin D_2 by ultraviolet (UV) irradiation. The enrichment of vitamin D in foods provides the advantage that calcium in foods can be more available for children, senior and postmenopausal women.

Further research on mushrooms and UV light is being conducted in the America and Australia. Tables 2 and 3 shows the effect of ultraviolet-C and ultraviolet-B irradiation on vitamin D_2 contents, respectively. After UV-C irradiation for 2 h, vitamin D_2 contents in *Agaricus bisporus* and *A. bitorquis* increased from 2.20 μg /g dry weight and 4.01 to 7.30 and 5.32, respectively. After UV-B irradiation for 2 h, the vitamin D_2 content in *A. bisporus* reached 12.48 μg/g. Mushrooms received UV-B irradiation resulted in higher vitamin D_2 conversion for *A. bisporus*. After UV-B irradiation for 2 h, vitamin D_2 contents in shiitake and straw mushrooms increased from 2.16 μg/g and 3.86 to 6.58 and 7.58, respectively. The increase rates in shiitake and straw mushrooms were not as high as that of *A. bisporus*.

- **Minerals**

Mushrooms contain high amount of potassium, copper and other minerals. Also, mushrooms are a good source of selenium (Se). Selenium is an essential micronutrient in human nutrition and it is incorporated into the antioxidant enzymes as glutathione peroxidase in the form of selenocysteine. These selenoproteins function in the prevention of cellular damage caused by free radicals. In addition, health benefits attributed to Se include anti-inflammatory and anti-carcinogenic properties. This element is found in soil where it is absorbed by plants and fungi and later accumulated in animals. Important sources of Se in foods are plants, meats, seafood and nuts.

Selenium can be accumulated in mushrooms through cultivation practice, when Se is supplied in the form of sodium selenite. This research is currently being conducted in the America.

- **Taste components**

Taste components present in mushrooms are soluble sugars and polyols, organic acids, free amino acids and 5'-nucleotides. Via a series of sensory evaluations on synthetic mushroom extracts prepared by the omission and addition of soluble components, major taste-active components in *A. bisporus* are found to be mannitol, oxalic, malic, citric, aspartic, glutamic acids, glycine, threonine, alanine, 5'-inosine monophosphate (5'-IMP), 5'-guanosine monophosphate (5'-GMP), and 5'-xanthosine monophosphate (5'-XMP). Mannitol and organic acids contribute most to the sweet and sour tastes, respectively. However, they are not the taste characteristic of mushroom flavor.

The predominant flavor of mushrooms is the umami taste, also called the palatable taste or the perception of satisfaction, which is an overall food flavor induced or enhanced by monosodium glutamate (MSG). In addition to four basic tastes such as sour, sweet, bitter and salty tastes as well as hot taste, the umami taste is the sixth taste in mouth perception. MSG can alleviate on salty, sour and bitter tastes, enhance the perception of sweet taste, and lower the sharp irritation of onion, the raw odor of meat and earthy note of potatoes. As a result of the properties of MSG, mushrooms can be widely used in most foods such as meat, seafood, soup, stew and cooked vegetables. Nevertheless, mushrooms cannot improve the taste of fruits, juices, desserts and cooked cereals.

Mushrooms contain considerably high amounts of free amino acids that impart the food taste with smooth feeling and thereby, compromising a sharp taste from some substances. Therefore, the combination of free amino acids always gives rise to a unique natural flavor. On the basis of their flavor characteristics, free amino acids are

grouped into four classes of taste components, including MSG-like, sweet, bitter and tasteless components. Besides, sweet components (alanine, glycine, and threonine) and MSG-like components (Aspartic (Asp) and glutamic acids (Glu)) were taste-active amino acids in mushrooms, whereas none of the bitter components were found to be taste-active. The bitter taste from bitter components might be eliminated or suppressed by the soluble sugars and polyols and sweet components.

Since they give the most typical mushroom taste, MSG-like components, Asp and Glu, are also called umami amino acids. Six 5'-nucleotides are usually detected in mushrooms, including 5'-adenosine monophosphate (5'-AMP), 5'-cytosine monophosphate (5'-CMP), 5'-GMP, 5'-IMP, 5'-uridine monophosphate (5'-UMP), and 5'-XMP. Among these 5'-nucleotides, four 5'-nucleotides including 5'-AMP, 5'-IMP, 5'-GMP and 5'-XMP are also called umami 5'-nucleotides. 5'-GMP gives a meaty flavor and is a much stronger flavor enhancer than MSG. In addition, the synergistic effect of umami 5'-nucleotides and umami amino acids may greatly increase the umami taste of mushrooms.

The equivalent umami concentration [EUC, mg monosodium glutamate (MSG)/100 g] is the concentration of MSG equivalent to the umami intensity of that given by the mixture of MSG and the 5'-nucleotide and is represented by the following addition equation:

$$Y = \Sigma a_i b_i + 1218\,(\Sigma a_i b_i)\,(\Sigma a_j b_j),$$

where Y is the EUC of the mixture in terms of mg MSG/100 g; a_i is the concentration (mg/100 g) of each umami amino acid [aspartic acid (Asp) or glutamic acid (Glu)]; a_j is the concentration (mg/100 g) of each umami 5'-nucleotide [5'-inosine monophosphate (5'-IMP), 5'-guanosine monophosphate (5'-GMP), 5'-xanthosine monophosphate (5'-XMP) or 5'-adenosine monophosphate (5'-AMP)]; b_i is the relative umami concentration (RUC) for each umami amino acid to MSG (Glu, 1 and Asp, 0.077); b_j is the RUC for each umami 5'-nucleotide to 5'-IMP (5'-IMP, 1; 5'-GMP, 2.3; 5'-XMP, 0.61 and

5'-AMP, 0.18); and 1218 is a synergistic constant based on the concentration of mg/100 g used.

Currently there are all varieties of fresh and dried mushrooms available in Taiwan. This paper summarized the results of mushroom taste components from a decade research, and calculated the EUC values of the fruit bodies and mycelia for each mushroom studied using the above additive equation, which are show in Tables 4 and 5, respectively. The EUC value is expressed as the percentages of dry matter by weight. An EUC value of 100% represents that the umami intensity of this fruit bodies or mycelia per 1 g dry matter is equivalent to the umami intensity given by 1 g of MSG and; in other words for short, 1 g MSG/g dry matter. EUC values are grouped into 4 levels: first level of > 1000% (> 10 g MSG/g dry matter), second level of 100-1000% (1-10 g MSG/g), third level of 10-100% (0.1-1 g MSG/g), and fourth level of 10% (<0.1 g MSG/g).

The EUC values at the first level are *V. volvacea* (paddy straw mushrooms) and *A. bisporus*. The EUC values of *V. volvacea* dramatically increased with cap opened. The EUC values of fruit bodies at the second and third levels are commercial mushroom products, including fresh or imported dried products. These mushrooms are famous not exclusively due to their umami taste, and rather partially due to different type of culinary quality such as unique flavor, consistency and texture. The EUC values of fruit bodies at the fourth level were mainly medicinal and ear mushrooms. Ear mushrooms for use in foods are dried products, and they provide bite and chew texture instead of the umami taste after dehydration and cooking.

As shown in Table 5, the EUC values of mycelia also varied and ranged from the highest value of 460% (*Termitomyces albuminosus*) to the lowest value of (*Agaricus blazei*). There are five species of mycelia at the second level, three species at the third level and one species at the fourth level. The EUC values of commercially available mushroom fruit bodies and mycelia summarized herein are valuable

for their further use as foods or food-flavoring materials or in the formulation of health foods.

- **Bioactive components**

In addition to the bitter triterpenoids well-known in *Ganoderma*, there are several biologically active components found in mushrooms including Lovastatin, γ–aminobutyric acid (GABA) and ergothioneine. Lovastatin, GABA and ergothioneine are secondary metabolites from fungal growth.

Lovastatin, also known as monacolin K, mevinolin or mevacor, is a kind of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), which inhibit the rate-limiting enzyme in the production of cholesterol, lowering total and LDL cholesterol levels and have been proven to reduce the risk of coronary *heart disease*. This class of statins has a well-studied lipid-lowering impact on serum cholesterol levels benefit. They have been shown to have effectively anti-inflammatory, antioxidant and pro-fibrinolytic properties and to prevent acute coronary syndromes and atherosclerotic disease.

GABA is a hypotensive agent, which is known to be produced by edible, medicinal mushrooms and *Monascus* species. In spontaneously hypertensive rats, GABA has an antihypertensive effect. Furthermore, the protection and hypotensive actions of some food products containing GABA were shown in hypertensive patients.

Ergothioneine is a thiol compound and a naturally occurring antioxidant of biological origin. In animals, ergothioneine provides several physiological benefits such as enhancement of metabolic energy, protection against formation of cataracts and molecular regulation of anti-inflammatory mechanisms in lungs. Synthesis of ergothioneine in nature is restricted to fungi and Mycobacterium. Ergothioneine synthesized by these organisms is taken up by plants from the soil and then passed to animals and humans where it accumulates at different concentrations in tissues and blood. In addition, ergothioneine is effective intrinsic anti-hydroxyl, anti-peroxyl

and anti-peroxynitrite radical antioxidant activity, as compared to classic molecules with antioxidant capacity as reduced glutathione, uric acid and trolox.

Tables 6 and 7 show Lovastatin, GABA and ergothioneine contents in fruiting bodies and mycelia, respectively. *Agaricus bisporus* and *B. edulis* contained high amount of Lovastatin (565 and 327 mg/kg, respectively) in 20 kinds of fruiting bodies. However, Lovastatin was not detected in mycelia of *G. frondosa*, and fruiting bodies of *L. edodes*, *G. frondosa* and *A. mesenterica*. However, *C. sinensis*, *A. salmonea* and *A. blazei* contained high amount of Lovastatin (1365, 1032 and 770 mg/kg, respectively) in 20 kinds of mycelia. Lovastatin contents can be grouped into four levels: first level of >1000 mg/kg dry matter, second level of 500–1000 mg/kg, third level of 100–500 mg/kg, and fourth level of < 100 mg/kg. Therefore, Lovastatin content in mycelia of *C. sinensis* and *A. salmonea* were at the first level, and those in *A. blazei*, *P. ostreatus* (Japan) and *A. camphorata* were at the second level. In addition, Lovastatin content in fruiting body of *A. bisporus* was at the second level. It seems that Lovastatin content in mycelia has high level than that in fruiting body. Furthermore, the result demonstrated the Lovastatin value in medicinal mushrooms was more than that in edible mushrooms.

Lovastatin and the HMG-CoA reductase activity are produced by fruiting bodies and mycelia liquid cultures of edible mushroom, especially in *Pleurotus* spp. The hypocholesterolemic influence of oyster mushroom (*Pleurotus* spp.), combined with inhibition of lipid peroxidation was shown in rats and rabbits. Oyster mushroom diet considerably reduced the numbers and size of atherosclerotic plaques in rabbits. Lovastatin could be detected in this species and was equally responsible for the observed effects.

Flammulina velutipes, *B. edulis* and *A. bisporus* contained high amount of GABA (230, 202 and 125 mg/kg, respectively) in 20 kinds of fruiting bodies. *Cordyceps cicadae*, *C. sinensis* and *A. blazei*

contained high amount of GABA (255, 221 and 200 mg/kg, respectively) in 20 kinds of mycelia. However, Lovastatin was not detected in mycelia of *G. frondosa* and *C. comatus*, and fruiting bodies of *A. mesenterica*, *I. obliquus*, *P. eryngii* (foot), *P. ostreatus* and *P. salmoneostramineus*. That GABA contents can be grouped into four levels: first level of > 200 mg/kg dry matter, second level of 100–200 mg/kg, third level of 10–100 mg/kg, and fourth level of < 10 mg/kg. Therefore, the values in mycelia of *C. cicadae*, *C. sinensis* and *A. blazei* were at the first level, and *A. salmonea*, *H. marmoreus* (white), *A. cylindracea*, *C. versicolor* and *H. marmoreus* were at the second level. GABA value in fruiting bodies of *F. velutipes* and *B. edulis* were at the first level, and *A. bisporus* and *H. marmoreus* were at the second level. Similarly, GABA content in mycelia is higher than that in fruiting body. Furthermore, the result demonstrated the GABA contents in medicinal mushrooms were more than those in edible mushrooms.

Pleurotus citrinopileatus contained the highest amount of ergothioneine (2850.7 mg/kg) in fruiting body. *Pleurotus eryngii* shows higher ergothioneine content in mycelia (1514.6 mg/kg) than fruiting body (840.4 mg/kg). This result shows that higher amount of ergothioneine are found in *Pleurotus* spp. Ergothioneine values can be grouped into four levels: first level of > 2000 mg/kg dry matter, second level of 1000–2000 mg/kg, third level of 200–1000 mg/kg, and fourth level of < 200 mg/kg. Therefore, ergothioneine value in mycelia of *P. eryngii* was at the second level. The ergothioneine value in fruiting bodies of *P. citrinopileatus* was at the first level, and *P. ostreatus* (korea), *P. ostreatus and P. salmoneostramineus* were at the second level. Generally, the ergothioneine values in fruiting bodies are higher than those in mycelial. Interestingly, the result demonstrated that ergothioneine contents in edible mushrooms are higher than those in medicinal mushrooms.

Conclusion

Mushrooms are not vegetables and are not even plants. Mushrooms are higher fungi which evolved between plants and animals. As a result of this, mushrooms have a much different nutritional profile from those of plants and animals. Mushrooms are known to be high in antioxidants and are in the top five highest antioxidant foods when compared to common vegetables. To know more information about their nutritional and medicinal values will facilitate the expansion and consumption of mushrooms.

Table 1. Proximate composition (% dry wt) of mushroom fruiting bodies

Fruit body	Ash	Carbohydrate (TRS + NRS)	Fat	Fiber	Protein
Agaricus bisporus	12.57	53.44	2.10	16.68	15.21
Agaricus bisporus Tainung 3	8.54	33.98 (14.48 + 19.50)	2.90	11.00	43.58
Agaricus blazei	8.85	45.47	1.87	7.95	35.86
Agaricus blazei	6.81	45.52 (10.71 + 34.81)	2.62	18.31	26.74
Agrocybe cylindracea B	8.19	29.02	2.18	16.37	44.24
Agrocybe cylindracea B	7.90	43.58 (14.73 + 28.85)	3.54	22.80	22.18
Agrocybe cylindracea B	6.06	23.00	4.26	26.55	40.13
Agrocybe cylindracea M	7.66	39.54	2.48	16.15	34.17
Agrocybe cylindracea W	8.59	27.06	2.71	16.70	44.94
Agrocybe cylindracea yellow	6.65	53.71 (7.88 + 45.83)	3.63	19.54	16.47
Antrodia camphorata	1.04	31.76	37.23	22.68	7.29
Antrodia camphorata	1.73	36.91	32.23	22.53	6.60
Auricularia fuscosuccinea brown	4.02	71.19 (9.87 + 61.32)	4.48	11.69	8.62
Auricularia fuscosuccinea white	5.54	68.88 (10.90 + 57.98)	4.54	8.51	12.53
Auricularia mesenterica	3.29	76.53 (17.81 + 58.72)	0.80	3.92	15.46

Table 1. continued

Auricularia polytricha	2.05	88.14 (17.62 + 70.52)	0.48	3.63	5.70
Boletus edulis	5.84	56.16 (8.31 + 47.85)	5.76	13.70	18.54
Clitocybe maxima cap	5.28	43.65 (22.21 + 21.44)	2.70	26.78	21.59
Clitocybe maxima stipe	3.67	50.92 (31.47 + 19.45)	1.24	31.76	12.41
Coprinus comatus	8.44	58.37 (15.91 + 42.46)	3.11	12.48	17.60
Cordyceps militaris	5.94	28.04 (14.03 + 14.01)	10.09	19.57	36.36
Coriolus versicolor	6.37	65.09 (28.27 + 36.82)	1.10	23.24	4.20
Dictyophora indusiata	6.25	67.02 (20.11 + 46.91)	2.98	9.16	14.59
Flammulina velutipes white	6.93	48.19 (13.88 + 34.31)	8.89	15.99	20.00
Flammulina velutipes white	7.84	48.02	10.08	11.40	22.66
Flammulina velutipes yellow	7.51	39.63 (12.03 + 27.60)	9.23	16.98	26.65
Flammulina velutipes yellow	9.41	45.82 (14.07 + 31.75)	3.90	23.98	16.89
Ganoderma lucidum	1.77	26.02 (15.12 + 10.90)	5.13	59.16	7.92
Ganoderma lucidum antler	1.70	27.78 (15.56 + 12.22)	3.85	59.49	7.18
Ganoderma tsugae	0.72	21.83 (15.81 + 6.02)	4.62	65.29	7.54

Table 1. continued

Ganoderma tsugae	1.69	10.41 (5.35 + 5.06)	5.72	73.37	8.81
Ganoderma tsugae baby	2.62	17.17 (5.86 + 11.31)	6.50	59.93	13.78
Grifola frondosa	6.99	58.78 (10.71 + 48.07)	3.10	10.05	21.08
Grifola frondosa	6.15	33.53 (20.11 + 13.42)	6.51	32.81	21.00
Hericium erinaceus	9.35	57.02 (17.39 + 39.63)	3.52	7.81	22.30
Hypsizigus marmoreus	6.85	47.45 (13.87 + 33.58)	3.62	24.75	17.33
Lentinula edodes 271	5.27	62.30 (25.81 + 36.49)	6.34	5.63	20.46
Lentinula edodes stipe	3.50	47.06 (36.14 + 10.92)	2.89	35.99	10.56
Lentinula edodes stipe	3.78	37.65 (19.68 + 17.97)	1.32	46.99	10.26
Lentinula edodes stipe	3.87	36.06 (11.11 + 24.95)	1.74	45.73	12.60
Lentinula edodes stipe	3.04	37.34	6.21	32.51	20.90
Lentinula edodes Tainung 1	5.85	63.90 (21.91 + 41.99)	5.71	4.88	19.66
Pleurotus citrinopileatus	6.72	45.82 (17.90 + 27.92)	3.44	18.03	25.99
Pleurotus cystidiosus	9.62	63.13 (21.00 + 42.13)	3.10	8.74	15.41
Pleurotus eryngii	4.96	66.21	2.65	7.42	18.76
Pleurotus eryngii base	5.15	77.33	0.81	7.59	9.12

Table 1. continued

Pleurotus eryngii large	5.76	64.55	1.57	5.97	22.15
Pleurotus eryngii small	7.21	61.12	1.81	9.15	20.71
Pleurotus ferulae	6.60	61.13	3.01	12.98	16.28
Pleurotus ostreatus	7.59	61.07 (19.65 + 41.42)	2.16	5.33	23.85
Pleurotus ostreatus gray	5.90	64.20	3.76	9.82	16.32
Tremella fuciformis	6.14	81.72 (31.64 + 50.08)	0.93	2.91	8.30
Tremella fuciformis	6.48	80.74 (29.73 + 51.01)	2.74	2.51	7.53
Tremella fuciformis	7.36	81.43	2.57	1.53	7.11
Tricholoma giganteum	5.03	70.07 (37.95 + 32.12)	4.28	4.50	16.12

TRS, Total reducing sugar; NRS, non-reducing sugar

Table 2. Effect of ultraviolet-C irradiation on contents of vitamin D_2 and ergosterol in *Agaricus bisporus* and *A. bitorquis*

	Content (μg/g dry mushroom) [relative %][a]			
	0 h[b]	0.5 h	1 h	2 h
Agaricus bisporus				
Vitamin D_2	2.20d[c]	4.49c	6.00b	7.30a
	[100]	[204]	[273]	[332]
Ergosterol	273.97a	165.62b	91.47c	33.93d
	[100]	[60]	[33]	[12]
Agaricus bitorquis				
Vitamin D_2	4.01c	4.59b	5.62a	5.32a
	[100]	[114]	[140]	[133]
Ergosterol	50.78c	43.53c	78.57b	254.88a
	[100]	[86]	[155]	[502]

[a]Based on the control (0 hr).

[b]Irradiation intensity: 0.2 mW/cm^2.

[c]Means with different letters within the same row are significantly different ($p < 0.05$).

Table 3. Effect of ultraviolet-B irradiation on contents of vitamin D_2 and ergosterol in *Agaricus bisporus*, *Lentinula edodes* and *Volvaria volvacea*

	Content (μg/g dry mushroom) [relative %][a]			
	0 h[b]	0.5 h	1 h	2 h
Agaricus bisporus				
Vitamin D_2	2.20d[c]	5.74c	8.51b	12.48a
	[100]	[261]	[387]	[567]
Ergosterol	273.97a	21.61b	40.39b	56.69b
	[100]	[8]	[15]	[21]
Lentinula edodes				
Vitamin D_2	2.16d	3.71c	4.69b	6.58a
	[100]	[172]	[217]	[305]
Ergosterol	297.09b	316.61b	373.15a	286.16b
	[100]	[107]	[126]	[96]
Volvariella volvacea				
Vitamin D_2	3.86d	4.98c	6.28b	7.58a
	[100]	[129]	[163]	[196]
Ergosterol	185.89b	96.92c	233.56a	215.81a
	[100]	[52]	[126]	[116]

[a]Based on the control (0 hr).

[b]Irradiation intensity: 0.14 mW/cm^2.

[c]Means with different letters within the same row are significantly different ($p < 0.05$).

Table 4. Equivalent umami concentration (EUC) for fruit bodies

Fruitnig body	EUC (% dry wt)
Volvariella volvacea (flat cap)	4465.00
Volvariella volvacea (stipe elongated)	2593.00
Volvariella volvacea (volva broken)	2198.00
Volvariella volvacea (egg shape)	1181.00
Agaricus bisporus (Tainung 3)	1144.00
Volvariella volvacea (bell shape)	1048.00
Pleurotus citrinopileatus	511.00
Flammulina velutipes (yellow)	363.00
Hypsizigus marmoreus	272.00
Agrocybe cylindracea (Brown)	164.00
Flammulina velutipes (white)	139.00
Agaricus blazei	136.00
Lentinula edodes (271)	132.00
Coprinus comatus	103.00
Agrocybe cylindracea (golden)	100.00
Pleurotus eryngii (small fruit body)	97.90
Pleurotus cystidiosus	85.20
Dictyophora indusiata	72.70
Pleurotus eryngii (large fruit body)	68.70
Agrocybe cylindracea (white)	68.10
Pleurotus ostreatus	48.00
Agrocybe cylindracea (yellow)	46.70
Tricholoma giganteum	38.70
Pleurotus eryngii (fruit body base)	32.10
Lentinula edodes (Tainung 1)	23.40
Grifola frondosa	12.70
Boletus edulis	10.50
Ganoderma lucidum	7.92
Coriolus versicolor	7.70
Ganoderma tsugae (baby Ling chih)	5.43

Table 4. continued

Auricularia fuscosuccinea (white)	4.11
Hericium erinaceus	3.00
Ganoderma lucidum (antler)	2.58
Auricularia fuscosuccinea (brown)	2.38
Auricularia mesenterica	1.52
Ganoderma tsugae	0.97
Ganoderma tsugae	0.66
Tremella fuciformis	0.49
Auricularia polytricha	0.12

Table 5. Equivalent umami concentration (EUC) for mycelia

Mycelia	EUC (% dry wt)
Termitomyces albuminosus	460.00
Grifola frondosa	375.00
Morchella esculenta	363.00
Hypsizigus marmoreus	128.00
Cordyceps militaris	124.00
Pleurotus citrinopileatus	37.10
Antrodia camphorata	21.20
Ganoderma tsugae	19.40
Agaricus blazei	1.92

Table 6. The Lovastatin, GABA and ergothioneine contents (mg/kg dry weight) of fruiting bodies

Fruit body	Lovastatin	GABA	Ergothioneine
Agaricus bisporus	565.4±18.5B	125.4±8.8C	932.7±5.0E
Auricularia mesenterica	ND	ND	149.4±1.0L
Boletus edulis	327.3±10.3C	202.1±4.9B	494.4±1.0HI
Clitocybe maxima (cap)	155.8±5.2GH	17.3±0.7EF	991.2±29.8E
Clitocybe maxima (stipe)	239.9±5.7D	22.7±0.7EF	664.4±3.6G
Flammulia velutipes	90.8±2.0J	229.7±13.3A	454.5±2.4IJ
Grifola frondosa	ND	17.8±2.4EF	553.2±1.3H
Hypsizigus marmoreus	257.9±8.9D	114.1±14.7 C	409.5±15.0J
Inonotus obliquus	22.0±0.3K	ND	47.7±0.6M
Lentinula edodes	ND	15.4±1.2F	412.3±9.2J
Pholiota nameko	185.5±3.6F	8.2±0.3F	228.8±7.8K
Pleurotus citrinopileatus	118.0±2.7I	17.8±3.3EF	2850.7±10.6A
Pleurotus cystidiosus	101.1±1.5IJ	37.1±1.5D E	258.9±12.4K
Pleurotus eryngii (foot)	151.8±3.2GH	ND	624.5±30.3G
Pleurotus eryngii	119.9±2.3I	25.5±0.9EF	840.4±19.7F
Pleurotus ferulae	142.2±2.5H	46.7±5.7D	464.1±6.9IJ
Pleurotus ostreatus (Japan)	606.5±5.6A	6.1±1.3F	944.1±43.1E
Pleurotus ostreatus (Korea)	165.3±3.8G	23.6±2.9EF	1829.4±49.9B
Pleurotus ostreatus	216.4±9.9E	ND	1458.4±35.3C
Pleurotus salmoneostramineus	ND	ND	1245.0±48.7D

Each value is expressed as mean ± standard error (n = 3). Means with different letters within a column are significantly different ($P < 0.05$).
ND, Not detected.

Table 7. The Lovastatin, GABA and ergothioneine contents (mg/kg dry weight) of mycelia

Fruit body	Lovastatin	GABA	Ergothioneine
Agaricus blazei	769.9±7.2C	200.4±21.0BC	79.6±7.3KLM
Agrocybe cylindracea	417.8±7.5F	123.0±10.3DE	279.4±7.3FG
Antrodia camphorata	543.6±6.5D	38.4±2.7G	281.6±13.8FG
Antrodia salmonea	1032.3±8.6B	133.0±9.1D	7.6±0.5M
Armillariella mellea	253.2±10.7G	34.2±3.4GH	219.6±9.3FGH
Coprinus comatus	109.6±5.4K	ND	399.0±4.6D
Cordyceps cicadae	134.3±4.2J	254.9±9.6A	588.0±42.3C
Cordyceps militaris (cm1)	37.7±0.7L	70.6±6.5F	215.0±10.5FGH
Cordyceps militaris (cm5)	47.9±3.5LM	68.6±5.4F	785.1±9.4B
Cordyceps militaris	57.3±3.2LM	180.1±8.4C	123.4±12.7JKL
Cordyceps sinensis	1365.3±7.9A	220.5±10.2B	142.0±38.5IJK
Coriolus versicolor	207.0±7.1H	115.8±4.2DE	13.0±1.9M
Ganoderma lucidum	10.6±0.3N	7.0±0.6I	16.5±2.0M
Grifola frondosa	ND	ND	296.2±25.1EF
Hericium erinaceus	187.5±7.3HI	6.2±0.7I	376.2±36.7DE

Table 7. continued

Hypsizigus marmoreus (white)	455.2±15.9E	125.3±1.8DE	221.4±4.6FGH
Hypsizigus marmoreus	424.3±13.6F	101.1±4.8E	206.7±12.6GHI
Inonotus obliquus	25.6±1.5MN	12.1±0.8HI	252.1±5.5FGH
Phellinus linteus	168.3±4.5I	67.8±4.8F	181.8±16.7HIJ
Pleurotus eryngii	44.5±0.9LM	ND	1514.6±15.7A

Each value is expressed as mean ± standard error (n = 3). Means with different letters within a column are significantly different ($P < 0.05$).

ND, Not detected.

Sawdust-bag cultivation technologies for mushroom production

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Introduction

Since its establishment a millennium ago in China, natural log culturing for mushroom production persists in many countries. Although it is a simple methodology of wood-decomposing mushroom cultivation, fruiting bodies require more than a year to form; moreover, much destruction occurs to the surrounding living forest stands. In 1970s, an innovative technique was developed in Taiwan in which a bag culture containing agricultural wastes and wood saw dusts were used in the replacement of logs. This has since brought about a revolutionary breakthrough in mushroom cultivation, the advantages of which not only enhance mushrooms particularly shiitake yield, but also to shorten cropping cycles of mushroom production. Not surprisingly, this technique quickly became pivotal to the cultivation of edible mushrooms.

To grow edible and medicinal mushrooms, sawdust derived from various trees is used. However, the kind of sawdust most suitable for growing these mushrooms depends on the individual mushroom species, such as *Lentinula edodes*, *Flammulina velutipes*, *Pleurotus* spp., *Auricularia* spp., *Ganoderma* spp., *Hypsizigus marmoreus*, *Agaricus braziliensis*, *Agrocybe cylindracea*, *Tremella fuciformis*, *Hericium erinaceus*, etc. Based on the species, fruiting conditions and substrate formulations for various mushrooms also differ. In this report, I introduce a general procedure which is useful as a benchmark for mushroom cultivation on sterilized sawdust with particular emphasis

on the use of these illustrate techniques of bag culture focusing specifically for oyster mushroom and shiitake cultivation.

Procedure of bag culture for mushroom cultivation

Bag culture for mushroom cultivation requires six steps, including mixing sawdust and other nutrients/ingredients, filling bags, sterilization, inoculation (spawning), incubation (spawn run), and fruiting. The general procedure is shown as Figure 1.

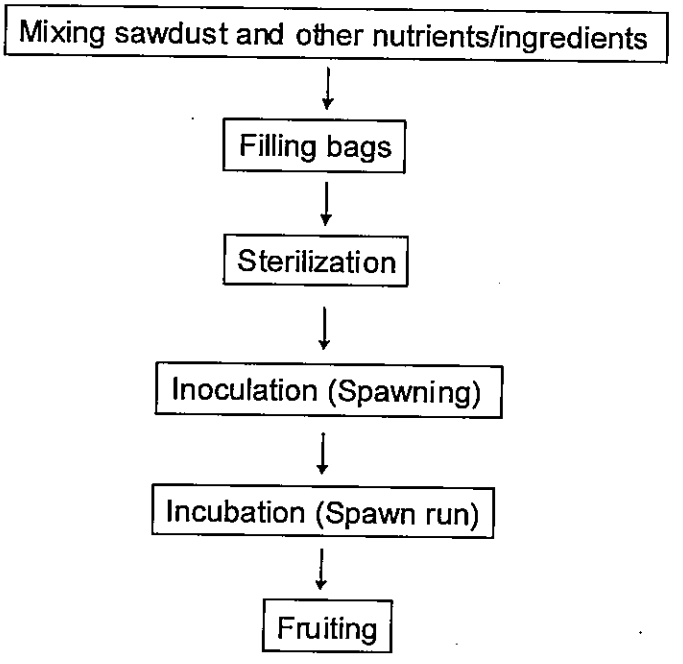


Fig 1. General procedure of mushroom cultivation on sterilized sawdust filled in plastic bags.

1. Preparation of a mixture of sawdust and other nutrients/ ingredients

In general, sawdust should be moistened then stacked for 2 to 3 days. If the fresh sawdust is not suitable for the desired mushroom growth, it should be stacked for several weeks or several months. The sawdust

pile should be turned regularly to remove detrimental components such as resins and phenolics.

To speed up spawn running and increase mushroom productivity, mushroom growers add nitrogen-rich additives such as rice bran, wheat bran, soybean meal, or corn flour to the sawdust. Using calcium carbonate or ground power of burned oyster shells, the pH value of the substrate is adjusted to 6.0–6.5.

Sawdust and other substrate ingredients are mechanically stirred in a mixer equipped with an apparatus that adds water. Based on the size of the sawdust and supplements, the water content of the substrate is adjusted to 60–67%.

2. Filling the bags

A cultivation substrate containing mixtures of saw dusts and other ingredients is filled into polypropylene bags by a semi-automatic filling machine or by hand. Different countries employ different types of bag with/without filter patches depending on their filling systems. For example, a cylindrical bag (9–10 cm in diameter and 30–40 cm in height) is most frequently used to match the filling machine in Taiwan. The bag is filled with 900–1500 g substrate mixtures based on the desired mushroom species. After the bag is full, a hole is made with a stick in the middle of the substrate mixture. This hole allows spawn inoculated to the button of bags. The wider distribution of spawn enables mycelia to grow both directions (from the top and button) to the substrates, which end up faster colonization during its spawn run in the bag culture. Finally, a plastic ring is secured outside the bags, the bag top is pulled through the ring, and the mouth is plugged with a cotton ball. Heat-sealing or plastic lids are acceptable alternatives.

3. Sterilization (heat treatment)

The objective of sterilization is to diminish contaminants (mostly keep away from microorganisms, e.g. bacteria and fungi) that hinder spawn grown in the substrate. Two heat-treatment methods are used for the sterilization of the substrates: high-pressure sterilization (121℃) and normal pressure sterilization (below 100℃). In normal pressure

sterilization, the temperature sterilization chamber should reach 98–100℃ in 3 hr, maintain this temperature for 4 hr using steam, and then end the steam treatment for 1 hr before pulling the bags from chamber for cooling.

During high pressure sterilization, the substrate temperature must reach 110℃ in 1.5 hr, bring the substrate temperature level to 100℃ for 30 min to remove cool air, then increase the temperature to 121℃ for 1 hr and preserve the same temperature for 1 hr with steam, and lastly end the steam treatment for 30 min and pull the bags from autoclave for cooling.

4. Inoculation (Spawning)

Spawn is the mushroom propagating material containing mushroom mycelia with a substrate mixture that promotes spawning. For the inoculation step, the sterilized substrate must first be cooled in a clean room equipped with an air shower, air filter, and mechanical cooling system. When the substrate temperature is cooled to 25–27℃, the substrate is ready for spawning. Prior to spawning, the spawn requires careful examination to ensure the substrate is free from contaminants. Depending on the desired mushroom species, the rates of spawn are 1–5% of the wet weight of the substrate.

5. Incubation (Spawn running)

For spawn running, the sealed inoculated bags are moved to an incubation room where the temperature can be adjusted according to the different stages. The spawn running time is different for each species of mushroom and also varied depending on the size of bag, the amount of spawn, strain used, and the temperature in the incubation room. The running of spawn usually takes 0.5 to 3 months for mycelia to fully spread throughout the substrate before the bags can be moved to a mushroom house or a cropping room for fruiting.

6. Fruiting

Initiating the fruit body and managing the mushroom crop varies according to mushroom species. The common strategies for inducing

fruiting of mushroom mycelia include temperature fluctuations, high humidity, water soaking, ventilation, light, and physical shocks in the mushroom house or cropping room. Changes in environmental conditions are necessary to make the growth of mushroom transition from a vegetative to reproductive stage. The mushroom house for cropping is constructed differently according to the mushroom species and the mushroom growers' capital. Some mushroom species, such as *Pleurotus* spp., *Auricularia* spp., and *Agaricus* spp., can be grown efficiently and less expensively in a mushroom house without equipped with any environmental controls; whereas, some mushroom species are prone to the natural climatic changes which can greatly affects its production stability. Thus, these environmental sensitive mushroom species can only be grown in some seasons of the year. With environmental controls, however, they can be grown throughout the year.

Cultivation of oyster mushroom

Oyster mushroom, *Pleurotus* genus, grows on a wide variety of ligninocellulosic of wood wastes. According to a survey by Poppe, J. (2004), about 90 kinds of agro- or forest wastes are useful to oyster mushroom production. For small scale farms on limited budgets, oyster mushrooms are a good choice for beginning mushroom cultivation because they are easier to grow and more productive than other species.

Species selection of oyster mushroom cultivation

More than eight kinds of oyster mushrooms are cultivated in the world. Before deciding which oyster species to grow, you should consider several factors, including available agro-waste materials, appropriate facilities, costs of necessary equipment, the level of skill required to manage the life cycle of the mushroom, and the market for that species (Beetz and Greer, 1999). Some oyster mushroom species, such as *P.* citrinopileatus, *P. sajor-caju*, and *P. cystidiosus*, do not require a temperature below 20℃ for initial fruiting. Therefore, they can be grown efficiently in a mushroom house without environmental

control. As for market conditions, *P. ostreatus* is one of the most popular mushrooms.

Sawdust sources

The frequently used sawdust is a mixture of different tree species from hardwoods. Without stacking, pine tree sawdust is not recommended because its resins inhibit mycelial growth. If sawdust is unavailable, rice straw/wheat straw and sugarcane bagasse serve as an alternative substrate for some *Pleurotus* species, such as *P. sajor-caju* or *P. citrinopileatus*, and produce a high biological efficiency as well.

Formula of cultivation substrate

The supplements are added to sawdust at 15% to 50% by dry weight of total substrate. Some species require lower nutrients while others require more nitrogen to produce a high mushroom yield. The additives used are mainly the by-products of agriculture, such as rich bran, wheat bran, or soybean meal.

Incubation temperature and time:

In Taiwan, most mushroom growers cannot afford expensive facilities and equipment to prepare substrates. They are not capable of producing their own spawn, either. To resolve this, several professional producers make bag substrate that has been inoculated with mushroom mycelium and sell such bags to mushroom growers. These bags are incubated at an optimum temperature of 20–25℃ in darkness since spawn running does not require light. Based on the bag size, substrate, spawn rate, strain, and temperature, full colonization takes 15–25 days.

Fruiting:

Once mycelia have fully grown through the substrates, it is ready for the formation of fruiting body – the mushrooms. In general, most oyster mushrooms do not require extra time to reach maturity. The bags with full grown mushroom mycelia may be treated two ways during the process of initiating of fruiting formation. One is to cut 4–6

slits on the sides of the bags; another is to take the cotton plug out of the mouth of the bag and strip the bag or make an opening. To establish optimal fruiting conditions, the air temperature should be maintained at 15–20℃ and the concentration of CO_2 in air is kept at the level of 1000–1500 ppm as manipulate/control by the ventilation of fresh-air. Meanwhile, the relative humidity is maintained at above 85% by the frequent addition of water in the mushroom house environment. The newly formed fruiting bodies/mushrooms can be firstly harvested within 7–10 days after the bag is cut for initiation of fruiting formation. Mushrooms are picked carefully without disturbing other developing mycelia. A continuation of 2 to 5 flushes can be harvested.

Cultivation of shiitake

Since shiitake is the third most cultivated edible mushroom in the world, I take shiitake as another good example to use sawdust-bag culture as an improved technology for mushroom production. Shiitake has earned considerable consumer demand as a fresh or dried mushroom product. It is cultivated in two ways: traditionally by natural logs and improved technology by using sawdust-bag culture. The growers can make their own decision to choose which cultivation substrate materials – pending on availability, cheapness and good recycling use of agro-wastes/leftovers for green production. Natural logs can produce high-quality shiitakes with good management, however, sawdust-bag culture has been developed and can be managed efficiently to keep high yield and with shortened fruiting cycles, thus a continuation of 3 to 6 flushes can be harvested in single cropping. This can be seen, despite a greater capital investment and more skillful management of sawdust-bag culture than natural log culture, sawdust has been evolving becoming the primary technology for shiitake production method in many countries. Figure 2 shows the pictures of the shiitake production on natural log and sawdust-bag.

Strain selection for optimal shiitake production:

Most shiitake production either cultivating in natural logs or sawdust-bag cultures is kept under natural climate without

temperature control. A key factor to running a successful business for shiitake production is selecting an appropriate strain which can well adaptive to local climate. Since each strain has its proper temperature range for to initial the primodia and the duration of production period, relevant information should be acquired prior to buying its culture or spawn. According to the preferred temperature during the fruiting period, shiitakes are classified to low temperature (<10 ℃), medium temperature (10–20 ℃), high temperature (>20 ℃), and wide-range temperature (5–35 ℃) strains. In addition, the performance of shiitake strains varies in terms of substrate selectivity, growth rate, fruiting quality, and yield. (Chen 2005)



Fig. 2. Shiitake mushrooms grow on logs (left) and on sterilized sawdust bags (right).

Substrate for shiitake fruiting production:

Non-aromatic hardwoods are usually preferably choice as raw substrates for shiitake production. Rapid-decomposing hardwoods (i.e. alder, poplar, cottonwood, willow, sweetgum, and aspen) are better than slow rotting woods (i.e. oak, ironwood, mahogany). However, denser hardwoods produce more shiitake flushes/harvests than those on faster decomposing hardwoods. In general, fresh sawdust does not

require go through stacking, if the sawdust contains components that would cause inhibition to mycelial growth, stacking should be conducted for up to several months.

Fresh sawdust from the trees of *Acacia confusa* Merr., *Trema orientalis, Liquidambar formosana* Hance may be used without prior fermentation. When the sawdust is moist enough, it is ready for substrate preparation.

The most commonly used substrate formulation/recipe for shiitake production is composition made of sawdust contains 15–25% rice bran (dry weight of sawdust) and 1% $CaCO_3$.

Spawn run and browning

The time for spawn running depends on the size of the sawdust bag culture, amount of spawn, strain used, and temperature. To achieve proper spawn run and browning, a period of 45 to 90 days for spawn running in the bag is necessary. After 30 to 45 days (1.5Kg/bag), mycelium shall be able to develop throughout the bag. At the end of incubation period, the entire surface of the substrate turns brown, indicating that mycelium is ready for fructification.

The browning process (oxidation of surface mycelium, also called skin formation) allows the mycelium on the bag surface to aggregate and toughen, thereby forming a layer of hyphae resistant to desiccation.

Fruiting and harvest

While the shiitake mycelium is ready for fruiting, growers take the cotton plugs out of the mouth of the bag and use a sharp knife to cut away the top of the plastic bag (partially), it should be careful not to cut too deeply and damage the mycelium. Another method is stripping the bags. The sawdust substrate block exposed to air is vulnerable to contamination under natural conditions.

Temperature fluctuations or water soaking is a common strategy to induce primordia. Soaking sawdust blocks or providing a physical shock (i.e. turning the blocks upside down) is required for subsequent flushes. Soaking allows water to rapidly displace carbon dioxide in the

substrate air spaces, providing enough moisture for one flush of mushroom. Many Taiwanese growers turn the blocks upside down after the first flush as a common practice.

During harvest, shiitakes are picked by hand and the residual stalk stubs are cut off from the substrate. Some shiitakes are sold as fresh while others are dried in an oven or under the sun as dried shiitakes which can be preserved for long shelf life for storage or sale in the market.

Preparation of media and spawn for mushroom cultivation

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Introduction

About 2,000 kinds of edible mushrooms exist worldwide. However, only about 80 of them can be artificially cultured. Mushroom growers have been cultivating several kinds of mushrooms for more than a hundred years in Taiwan, such as white button mushroom, straw mushroom, shiitake, enokitake, and Jew's ear. In recent decades, more newly developed mushrooms such as king oyster mushroom, oyster mushroom, brown swordbelt mushroom, and maitake have been added to the production lists. These cultivable mushrooms can be divided into two types based on their difference of cultured material. One of them is cultured on fermented substrates and the other uses sawdust or natural logs. No matter which type of cultivable mushrooms, the good quality of spawn is important warrant for the success of cultivation.

The spawn preparation procedure

Spawn is similar to seed for a crop. However, spawn is not the spores of a mushroom. Spawn is the mycelia of a mushroom growing on grain or sawdust. Spawn quality is the key factor for a successful mushroom

business. Producing spawns for mushroom production requires isolating the mycelium from the mushroom fruitbody on a potato dextrose agar (PDA) medium in petri dishes or tested tubes. After multiplying the culture by subculture, the strain is incubated among a spawn substrate such as grain or sawdust. The spawn preparation consists of four common stages. Stage 1 is the mother culture isolated from spores, the fruitbody (mushrooms), or the wood log. Stage 2 is strain cultured on PDA medium. Stage 3 is mycelial culture transferred to the spawn substrate. Stage 4 is to multiply the spawn for mushroom production.

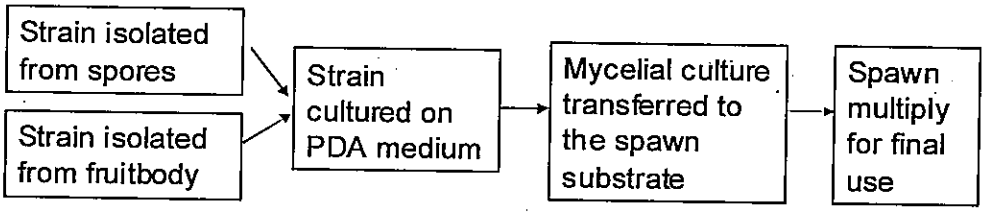


Fig 1. Four stages for making spawn.

Preparation of media suitable for most mushroom species

Preparation of agar slants

Most mushroom species grow on potato dextrose agar (PDA). The ingredients of PDA consist of 200 g diced potato, 20 g agar powder, 20 g dextrose or sugar, and 1 liter of water.

1. Wash and weigh the potatoes.
2. Dice potatoes into small pieces.
3. Boil for about 15 to 20 minutes until potatoes are soft.
4. Remove the potatoes through two layers of cheesecloth and add water to the broth to make exactly 1 liter.
5. Add the dextrose and the agar.
6. Stir occasionally and heat gently until the agar has melted.
7. Pour out the medium into test tubes when the agar is still hot and fill about one fourth of the test tubes (about 10 cc) or bottles.
8. Seal the tubes or bottles with cotton or silica plugs.

9. Sterilize the medium under pressure for 15 min.
10. Place the test tubes or bottles in an inclined position when the agar is still fluid.

Preparation of spawn

Grain spawn

Ingredients: 100% grains (wheat, sorghum, millet), 1% $CaCO_3$

1. Weight and wash the grain.
2. Boil the grain for about 15 to 20 minutes.
3. Cool down and add 1% $CaCO_3$.
4. Fill in bottles and seal with cotton plugs.
5. Sterilize at 121℃ for 40–60 min.
6. Mycelial culture is transferred to sterilized grain and incubate at 24 ℃ until mycelia grown through the substrate.

Sawdust spawn

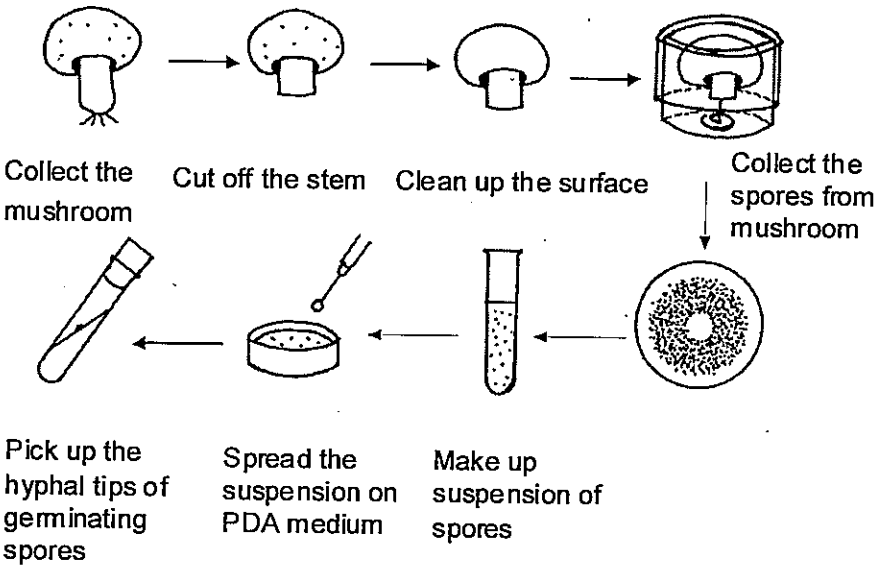
Ingredients: 80% sawdust, 20% rice brain, 1% $CaCO_3$

1. Weight and mix the sawdust, rice brain, and $CaCO_3$.
2. Add water and adjust the moisture content of the substrate to around 60~65%.
3. Fill bottles and seal with cotton plugs.
4. Sterilize at 120℃ for 40–60 min.
5. Mycelial culture is transferred to sterilized sawdust and incubate at 24℃ until mycelia grown through the bottle.

Isolation of mother culture from mushrooms

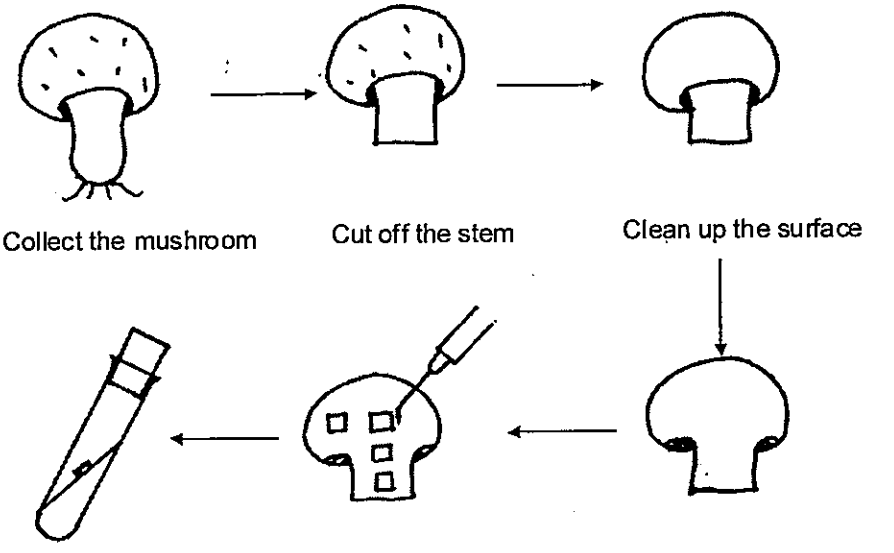
Isolation from spores

1. Collect the mushroom.
2. Cut off the stem and clean up the surface of mushroom.
3. Collect the spores from mushroom.
4. Make up suspension of spores.
5. Spread the suspension on PDA medium.
6. Pick up the hyphal tips of germinating spores.
7. Crossbreed monokaryotic mycelium to obtain a dikaryotic stain (hybrid).



Isolation from fruit body

1. Collect the mushrooms.
2. Cut off the stem and clean up the surface of mushroom.
3. Flame a scalpel until red-hot, then dip it in 95% alcohol for cooling, and flame it again to remove the alcohol.
4. Break the mushroom in half by hand and do not touch the inner surface.
5. Use the heated scalpel to take out a small piece of the inner tissue.
6. Heat the mouth of the tube in the flame and put the tissue in the middle of agar.



Preservation of mother cultures of mushroom

Spawn variations always affect the yield and quality of mushroom production. To prevent the effect of spawn variation, it is important to preserve mother cultures. Several methods have been developed for this purpose, such as subculture, refrigerating in 4℃, -20℃or -80℃, storage in liquid nitrogen, mineral oil, and sterilized water. This practicum includes three simple methods as following.

Preservation with mineral oil on agar slant

1. Subculture on PDA medium and incubate at 25℃ for 7~14 days.
2. Sterilize the mineral oil with autoclave.
3. Put sterilized mineral oil into oven overnight.
4. Add the sterilized mineral oil to agar slant until the cultured is flooded.
5. Store at 16℃.

Preservation with sterilized water

1. Subculture on PDA medium and incubate 25℃ for 7~14 days.
2. Mycelial disks are punched out with a 5-mm cork border form the margin of the colony.
3. Five mycelial disks are put into a vial containing sterilized distilled water.
4. Store at 16℃.

Preservation of stock cultures by deep-freezing

1. Subculture on PDA medium and incubate at 25℃ for 7~14 days.
2. Cut the mycelial colony by 3 X 3 X 3 mm for the agar blocks or with a 5-mm cork border.
3. Place agar blocks onto vial containing 10% sterilized glycerol.
4. Incubate at 25℃ for 2 days.
5. Vials are stored at -20℃, -80℃ or liquid nitrogen.
6. Thaw the frozen culture for 1-3 hr at room temperature before using.

Practical training for white button mushroom

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Introduction

The white button mushroom (*Agaricus bisporus*) is one of the most commonly and widely consumed mushrooms in the world. *A. bisporus* is now cultivated in at least 70 countries around the world, and global production in the early 1990s was reported to be over 1.5 billion kg, worth over US$ 2 billion. While the white button mushroom retains the highest overall consumption worldwide, it is one of edible mushrooms cultivated globally. Large-scale white button mushroom production is centered in Europe (mainly the western part), North America (USA, Canada), and S.E. Asia (China, Korea, Indonesia, Taiwan, and India). The estimated national annual production of mushrooms is approximately 50,000 tons with button mushrooms comprising 85 percent of this production. Button mushroom farming is a complex business, which requires technical skills. The major process of mushroom production involves the following steps:

I. Spawn production
II. Compost preparation
III. Spawning
IV. Spawn running
V. Casing
VI. Scratching
VII. Fruiting
VIII. Harvesting

This practicum provides practical training about spawning, casing, and scratching in mushroom cultivation. The following sections provide detailed information from step III spawning to step VIII harvesting.

Spawning

The process of mixing spawn with compost is called spawning. Before spawning, the mushroom spawn should be checked for quality without contamination. Mushroom spawn can be preserved at 4℃, but it should be taken out from refrigerator to restore its vigor under room temperature one day before it is used as inoculum or as a planting spawn. Vigorous growth of the planting spawn is a prerequisite to good growth and high yield. Old spawn is not acceptable because of possible decreased vigor and leads to low yield consequently.

The method followed for spawning is given below:

Layer Spawning:

Evenly mix 3–4 layers of spawn with the prepared compost. Cover the top portion with the whole mass of compost at the rate of 5 ml / kg.

Spawn running

After the spawning process, fill the compost in trays (plastic or wooden trays accommodating 15–20 kg compost) / shelves covered with a newspaper sheet or polythene. Spawn running is the phase during which mycelium grows from the spawn and permeates into the substrate and the time from spawn to colonization takes about two weeks. Maintain the temperature in the cropping room at 24 ± 2℃ and maintain the compost temperature for the spawn running phase no higher than 27℃. A higher temperature is detrimental for mycelial growth. Relative humidity should be around 90% and a higher CO_2 concentration (>3000ppm) is beneficial for spawn running.

Casing

While the compost beds are complete grown through by mycelium, cover the beds' surface with a layer of soil (casing) about 2.5 cm thick

to induce fruiting. Casing material characteristics include high porosity, water-holding capacity, disinfected, and a pH should range between 7–7.5. Although peat moss is considered the best casing material, it is not popular in Taiwan. The price of peat moss is expensive, so most farmers favor using lateritic soil for mushroom casing.

The casing soil before application should be pasteurized at 66–70℃ for 7–8 hours, treated with 2% formaldehyde, or sterilized with steam. The treatment needs to be done at least 15 days before the material is used for casing. After the casing is finished, maintain the room temperature again at 23–27℃, keeping the compost temperature no higher than 27℃ and relative humidity at 90–95%.

Scratching

When 70–80% of the casing layer surface is covered with mushroom mycelium, it is time for scratching. Before scratching, the casing layer should be heavily wetted. The scratching is done from the casing surfaces down to the compost interfaces with an iron-toothed rake. After scratching, maintain high relative humidity and CO_2 concentration in the mushroom house for mycelial fragment regrowth, and do not introduce fresh air or spread water onto casing surfaces.

Fruiting

Three days after scratching and heavily wetting the casing layer, fresh air can be introduced to cropping room and start re-circulating air. Under favorable environmental conditions viz. temperature (initially 24 ± 2℃ for about a week and then 16 ± 2℃), moisture (2–3 light sprays per day for moistening the casing layer), humidity (above 85%), proper ventilation and CO_2 concentration (900–1200 ppm), the fruit body initials which appear in the form of pin heads start growing and gradually develop into the button stage.

Harvesting

Mushroom caps at the button stage measuring 2.5 to 4 cm across and closed, are ideal for harvesting. The first crop appears about three weeks (20–25 days) after casing. Mushrooms need to be harvested by

light twisting without disturbing the casing soil. Once the harvesting is complete, gaps in the beds should be filled with fresh sterilized casing material and then watered. In Taiwan, about 15–20 kg fresh mushrooms per 100 kg fresh compost can be obtained in a two-month crop.

Practical training for substrate preparation techniques for mushrooms

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Introduction

The culture media (substrate) for mushrooms is as important as soil is for plants. Mushrooms are the fruiting bodies of macro-fungi. Fungi lack chlorophyll, which makes them different from plants that obtain energy from the sun through photosynthesis. Instead, mushrooms can grow on ligninocellulosic wastes, degrading and taking nutrients from those materials for their growth. Agro-wastes or leftovers either from agricultural practices from forest stands, such as cereal straws are largely burnt or discarded by the farmers. However, these from agro-wastes can be further treated and recycled as useful raw materials for use in the cultivation of value-added edible mushrooms. This type of bioconversion by mushroom mycelia exercise can greatly reduce environmental pollution (S. T. Chang 2007). Therefore, mushroom cultivation is a friendly green technology for extending recycling use of agro-wastes/leftovers and eco-friendly resource management beneficial to the environment.

Preparation of substrate materials and additives

Commonly used substrate materials for mushroom cultivation are paddy straw, sawdust, cottonseed hull, cereal straw, corncob, coffee waste, sugar cane bagasse, wild grasses, and other plant fibers with high lignin or cellulose contents (K. Hyungjong and Byung S. K. 2004). These culture substrates are considered agricultural wastes, as substrates are very inexpensive and sometimes free.

Various edible mushrooms can be grown on different agricultural wastes and leftovers which, if not used, usually end up discarded, such as growing oyster mushrooms on a wider variety of substrates than any other cultivated mushrooms. Readily available additional nitrogen sources such as wheat bran, rice bran, or corn are necessary for mushroom growth during their vegetative growth stage. Additional substrates typically affect mushroom yield. In addition, gypsum, calcium carbonate, or chlorinated lime is added to the substrate and function as buffers for controlling pH value in the substrate.

Sawdust is currently the most commonly used basic substrate for mushroom cultivation, except for some mushrooms cultivated on fermented substrates (ie. the white button mushroom). Various types of trees available in each region can be used as sawdust sources. The frequently used hardwood tree species for mushroom cultivation include *Trema orientalis, Machilus kusanoi,* oak, poplar, alder, maple, mango, elm, and etc. Selecting tree species that are both favorable for mushroom growth and that are easily available is important for mushroom production. Pine, cedar, and redwood trees should be avoided to use for as mushroom culture substrates because resins in sawdust inhibit mycelial growth and colonization.

Bag cultivation

Bag cultivation is the most commonly used method for mushroom cultivation in many countries around the world. Plastic bags (polythene and polypropylene) are most frequently used for mushroom cultivation.

Sawdust preparation

Fresh sawdust is not suitable for mushroom culture because it can not absorb water immediately. To make sure the sawdust is moisten enough, the sawdust is piled up and the stacking needs to be turned over with water once every two months. After one to four months, the sawdust could be used for mushroom cultivation.

Additives :

Rice bran is a by-product of the rice milling process (converting brown rice to white rice).

Wheat bran is the hard outer layer of grain and consists of combined aleurone and pericarp.

Sample Formulation for *Pleurotus eryngii*

60% Sawdust (w/w), 24% rice bran, 15% wheat bran, and 1% calcium carbonate.

Substrate mixing

Sawdust is mixed with water and other supplements either manually or mechanically. During mixing, attention should be paid to evenly distributing water throughout the entire substrate. Generally, the moisture content of the substrate is typically adjusted to 65%. The palm test method is a simple way to check whether the mixture has proper water content.

Filling and compressing

The prepared substrate mixture is filled into bags before heat-treatment. The filled bags are then properly compressed for quick mushroom mycelia colonization. After filling and compressing, a 2–3 cm-diameter hole is made in the center, which allows for spawn down at the bottom of the bag. The hole provides a deeper inoculation and a better oxygen supply, enhancing faster colonization. Mushroom mycelia need some oxygen for respiration during growing stage; therefore, ventilative plugs or stoppers are recommended for free air

exchange. Farmers in Taiwan use plastic neck-rings and plugs with cotton balls to close the open–side of the bag.



Fig 1. The process of substrate preparation for bag culture: A) substrate mixing, B) substrates filling, C) holing, D) plugging with cotton balls.

Heat treatment

Before spawning, the substrate needs to be treated to kill or reduce pests and microorganisms by using sterilization or pasteurization. Sterilization kills all microorganisms while pasteurization only reduces the number of microorganisms. The sterilization method is recommended to minimize the risk of contamination.

Normal-pressure sterilization

Once the substrate bags are done, they are then placed into the autoclave or sterilizer. The desired temperature for normal-pressure sterilization is maintained at 100℃ for 6 hours, measured from the time the inside temperature reaches the target temperature. After sterilization, the substrate bags are then taken out and moved to a cooler room overnight.

High-pressure sterilization

The desired sterilization pressure and temperature are under the pressure of 1.5 kg/cm^2 and the temperature inside the sterilizer rises up to 121℃. When it reaches 121℃, the temperature is maintained for 60 minutes. After steam is released, the substrate bags are moved from the sterilizer to a cooler place. The sterilized substrates are cooled to room temperature for spawn inoculation.

Spawning

Mushroom spawn is a medium that carries mushroom mycelia. Most growers use spawn produced by themselves or commercial spawn providers. The following describes how to inoculate substrate bags.

Bottle cultivation

The bottle culture system has been successfully adopted for use in commercial production of spawn and mushrooms for many years in the past in Japan, Taiwan, Korea, and many other countries. The initial set-up cost for the system is higher for most beginning growers due to some automatic equipment and facilities are used. Some spawn suppliers favor propylene bottles since they are heat-resistant and thus, autoclavable, endurable, re-usable, and easy to handle (K. Hyunjon 2004). The major process of bottle culture system for mushroom production is illustrated as fig 1.

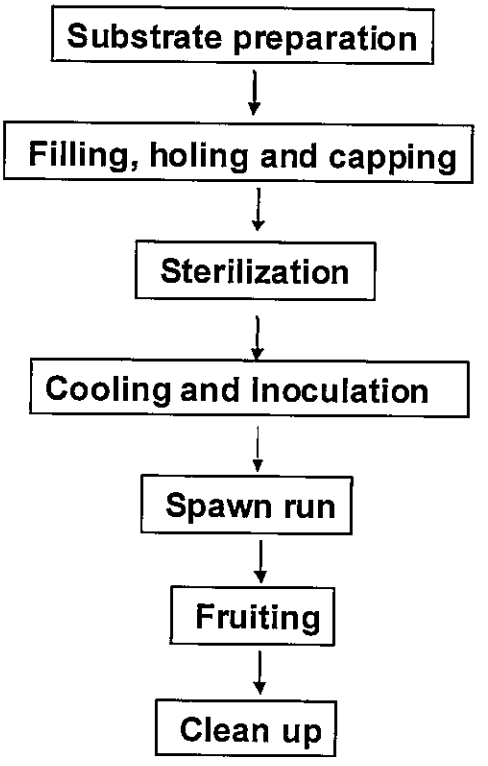


Fig 2. The major process of bottle culture system.

The section above describes **substrate preparation.**

Filling, holing and capping

The prepared substrate is loaded into the bottle feeder which fills bottles with the mixture. Once bottles are filled, compactors press the bottle mixture down to the pre-set height, and then hole-makers go through the compacted mixture. When the filling and the holing steps are complete, the bottles are transferred to another machine that automatically caps the bottles.

The section above describes **heat treatment (sterilization).**



Fig 3. The process of substrate preparation for bottle culture: A) sawdust stacking, B) substrate mixing, C) substrates automatic filling, D) holing, E) capping, F) bottles loading in the autoclave.

Cooling and Inoculation

After sterilization, the bottles are removed from the autoclave and cooled to 25℃ in the cooling room. Bottles ready to inoculate are moved from the cooling room through a small window onto the clean bench on the skate wheel conveyor. Before inoculation, the inside of the inoculating room must be disinfected with an ultraviolet lamp and 70% alcohol. The semi-automatic inoculating machine allows the operation to be much faster and effective.

Spawn run

Inoculated bottles are transferred to an incubation room, where the temperature and humidity are maintained at 20–21℃ and 65–70%. The spawn run is strain-dependant, and typically takes 30–35 days. During incubation, the maximum limit of CO_2 concentration for mycelia growth is 5000ppm.

Fruiting

Once the spawn run is complete, the bottles are brought to a growing room or exposed to a lower temperature. Low temperature or high humidity is controlled and maintained as the mycelia shift into reproductive growth from vegetative growth to induce fruiting body formation.

Clean up

After harvest, the bottles are loaded into an automatic machine to remove spent substrates. The emptied bottles are washed with water, dried, and arranged to be recycled for further use. The emptying area should be far from the growing and incubation room since the spent substrates might harbor pathogens or pest spores.

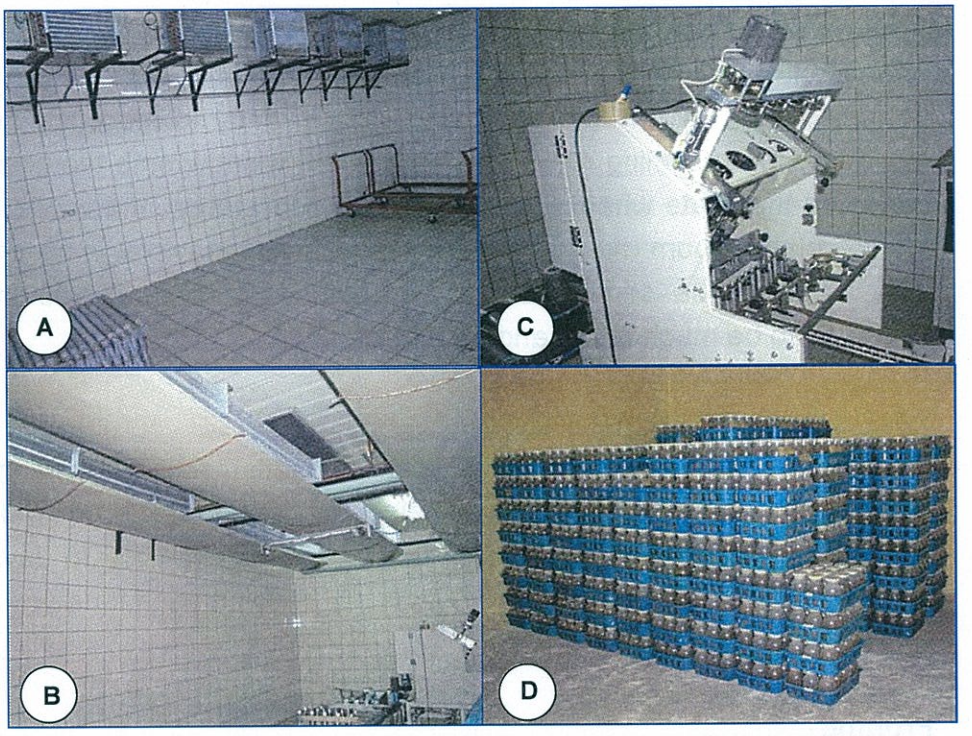


Fig. 4. A) A cooling room with mechanical cooling system, B) an inoculating room with air filter, C) a semi-automatic inoculating machine, D) incubation room.

Appendix



Common name: Enokitake, the winter mushroom, the golden mushroom

Scientific name: ***Flammulina velutipes*** (Curtis) Singer

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:4-16 ℃

Relative humidity: 95-100%

CO_2: 2000-4000 ppm

Light requirement: 20-50 lux

Fruiting body development:

Temperature:10-16 ℃

Relative humidity: 90-95%

CO_2: 2000-4000 ppm

Light requirement: 20-50 lux



Common name: Nameko, Slime Pholiota, Viscid mushroom,
Scientific name: *Pholiota nameko* (T. Ito) S. Ito et Imai
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:10-16 ℃
Relative humidity: 95-100%
CO_2: 500-1000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:12-18 ℃
Relative humidity: 90-95%
CO_2: 800-1200 ppm
Light requirement: 500-1000 lux



Common name: The oyster mushroom, Hiratake

Scientific name: *Pleurotus ostreatus* (Jacquin:Fr.) Kummer

Growth parameters:

Incubation temperature: 22-24 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: ＜1000 ppm

Light requirement: 1000-1500 lux

Fruitbody development:

Temperature:10-21 ℃

Relative humidity: 85-90%

CO_2: ＜1000ppm

Light requirement: 1000-1500 lux



Common name: The Indian mushroom, the Phoenix mushroom,
Scientific name: *Pleurotus sajor-caju* (Fr.) Singer
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:21-24 ℃
Relative humidity: 95-100%
CO_2: 400-800 ppm
Light requirement: 1000-1500 lux
Fruitbody development:
Temperature:21-24 ℃
Relative humidity: 90-95%
CO_2: 400-800 ppm
Light requirement: 1000-1500 lux



Common name: king oyster mushroom,
Scientific name: *Pleurotus eryngii* (DC.:Fr.) Quél
Growth parameters:
Incubation temperature: 21-24 ℃
Primordia formation:
Temperature:16-18 ℃
Relative humidity: 95-100%
CO_2: 1000-2000 ppm
Light requirement: 20-50 lux
Fruitbody development:
Temperature:14-16 ℃
Relative humidity: 90-95%
CO_2: 2000-3000 ppm
Light requirement: 20-50 lux



Common name: Yuhuanmo, the golden oyster mushroom
Scientific name: *Pleurotus citrinopileatus* Singer
Growth parameters:
Incubation temperature: 24-29 ℃
Primordia formation:
Temperature:21-27 ℃
Relative humidity: 95-100%
CO_2: ＜1000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:21-29 ℃
Relative humidity: 90-95%
CO_2: ＜1000 ppm
Light requirement: 500-1000 lux



Common name: Brown swordbelt, the black poplar mushroom, Pioppino,

Scientific name: *Agrocybe chaxingu* Huang

Growth parameters:

Incubation temperature: 21-27 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:13-18 ℃

Relative humidity: 90-95%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux



Common name: The beech mushroom, Bunashimeji
Scientific name: *Hypsizygus marmoreus* (Peck) Bigelow
Growth parameters:
Incubation temperature: 21-24 ℃
Primordia formation:
Temperature:10-15 ℃
Relative humidity: 95-100%
CO_2: 500-1000 ppm
Light requirement: 400-600 lux
Fruitbody development:
Temperature:13-18 ℃
Relative humidity: 90-95%
CO_2: 2000-4000 ppm
Light requirement: 400-600 lux



Common name: Wood Blewit

Scientific name: *Clitocybe nuda* (Bull.:Fr.) Bigelow & Smith

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-15 ℃

Relative humidity: 95-100%

CO_2: ＜1000 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:10-15 ℃

Relative humidity: 90-95%

CO_2: ＜2000 ppm

Light requirement: 500-1000 lux



Common name: Sunny mushroom, Brazil mushroom, Royal sun Agaricus, ABM, Almond Portobello

Scientific name: *Agaricus blazei* Murrill

Growth parameters:

Incubation temperature: 21-27 ℃

Primordia formation:

Temperature:21-24 ℃

Relative humidity: 80-90%

CO_2: 400-800 ppm

Light requirement: 100-200 lux

Fruitbody development:

Temperature:24-27 ℃

Relative humidity: 75-85%

CO_2: ＜2000 ppm

Light requirement: 100-200 lux



Common name: Reishi, Ling Zhi
Scientific name: *Ganoderma lucidum* (Curtis:Fr.) P. Karst
Growth parameters:
Incubation temperature: 21-27 ℃
Primordia formation:
Temperature:21-27 ℃
Relative humidity: 95-100%
CO_2: 2000-5000 ppm
Light requirement: 500-1000 lux
Fruitbody development:
Temperature:21-27 ℃
Relative humidity: 90-95%
CO_2: ＜2000 ppm
Light requirement: 750-1500 lux



Common name: Maitake (Dancing mushroom), Hen-of-the-woods

Scientific name: *Grifola frondosa* (Dicks:Fr.) S. F. Gray

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-20 ℃

Relative humidity: 90-95%

CO_2: 2000-5000 ppm

Light requirement: 100-500 lux

Fruitbody development:

Temperature:13-18 ℃

Relative humidity: 75-85%

CO_2: ＜1000 ppm

Light requirement: 500-1000 lux



Common name: Lion's mane mushroom, Monkey's head

Scientific name: *Hericium erinaceus* (Bulliard :Fries) Persoon

Growth parameters:

Incubation temperature: 21-24 ℃

Primordia formation:

Temperature:10-16 ℃

Relative humidity: 95-100%

CO_2: 500-700 ppm

Light requirement: 500-1000 lux

Fruitbody development:

Temperature:18-24 ℃

Relative humidity: 90-95%

CO_2: 500-1000 ppm

Light requirement: 500-1000 lux



Common name: Wood ear, Jew's ear

Scientific name: *Auricularia polytricha* (Montagne) Saccardo

Growth parameters:

Incubation temperature: 24-30℃

Primordia formation:

Temperature: 12-24℃

Relative humidity:90-100%

CO_2:600-1000 ppm

Light requirements: 500-1000 lux

Fruitbody development:

Temperature: 21-30℃

Relative humidity:85-90%

CO_2:1000-1500 ppm

Light requirements: 500-1000 lux

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