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ORIGINAL ARTICLES

Utilization of Agro. and Agro-industrial Wastes for Cultivation of Shiitake (*Lentinus edodes*) an Edible and Medicinal Mushroom and Their Drying Aspects in Egypt.

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ABSTRACT

Shiitake mushroom *Lentinis edodes* is an edible and medicinal mushroom that is highly appreciated and has a commercial potential in many countries especially China, Japan and United States. This type of mushroom was newly introduced to Egypt from China. *L. edodes* mushroom was grown using different media substrates during three consecutive seasons. Incubation period, early of harvesting, yield and biological efficiency were estimated as well as drying parameters for fruit bodies. The incubation period ranged from 36 to 57 days and was affected mainly by media type. Sawdust recorded the shortest incubation time and first harvesting day time, while bagasse showed the longest ones. Also, Sawdust produced the maximum yield 297g/ kg wet media with the highest biological efficiency%, while bagasse recorded the lowest values. Rice straw and corn cobs lies in between. The shiitake fruit bodies contained (86.37-87.52%) moisture content, (21.49-23.17%) crude protein, (3.62-4.15%) ether extract and (7.2-8.35%) ash content. The shiitake fruit bodies were used for drying process. Drying time was affected mainly by drying method and pre-drying treatments which ranged between 40-60 hrs. for sun drying and 10-16 hrs. for oven dehydration. Moisture content of dried mushroom ranged from 5.16 to 8.96% depending upon drying method and pre-drying treatments. Rehydration ratio of dried shiitake was greatly affected by pre-drying treatment. Steam blanched dried samples attained the lowest rehydration ratio, while dried control sample had the highest rehydration ratio.

Key words: shiitake mushroom, *Lentinus edodes*, sawdust, bagasse, rice straw, corncobs, cultivation, growing parameters, incubation time, yield, drying ratio, rehydration ratio, chemical composition

Introduction

Mushrooms are widely consumed due to their delicious flavour, adequate protein content and exotic preference. In addition to their nutritional value, many edible large mushrooms have long been used for medicinal purposes.

Due to the documented probiotic properties of Shiitake mushroom (Stamets 1993, Wasser and Weis 1999, Manzi and Pizzoferrato, 2000) and their relatively high nutritive value, it is recommended in numerous countries as an addition to the daily diet (Kalac and Svoboda, 2000, Isiloglu *et. al.* 2001, Bernas *et al*. 2006). Also, Regula and Siwulski (2007) stated that, dried shiitake and oyster mushrooms can be used as additives in food products.

Shiitake mushroom *(Lentinus edodes)* has been presented in the literature as a microorganism with potential for food industry, medicinal application, enzyme production and effluent treatment. This fungus is the source of bioactive polysaccharides. Lentinan is a cell wall polysaccharide- extractable from both fruit body and mycelium, and LEM - a protein bound polysaccharide derived only from the mycelium (Chihara, 1992). Both compounds have demonstrated anti-cancer activity. Also both compounds have antiviral and antimicrobial activity (Wasser & Weis, 1999). Besides, Eritadenine, a compound extracted from Shiitake fruit bodies, is able to lower blood serum cholesterol (BSC) by the acceleration of the excretion of ingested cholesterol and its metabolic decomposition (Susuki &Oshima, 1974).

Shiitake mushroom have been cultivated in China on notched logs stacked in evergreen forests since as early as A.D. 1100. It is believed that Chinese growers introduced shiitake cultivation techniques to Japanese farmers, who named the mushroom and were later responsible for its spread eastward. Shiitake can be grown on synthetic as well as natural logs. Composed of sawdust, straw and corncobs supplemented with millet and wheat bran, synthetic logs may produce three to four times as many mushrooms as natural logs—in one-tenth of the time. The popularity of the culinary characteristics offered by shiitake bode well for the continued growth and development of the industry worldwide **(**Royse, 2001).

Kurtzman, (2007, 2011) recorded that, different places require different strategies for growing or possibly preventing fungal growth. Egypt is a very hot country, but since it is also a very dry country, so 30°C outside,

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may allow the humidified growing room to be only 21°C which mushroom can generally grow. Also, the author stated that, Egypt started growing mushroom and will have a real future in mushroom growing.

There are many reasons why shiitake is popular. When cooked, it imparts a full-bodied aromatic with distinctly pleasant flavor to the dish while maintaining its own original color and chewy texture. Fresh shiitake resists both bruising and spoilage remarkably well. Shiitake is easily dried. Dried shiitake is both convenient for use and inexpensive for industry to store and transport. Heat used to dry shiitake enhances certain popular flavor characteristics. Dried shiitake mushroom dehydrates well, after which it rivals fresh ones for color, shape, and texture (Leatham, 1982).

This investigation was carried out to determine the possibility of cultivation of shiitake mushroom (*Lentinus edodes*) on some agro and agro-industrial wastes available in Egypt. Besides, studying the growing parameters of shiitake mushroom on different media and to estimate the macro nutrients of shiitake fruit bodies and their drying properties.

Materials And Methods

Fungal Strain:

Shiitake mushroom *Lentinus edodes* LC 2141 was kindly obtained from Jun-Cao Research Institute of, Fujian Agricultural & Forestry Univ.(FAFU), China. The culture was maintained on Potato Dextrose Agar (PDA) medium and stored in refrigerator at 5 -7 °C after growth. The culture was used for producing the grain spawn by the convenient method. The prepared spawn were stored at 5°C until using them for cultivation.

Growing Media and Cultivation:

Hard wood sawdust, rice straw, crushed corn cobs and crushed bagasse were used as the base of growing media. To each of the previous cellulosic wastes 20% wheat bran, 1% soy bean flour, 2% gypsum were added and mixed well. The moisture content of the aforementioned media formulae were adjusted to approximately 6o – 63%. Each formula was filled in polypropylene bags (1kg each) and autoclaved at 121°C for 1 hour. After the sterilized media was cooled down, the bags were inoculated by the previously prepared grain spawn 2%(w/w), then being incubated at 22 - 27°C for spawn run (mycelium growth).

At the end of incubation time (fully mycelium growth) the polypropylene bags were cut off and completely removed and subjected to the fruiting conditions i.e. exposure to scattered light, watering by daily water spraying, good ventilation to be sure to eliminate the dense carbon dioxide in growing rooms (Kurtzman, 2010). Adjusting relative humidity to 85–90% and temperature around 20°C. These conditions encourage the formation of brown protective layer. The crop was picked after 2- 4 weeks from the end of incubation time in consecutive 2- 3 flushes at intervals of 15-20 days. After each flush the media blocks were soaked in cold water for 24 hrs.

Since this mushroom type is newly introduced to Egypt from China and their cultivation techniques are not adopted, so this experiment was carried out in three consecutive trials to get actual and reliable results.
The first season started in 13 Nov. 2008 until 24 March 2009.
The second season started in15 Nov. 2009 until 25 March 2010.
The third season started in 11 Nov. 2010 until 23 March 2011.

Fresh fruiting bodies of shiitake *L. edodes* mushrooms were preserved by drying as follows:

Drying of mushrooms:

Mushroom sample was divided into 3 equal parts; each of which was subjected to one of the following pretreatments before the dehydration process.
A- Untreated sample (control)
B- Steam blanching at 96 °C for 5 minutes.
C- Soaking in 0.2 % $Na_2S_2O_5$ for 10 min. (sulfuring)

All the pretreated mushroom samples were dried by the following methods.

Sun drying:

Pre-treated samples of the tested mushrooms were sun-dried according to Suguna *et. al.* (1995). Samples were spread in single layer in wooden trays (100 x 50 x 2.5 cm) and dried in direct sun light. Sun drying was continued until the samples reached a constant weight. Sun drying day was expressed as 10 hours.

Oven drying:

The perforated Stainless Steel trays (50 x 50 x 2cm) were loaded by pre-treated mushroom samples and dried in an air ventilation oven at 60 ^{o}C for 2 hrs, then temperature was reduced to 50^{o}C. Dehydration was continued until samples reached constant weight according to the method of Bhatti *et. al.* (1989).

All dried samples were analyzed immediately after drying for moisture content and rehydration ratio (as fast and good quality parameters for dried products)

Analysis:

All determinations were carried out in triplicates. Moisture, crude protein (N x 6.25), fat, and ash contents were estimated according to the A.O.A.C(1990). Total carbohydrates were calculated by differences. Biological efficiency (BE%) was calculated as reported by Stamets(1993) as follows:

BE% = [Fresh fruiting bodies (g) / dry weight of medium substrate (g)] 100

Rehydration ratio was estimated according to Von- Loeseck(1955).

Data of spawn run time (days) and yield were statistically analyzed using ANOVA procedure of the SPSS statistical package at confidence level of 5% (0.05) (SPSS, 1990).

Results And Discussion

Media moisture content:

The moisture content of different media used for *L. edodes* cultivation in three seasons ranged from 60.64 to 62.55% (table, 1). No clear trend for moisture content differences was recorded according to substrate or season. The moisture content of the tested media met the optimum range stated by many authors , Yong & Man (1992*),* who found that *L.edodes* N.85 strain grew very weak at 50% and 70% medium moisture content, while the optimum was 59.2%.; Lin *et al.* (2000*)* recorded that the most favorable medium moisture content for the growth of *L.edodes* was 60%; Medany (2004) who found that at 70.29%moisture content, no growth was detected for *L.edodes* mycelium, and at 54.37% the growth started slowly for 5 days then stopped and could not colonize the media. Meanwhile at 60.97% the growth of *L. edodes* was faster, denser and only required 40 days for complete growth.

Table 1: Media moisture content% (on fresh basis)

	First season	Second season	Third season
Saw dust	61.77^{c}	62.40^{ab}	60.64^{cd}
Rice straw	62.38^{ab}	62.11^{b}	61.33^{d}
Corn cobs	62.55^{a}	60.89^{e}	61.58^{cd}
Bagasse	60.91^{e}	61.46^{cd}	60.83^{e}

Means within columns have different superscript are significantly different.

Growing parameters:

The data presented in Table (2) show that, the incubation period for *L. edodes* grown on different media ranged from 36 to 57 days as affected mainly by media type. The shortest incubation period was recorded for sawdust medium with no significant differences between the three seasons. Meanwhile, the incubation period for bagasse medium reached to 57 days. No or little significant differences were detected in the incubation period among rice straw, corn cobs and bagasse media for all the tested seasons. No obvious significant differences in the incubation period were caused by season for the same medium.

The present results are shorter than those for incubation period of *L. edodes* reported by Yong and Man (1992) who recorded 80 days on saw dust. While, the present results are within the range of the data obtained by Stamets & Chilton (1983) 30-60days, Kaura & Lakhanpal (1995) and Zhang (1997) 40-70days *;*Philipoussis *et.al* (2000) 46 days when grown on wheat straw or poplar saw dust under most favorable conditions was 46 days; Medany (2004) recorded incubation period of 40- 62 days for *L.edodes* grown on different media.

After the complete mycelium growth of *L. edodes* the bags were subjected to the conditions which induce the protective brown layer coating, then start fruiting. The data listed in Table (2) show that fruit bodies initiation and development time including brown layer coating lasted from 16 – 27 days for all tested growing media. It could be seen that, saw dust recorded the shortest time for pin heads initiation and fruit bodies development being 16 – 20 days followed by those grown on rice straw (17 -21 days). The initiation of pins and development of fruit bodies grown on saw dust and rice straw did not differ significantly. Meanwhile, bagasse recorded longer time for pin head initiation and fruit development. No significant differences were detected in these values caused by season for each media. Different times for *L.edodes* pin initiation were recorded by many authors, Stamets & Chilton (1983) 1-2 weeks ; Kawai *et al.* (1995) 20 days; Kaura and Lakhanpal (1995) 32 days; Pire *et al.* (2001) who recorded rather shorter appearance time for *L.edodes* pin head being 7-10 days. Medany (2004) 8 -20 days.

Table 2: growing parameters of L edodes on different media

	Incubation time (days)			Fruit bodies initiation& develop(days)			First harvest day (earliness)		
	1st season	2nd season	3rd season	1st season	2nd season	3rd season	1st season	2nd season	3rd season
Saw dust	38^{A}	36^{A}	40^{A}	16^{a}	20^{abc}	17^{ab}	54	56	57
Rice straw	49^{B}	52^{BC}	50^{B}	18^{ab}	17^{ab}	21^{abcd}	67	69	71
Corn cobs	51^{BC}	49^{B}	53^{BC}	22^{abcd}	25^{cd}	23^{bcd}	73	74	76
Bagasse	55^{BC}	53^{BC}	57^{C}	23^{bcd}	25^{cd}	27^{d}	78	78	84

Means within columns have different capital superscript are significantly different.

Regarding the first harvest day (earliness), the data in Table (2) reveal that, it differ according to the media type and ranged between 54 – 84 days. Saw dust medium recorded the shortest period being 54, 56 and 57 days for first picking day in first, second and third seasons, respectively. Rice straw come in second order (67 – 71 days) after sawdust, and then corn cobs 73- 76 days. Meanwhile, bagasse lasted the longest time to reach harvesting day being 78- 84 days in all tested seasons. Lin *el al.* (2000) recorded that 60-70 days passed from spawning to get the first harvest for *L.edodes.*

Yield and biological efficiency of L. edodes:

The data in Table (3) reveal that, the yield of shiitake mushroom ranged between 122 – 297 g /kg wet media, while biological efficiency (BE%) ranged from 31.66- 76.64%. Among the different media used for cultivation, sawdust produced the highest yield being 293, 268 and 297 g / kg wet medium for first, second and third season, consecutively which highly differed significantly than the other media. Growing season has no significant differences in yield for the same medium. Rice straw and corncobs media followed sawdust and gave adequate yield ranging from 181 to 221 g / kg wet medium in all tested seasons with approximately no significant differences between them.

Table 3: Yield and biological efficiency of *L. edodes* grown on different media

	Yield g / kg wet media			Biological efficiency (BE%)		
	1st season	2nd season	3rd season	1st season	2nd season	3rd season
Saw dust	293^{a}	268^{a}	297^{a}	76.64	71.28	75.46
Rice straw	212^{bcd}	218^{bc}	221^{b}	56.35	57.53	57.15
Corn cobs	187^{cd}	190^{bcd}	181^{d}	49.93	48.58	47.11
Bagasse	130^{e}	122^{e}	137^{e}	33.26	31.66	34.98

Means within columns have different superscript are significantly different.

Meanwhile, bagasse lasted the end of descending order in yield of shiitake mushroom (122- 137 g / kg wet medium), which highly differed significantly in yield values compared to other media. Also, the data show that, values of BE% differed according to the media used in growing and has the same trend of yield. Sawdust medium recorded the highest BE% being 71.38- 76.64%, while bagasse gave the lowest ones being 31.66- 34.98% in all tested seasons. The present results are within the range of the data obtained by Yong and Man (1992), Lin *et. al.* (2000) and Philippoussis et al. (2000) who found that, the biological efficiency of *L.edodes* grown on different media ranged between 12.78 and 93.9%. Also Royse and Sauchez –vazquez (2000) got a biological efficiency for *L.edodes* ranged from 107.2 to 121.8%. Medany (2004) recorded 283g / kg wet oak sawdust medium.

Morphological Characteristics Of Shiitake Mushroom:

The morphological characteristics of shiitake mushroom fruit bodies are listed in Table (4). The fruit bodies had cream to dark brown color caps with 5.2 to 10.4 cm diameter, caps mostly regular shape, sometimes irregular. The stems and gills are white to off white, and fruits usually grow individually. The average fruit body weight was 12.85g. The present results are in accordance with the data reported by Stamets & Chilton (1983); Jie (2002) and Medany (2004)

Table 4: Morphological characteristics of *L. edodes* fruit bodies

Character	Description
Fruit body weight	8.3 – 17.4 g
Cap diameter	5.2 – 10.4 cm
Stem diameter	0.5 – 1.7 cm
Stem length	2.2 – 5.0 cm
Cap surface color	Cream to dark brown
Flesh color	white
Stem color	Off white
Gills color	Off white
Fruit body shape	Mostly regular cap but some times irregular standing centrally over cylindrical stem, with straight radial gills. The caps have some cracks on the surface showing the white flesh. The fruit bodies usually grew single.

Chemical Composition Of Shiitake Mushroom:

Moisture content of shiitake mushroom grown on different media in the third season ranged from 86.37 – 87.52% with no significant differences between all values (table,5). As for protein content it was ranged between 21.49 and 23.17% on dry weight basis. No significant differences in protein content were detected. Shiitake mushroom grown on different media contained relatively low amounts of ether extract being 3.62-4.15% which did not differ significantly according to growing media.

Table 5: Macronutrients profile (%) of *L. edodes* fruit bodies grown in the third season (on dry basis)

	*Moisture content	Crude protein	Ether extract	Ash	Total carbohydrates
Saw dust	86.37^{a}	23.17^{a}	3.79^{a}	8.35^{a}	64.69
Rice straw	87.21^{a}	22.74^{a}	4.15^{a}	7.68^{a}	65.43
Corn cobs	86.93^{a}	21.49^{a}	3.88^{a}	7.20^{a}	67.43
Bagasse	87.52^{a}	22.08^{a}	3.62^{a}	8.08^{a}	66.22

moisture content was estimated on wet basis.
Means within a column have different superscript are significantly different.

Regarding ash content, the data in table (5) show that , the values of ash content ranged from 7.2 to 8.35% . No significant differences in ash values caused by media type. Total carbohydrates were calculated by differences and represent a high proportion among chemical constituents in shiitake mushroom being varied from 64.69 to 67.43%. The results of chemical constituents of shiitake obtained coincide with those reported by many authors, Crisan & Sands (1978), Stamets (1993), Mizuno (1995), Manzi *et. al*.(1999), Lin *et. al.* (2000), and Morais *et. al.* (2000), Medany (2004).

Drying Parameters Of Shiitake Mushroom:

The fruit bodies of *L. edodes* grown on sawdust medium in third season were used for drying process. Drying time (table, 6) was distinctly affected by pre-drying treatment and drying method. Regarding sun drying, control (untreated) sample required 40 hrs (4 days). Sulfured sample tooke 55 hrs, while steam blanched sample needed 60 hrs. Oven dehydration for shiitake samples have the same trend of sun drying and required 10, 13 and 16 hrs for control, sulfured and steam blanched samples, consecutively .The longer drying time of pretreated sample especially blanched one than the control sample. This might be attributed to the absorbance of much water during blanching and sulfuring process as reported by Hassan (2002). He also recorded that control (untreated) samples of *P.ostreatus* and *A. bisporus* needed shorter sun-drying time being 35 and 40 hours, while pretreated samples recorded (40-50 hours), respectively. While, oven-dehydration required 9-13.5 hours. Medany (2004) recorded that, oven-dehydration time of *L. edodes* was 9.5-13.5 hours.

Table 6: drying parameters of *L edodes* fruit bodies grown on saw dust at the third season.

	Drying time (hrs)	Drying ratio	Moisture content %	Rehydration ratio	Appearance
Sun drying					
Control	40	7.86 : 1	8.31^{c}	7.32^{b} : 1	+
Sulfured	50	8.12 : 1	8.75^{c}	6.51^{c} : 1	+
Steam blanched	60	8.70 : 1	8.96^{c}	3.44^{e} : 1	-
Oven dehydration					
Control	10	8.11 : 1	5.16^{a}	7.97^{a} : 1	++
sulfured	13	8.16 : 1	6.27^{b}	7.13^{b} : 1	+
Steam blanched	16	8.97 : 1	6.47^{b}	3.25^{e} : 1	-

+ Appearance acceptability degree
- Bad appearance (not acceptable)
Note: sun drying day represent 10 hrs.
Means within a column have different superscript are significantly different.

As for drying ratio, the data reveal that, drying ratio differed slightly according to pre-drying treatments and drying method. Control sample exhibited a high drying yield (low drying ratio) being 7.86 :1 and 8.11 :1 for sun and oven drying, respectively. Sulfured sample gave a moderate drying yield when dried by either sun or oven drying. On the other hand, steam blanched sample resulted a low drying yield (high drying ratio) when dried either by sun or oven. Pre-drying treatments of mushroom samples reduced the drying yield. This might be due to the loss of soluble solids by leaching out and/or absorption of water through blanching and sulfuring process. In addition, it could be noticed that, oven drying method resulted low drying yield compared to sun drying one. This might be due to low moisture content of oven-dried samples than sun dried ones. Hassan (2002), found that drying ratio of *P.ostreatus* and white *A.bisporus* ranged from (9.16: 1 to 10.88: 1) and (8.81: 1 to 9.94: 1), respectively.

Regarding moisture content of dried shiitake samples the data show that, it was affected mainly by drying method rather than pre-drying treatments. Moisture content of sun dried samples (8.31 – 8.96%) was higher than the oven dried ones (5.16 – 6.47%). No significant differences between sundried samples were detected. Oven dried control sample had the lowest moisture content (5.16%) and differed significantly than all the other samples. Also, sulfured and steam blanched samples dehydrated by oven had 6.27 and 6.47%, consecutively without significant differences between them.

Rehydration ratio reflects the quality attributes of dried products. The high reconstitution values, the more water absorption, reflecte a good quality of dried products. The rehydration ratio was affected mainly by pre-drying treatments (table, 6). Oven dehydrated samples had higher rehydration ratio than the sun dried ones. The control (untreated) sample showed the highest rehydration ratio specially when dried by oven (7. 97: 1) and differed significantly than the other samples and followed by the sulfured sample. On the other hand, the steam blanched sample dried either by sun or oven had the lowest rehydration ratio (3.25 – 3.44 : 1). Hassan (2002) stated that, oven dried *P.ostreatus* and *A.bisporus* had higher rehydration ratio compared to sun-dried ones.

Among all dried samples, the control sample dried by the oven had the best appearance and followed by those sulfured samples (sun or oven dried) then, sun dried control sample. On the other hand, steam blanched samples dried either by sun or oven had the worst appearance and were unacceptable.

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