Sunflower Seed Hulls as Substrate for the Cultivation of Shiitake Mushrooms

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SUMMARY. Nutritive agar formulations with additions of poplar (*Populus alba*) sawdust, wheat (*Triticum durum*) bran, or milled sunflower (*Helianthus annuus*) seed hulls (SSH) were evaluated for mycelium cultivation of shiitake (*Lentinula edodes*), in petri dishes. Sawdust, 2, 3 and 4 g·L^{-1} (0.27, 0.40 and 0.53 oz/gal) added to MYA (malt, yeast extract and agar) medium did not improve the mycelium growth rate, while media that included 1, 2, and 3 g·L^{-1} (0.13, 0.27, and 0.40 oz/gal) wheat bran or 2, 3, and 4 g·L^{-1} (0.27, 0.40, and 0.53 oz/gal) milled SSH exhibited a significant increase in the mycelium growth rate, at 25 °C (77 °F). The use of SSH obtained directly from the oil industry was evaluated as a substrate for the cultivation of shiitake mushroom via synthetic logs in plastic bags. A linear growth test was used to previously assay the mycelium growth rate in substrate compositions with different contents of SSH, wheat bran, and poplar sawdust, at 25 °C. The largest mycelial growth rates were 2.75, 2.88, and 2.93 mm·d^{-1} (0.108, 0.113, and 0.115 inch/day) for the substrates formulated with 8 SSH : 2 wheat bran, 9 SSH : 1 poplar sawdust, and 8 SSH : 1 wheat bran : 1 poplar sawdust by weight, respectively. The synthetic logs showed a daily production rate of 2 kg shiitake/100 kg dry substrate for a 55 days cycle production with a simple formula containing 37.5% SSH, 0.5% calcium carbonate ($CaCO_3$), 2% calcium sulfate ($CaSO_4$), and 60% water. Addition of wheat bran to the SSH-based synthetic log produced no significant differences on biological efficiency, mushroom production, or productivity.

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Shiitake mushrooms are consumed worldwide and are the most popular mushrooms in Asian countries and are valued both for high nutritional and medicinal properties. Several polysaccharides and other compounds from shiitake mushrooms (Chihara, 1978) were identified as having important antitumor (Chen and Ho, 1986), antiviral (Suzuki et al., 1979), and cholesterol reducing effects (Kim et al., 1992), and can greatly stimulate the immune system (Chihara, 1990, 1992).

Shiitake is a white rot fungus able to degrade the main components of wood, including lignin and cellulose. It produces enzymes capable of hydrolyzing cellulose and hemicellulose, and oxidizing lignin (Buswell and Oider, 1987; Morais et al., 2000; Zadrazil, 1985). Although a great proportion of shiitake production still comes from cultivation on natural logs, techniques with specially formulated substrates in plastic bags, known as synthetic logs, have been developed in countries—China, Japan, rest of Asia—known as traditional producers of shiitake (Chang and Miles, 1989). The synthetic log technique avoids the prolonged time, usually more than six months, needed by the traditional cultivation on hardwood logs (Sabota 1994).

Many agricultural and industrial residues can be used as alternative substrates to manufacture these artificial logs: rice (*Oryza sativa*) straw, sawdust, wheat grain, rye (*Secale cereale*), oat (*Avena sativa*), and sorghum (*Sorghum* spp.) grains, used tea (*Thea sinensis*) leaves, cotton (*Gossypium* spp.) wastes, cotton pulp and seed husks (Chang and Quimio, 1986), and hardwood sawdust (Pacumbaba and Pacumbaba, 1999a, 1999b). Sunflower seed hulls (SSH), however, have not been tested in a substrate formulation for shiitake mushroom cultivation. Sunflower seed hulls is an abundant residue of the edible oil production in many countries. The sunflower seed is transformed during the oil extraction procedure into oil and flour, and seed hulls are a byproduct. The SSH constitutes about 18% to 20% of the processed seeds (Helgeson et al., 1977), and cannot be used in animal feeding or other applications. The main organic macronutrients of sunflower seed hulls are lipids, carbohydrates, and proteins. The highest content of organic components is in the lignin and cellulose–hemicellulose portion, the lignin being about 20% to 25% of total weight (Dorrel and Vick, 1997). Reduced sugars are also an important part of the seed coating amounting to about 25%. Lipids and protein content are around 5% and 4%, respectively, and almost 3% of the lipids are waxes (Cancalon, 1971). This chemical composition of sunflower seed hulls makes it an attractive material for growing microorganisms. The high lignin content, however, limits the possibility of rapid biodegradation. The white rot fungi, basidiomycetes, are considered the primary agents in nature for lignin degradation (Buswell and Oider, 1987; Zadrazil and Reinger, 1988). The ability of shiitake to colonize substrates is limited by several factors, including physicochemical and nutritional factors (Kahn and Chaudhary, 1989; Song et al., 1987). The growth rates of shiitake mycelia in different formulations of nutritive agar was studied and a linear growth test was used to evaluate the mycelial colonization rate on different SSH substrate formulations containing different wheat bran and poplar sawdust ratios.

The goal of the present study was to evaluate the feasibility of using SSH as a substrate for the cultivation of shiitake mushrooms in synthetic logs in plastic bags.

Materials and methods

FUNGI, CULTURE MEDIA, AND MYCELIAL GROWTH TEST. The shiitake strain used was Somycel 4055 (Somycel S.A., Langeais, France). Shiitake mycelium was maintained in 10-cm (4-inch) diameter petri dishes containing MYA (malt–yeast–agar) medium consisting of 20 g·L^{-1} malt extract, 2 g·L^{-1} yeast extract, 20 g·L^{-1} agar, and 10 g·L^{-1} glucose, pH 5.5 (Stamets, 1993). Medium was sterilized at 121 °C (250 °F) for 20 min, plates were incubated in

darkness at 25 °C for 10 d. After this incubation, mycelium was ready for use. Different additives were evaluated in order to increase the mycelial growth rate on MYA medium. Poplar sawdust and completely milled SSH were added to MYA at concentrations of 0, 2, 3, and 4 $g \cdot L^{-1}$, and wheat bran at 0, 1, 2, and 3 $g \cdot L^{-1}$. The mixture of additives and agar medium was autoclaved, and then poured into the plates. Colonized agar plugs [4-mm (0.16-inch) diameter] from the culture stocks were placed on the center of the petri dishes. They were incubated at 25 °C in darkness for 7 d and the growth rates of the mycelium were evaluated by measuring the mean colony diameter, with five replications per treatment.

Linear growth test. A linear growth test (Duncan, 1997) was used to evaluate the rate of shiitake mycelial growth on SSH substrate supplemented with various additives, all containing 37.5% lignocellulosic material. Treatments were SSH, SSH : wheat bran (9:1 and 8:2 by weight), SSH : poplar sawdust (9:1 by weight), and SSH : poplar sawdust : wheat bran (8:1:1 by weight), and oat straw. All these substrate mixtures contained the following salt and water levels: 0.5% $CaCO_3$, 2% $CaSO_4$, and 60% water, by weight. The substrate was packed to an approximate density of 0.5 $g \cdot cm^{-3}$ (0.3 $oz/inch^3$) in glass tubes 20 cm long and 1.6 cm in diameter (8 × 0.63 inches). An agar disk with mushroom mycelium and the same diameter as the tube, was placed on one end of the tube and was pushed until it touched the substrate. Both ends of the tubes were plugged with cotton, and 10 tubes per treatment were incubated at 25 °C in darkness for 12 d.

Spawn production, substrate preparation, and spawn running. Spawn was prepared in 1-L (0.26-gal) bottles filled with a mixture of 59.1% wheat grain, 40% water, 0.1% $CaCO_3$, and 0.8% $CaSO_4$, by weight. Bottles of media were sterilized at 15 $lb/inch^2$ (6.82 $kg \cdot cm^{-2}$) for 90 min and were inoculated with *L. edodes* mycelium (two wedges per bottle). The spawn was incubated at 25 °C in darkness for 15 to 20 d with periodic shakings.

Three substrate formulations were assayed: SSH, 8 SSH : 2 wheat bran, and 9 SSH : 1 wheat bran, by weight . All formulations contained 60% water, 0.5% $CaCO_3$, 2% $CaSO_4$, by weight, and 2 $mg \cdot L^{-1}$ (ppm) Bavistin, FL (BASF Aktiengesellchaft, Ludwigshafen, Germany), a fungicide containing 50% wt/vol carbendazim (2-methoxycarbomyl benzimidazole). Final dry lignocellulosic material content was 37.5% for all formulations. Substrate formulations were decontaminated following a technique previously developed in our laboratory. This method makes use of a plaster-making machine with 180-L (50-gal) metallic rotary drum, with 40-L (11-gal) useful substrate capacity. A gas heater with 2 ring-shaped burners of 0.21 m (8.27 inches) and 0.08 m (3.15 inches) diameter was placed at 0.04 m (1.60 inch) distance from the lower base of the drum. An electric motor of 560 W rotated the drum and rotation was automatically interrupted every 15 min. The rotation speed was 32 rpm. This treatment was enough to produce an effective substrate decontamination after about 2.5 h. The substrate temperature was allowed to fall to 35 to 40 °C (95 to 104 °F), with the drum rotating for 2 h, then the spawn was added to the substrate and rotation continued for 15 to 20 min until homogenous mixing of spawned substrate (Curvetto, 1997; Curvetto et al., 1997).

The substrate formulations were inoculated with shiitake spawn (7% by weight) and 1.5 kg (3.3 lb) substrate were packed to an approximate density 0.5 $g \cdot cm^{-3}$ into 13 × 20-cm (5 × 8-inch) high-density polypropylene bags (n = 10). Bag ends were tightly closed and microperforations were made in all the bags with a special rod resulting in about 7000 holes per square meter. Bags were then placed in a growth chamber at 24 °C (75 °F) and 70% to 80% relative humidity (RH) for 25 to 30 d in darkness, to allow the substrate colonization.

Fruiting, cropping, and production. Shiitake mycelium grew vegetatively until full colonization of the synthetic log in plastic bags and reached the blistering stage (with irregular blister-like surface topography formations, which are the precursors to primordia) at 25 to 30 d from spawning. At this time, the plastic bags were removed and the logs were immersed in tap water at 4 to 6 °C (39 to 43 °F) in darkness for 48 h, to initiate the fruiting stage. Then the synthetic logs were kept in a controlled environment with 12 h photoperiod under 1500 to 2000 lux (140 to 186 fc), 80% to 90% RH, 22 °C (72 °F) and adequate ventilation. Fruiting was thus stimulated, and after 3 to 5 d mushrooms were ready for harvest. After this first flush, and with the purpose of obtaining a second flush, synthetic logs were allowed to dry for 7 to 10 d in a room at about 50% RH, and 25 °C and then immersed again in water at 4 to 6 °C for 48 h before put them back in the growing room.

We calculated the biological efficiency (BE) (% BE = kg fresh mushrooms/kg dry substrate × 100), mushroom production (MP) (% MP = kg fresh mushrooms/kg wet substrate × 100) for both flushes, and the accumulated productivity (kg fresh mushroom/100 kg dry substrate per day) of shiitake mushrooms at the end of the second crop.

Statistical analysis. The results from the mycelial growth test in petri dishes were evaluated by analysis of variance (ANOVA) test, and mean values separation was done by Tukey's test (Ott, 1984), $P < 0.001$. For linear growth test, 10 replications per treatment, the measurements were evaluated by ANOVA test. Separation of mycelial growth rate mean values was performed by the test of Tukey, $P < 0.05$. For fruiting parameters, a completely randomized design with 10 replications per treatment was used and results were analyzed as mentioned for the other tests ($P < 0.05$).

Results and discussion

Formulation of the solid culture media. Table 1 shows the colonization rates of different culture media by shiitake mycelium. The addition of poplar sawdust did not improve the mycelium growth rate, while the media prepared with either wheat bran or milled sunflower seed hulls, in all the concentrations evaluated, exhibited the highest mycelium growth rate, and both additives were appropriate for growing this shiitake strain. For our experiments we chose 4 $g \cdot L^{-1}$ milled sunflower seed hulls in the MYA medium.

Linear growth test. The effect of different substrate formulations on shiitake mycelium growth is shown in Table 2. The mycelial growth was 2.41 $mm \cdot d^{-1}$ (0.10 inch/d) on SSH alone (treatment 1). The highest growth rates were obtained on substrates formulated with SSH and wheat bran (8:2 by weight, treatment 3), SSH and

Table 1. Shiitake (*Lentinula edodes*) mycelial growth in malt, yeast extract and agar (MYA) medium supplemented with different contents of poplar sawdust (PS), wheat bran (WB) or milled sunflower seed hull (SSH): 1) 2 g·L^{-1} PS, 2) 3 g·L^{-1} PS, 3) 4 g·L^{-1} PS, 4) 1 g·L^{-1} WB, 5) 2 g·L^{-1} WB, 6) 3 g·L^{-1} WB, 7) 2 g·L^{-1} SSH, 8) 3 g·L^{-1} SSH, 9) 4 g·L^{-1} SSH. Growth was measured as the mean of two diameters of the colony taken at right angles, after 7 d incubation in darkness at 25 °C (77 °F).

Treatment	PS (g·L^{-1})[z]	WB (g·L^{-1})	SSH (g·L^{-1})	Mean colony diam (cm)[y]
Control (MYA)	0	0	0	5.42 a[x]
1	2	0	0	5.36 a
2	3	0	0	5.34 a
3	4	0	0	5.32 a
4	0	1	0	6.50 b
5	0	2	0	6.24 b
6	0	3	0	6.36 b
7	0	0	2	6.38 b
8	0	0	3	6.30 b
9	0	0	4	6.52 b

[z]1 g·L^{-1} = 0.13 oz/gal.
[y]1.00 cm = 0.394 inch.
[x]Means followed by the same letter are not significantly different ($P < 0.001$).

Table 2. Linear growth rate of shiitake (*Lentinula edodes*) mycelium on six substrate formulations with different contents of sunflower seed hull (SSH), wheat bran (WB), poplar sawdust (PS), and/or oat straw (OS). All formulations contained 0.5% calcium carbonate, 2% calcium sulfate, and 60% water. Treatments were: 1. 37.50% SSH, 2. 33.75% SSH + 3.75% WB, 3. 30.00% SSH + 7.50% WB, 4. 33.75% SSH + 3.75% PS, 5. 30.00% SSH + 3.75% WB + 3.75% PS, 6. 37.50% OS. Final content of lignocellulosic material was 37.5%.

	Substrate composition				Mycelial
Treatment	SSH (%)	WB (%)	PS (%)	OS (%)	growth rate (mm·d^{-1})[z]
1	37.50	0	0	0	2.41 a[y]
2	33.75	3.75	0	0	2.63 b
3	30.00	7.50	0	0	2.75 bc
4	33.75	0	3.75	0	2.88 c
5	30.00	3.75	3.75	0	2.93 c
6	0	0	0	37.50	2.59 b

[z]1.00 mm·d^{-1} = 0.039 inch/d.
[y]Means followed by the same letter are not significantly different ($P < 0.05$).

Table 3. Yield parameters of shiitake (*Lentinula edodes*) mushroom grown on three sunflower seed hulls (SSH) substrate formulations in synthetic logs.

Substrate formulation (by wt)	BE 1st flush[z] (%)	BE 2nd flush[y] (%)	Accumulated BE[x] (%)	MP[w] (%)	Productivity[v] (%)
SSH	46[u]	62[u]	108	43	2.0
8 SSH : 2 wheat bran	49	64	112	47	2.0
9 SSH : 1 wheat bran	45	57	102	41	1.9

[z]Biological efficiency (BE) for the first flush at day 35 from spawn inoculation.
[y]Biological efficiency for the second flush at day 55 from spawn inoculation.
[x]Accumulated biological efficiency.
[w]Mushroom production (MP) at the second crop.
[v]Productivity (mushroom production per day).
[u]Biological efficiency within each flush are not significantly different ($P < 0.05$).

poplar sawdust (9:1 by weight, treatment 4) and SSH, wheat bran and poplar sawdust (8:1:1 by weight, treatment 5).

FRUITING, CROPPING, AND PRODUCTION. We used the simplest substrate to evaluate the effectiveness of the additives on the fruiting yields; i.e., 37.5% sunflower seed hulls, 0.5% $CaCO_3$, 2% $CaSO_4$ and 60% water. Since wheat bran is cheap and easily available, we used two formulations of sunflower seed hulls and wheat bran (8:2 and 9:1 by weight). Carpophores appeared with typical morphology and normal development, resulting in harvested mushrooms mainly with 6 to 8 cm (2.4 to 3.1 inches) diameter.

BE for the first two flushes (at day 35 and day 55 from spawn inoculation), and the accumulated BE, MP and productivity, at the second crop for the substrate formulations are given in Table 3. The addition of wheat bran to the sunflower seed hull substrate did not produce significant differences in parameter yields or productivity ($P < 0.05$). In fact, the formulation containing only SSH as lignocellulose material had a relatively high yield of 108% in 55 d. This represents a daily production rate of 2 kg mushrooms/100 kg dry substrate comparable to and even greater than that reported with other substrates that have longer cultivation periods (Kalberer, 1989; Pacumbaba and Pacumbaba, 1999a, 1999b; Przybylowicz and Donoghue, 1990; Rinker, 1991). Recently, Morais et al. (2000) working with four strains of shiitake and using both the best productive substrate formulation and the best strain, obtained a BE of 60% after running for about a 100-d production cycle. This formulation resulted in a daily production rate of 0.6 kg shiitake/100 kg dry substrate. Hence, it appears that if some nutrient deficiency was present in our substrate and culture conditions, it was not strongly limiting the shiitake growth performance.

Conclusions

Our results showed that a plastic bag production system using sunflower seed hull as substrate allows a high yield of shiitake mushroom in a shorter cycle of production than that reported with other substrates. A simple substrate formula like the one presented in this study, composed of an abundant and cheap residue from the edible oil

industry would have a positive impact on production costs. The short production cycle and high yield obtained with this substrate and the Somycel 4055 strain of shiitake clearly shows the advantage of using the synthetic log system instead of the traditional method using hardwood logs.

Literature cited

Buswell, J.A. and O. Oider. 1987. Lignin biodegradation. Crit. Rev. Biotechnol. 6:1–60.

Cancalon, P. 1971. Chemical composition of sunflower seed hulls. J. Amer. Oil Chem. Soc. 48:629–632.

Chang, S.T. and T.H. Quimio. 1986. Guide to low cost mushroom cultivation in the Tropics. Univ. Phillipines Press, Los Banos.

Chang, S.T. and P.G. Miles. 1989. Edible muhsrooms and their cultivation. CRC Press, Boca Raton, Fla.

Chen, C.C. and L.T. Ho. 1986. Identification of sulphurous compounds of shiitake mushroom. J. Agr. Food Chem. 34:830–833.

Chihara, G. 1978. Antitumor and immunological properties of polysaccharides from fungal origin. Mushroom Sci. 9(2):797–814.

Chihara, G. 1990. Lentinan and its related polysaccharides as host defence potentiator: Their application to infectious diseases and cancer, p. 9–18. In: K.N. Masihi and W. Lange (eds.). Immunotherapeutic prospects of infectious diseases. Springer Verlag, New York.

Chihara, G. 1992. Immunopharmacology of lentinan, a polysacharide isolated from *Lentinus edodes*: Its application as a host defence potentiator. Intl. J. Oriental Med. 17:55–77.

Curvetto, N. 1997. Method for decontaminating sunflower seed hull-based substrate in the cultivation of *Pleurotus* edible mushroom. Patent No. P 97 01 01734, AR 006879 A1. Bul. Trade Marks and Patents, 29 Nov. 1999, p. 48. Natl. Inst. Trade Marks and Patents (INPI), Buenos Aires, Argentina.

Curvetto, N., S. Delmastro, R. Devalis, and D. Figlas. 1997. A low cost method for decontaminating sunflower seed hull-based substrate in the cultivation of *Pleurotus* edible mushroom. Mushroom Res. 6(1):25–28.

Dorrel, D.G. and B.A. Vick. 1997. Properties and process of oilseed sunflower. In: A.A. Schneiter (ed.). Sunflower technology and production. Agron. Monogr. 35:709–745.

Duncan, K.W. 1997. An ecophysiological approach to the evaluation, modification and production of mushroom growing media. Mushroom News 45(2):12–27.

Helgeson D.L., D.W. Cobian, R.C. Coon, W.C. Hardie, L.W. Schaffner, and D.F. Scott. 1977. Economic feasibility of establishing oil sunflower processing plants in North Dakota. N. Dak. State Univ. Agr. Expt. Sta.–Econ. Dev. Admin.–U.S. Dept. of Commerce, Bul. 503.

Kalberer, P.P. 1989. The cultivation of shiitake on supplemented sawdust. Proc. 12th Intl. Cong. Science Cultivation of Edible Fungi. Mushroom Sci. XII(II):317–325.

Kahn, S.M. and Y.A. Chaudhary. 1989. Some studies on oyster mushroom (*Pleurotus* spp.) on the waste material of corn industry in Pakistan. Proc. 12th Intl. Congr. Science Cultivation of Edible Fungi. Mushroom Sci. XII(II):23–29.

Kim, G. J., H.S. Kim, and S.Y. Chung. 1992. Effects of varied mushrooms on lipid composition in dietary hypercholesterolemic rats. J. Kor. Soc. Food Nutr. 21:131–135.

Morais, M.H., A.L. Ramos, N. Matos, and E.J. Santos Oliveira. 2000. Production of shiitake mushroom (*Lentinus edodes*) on lignocellulosic residues. Food Sci. Technol. Intl. 6(2):123–128.

Ott L. 1984. An introduction to statistical methods and data analysis. 2nd ed. Duxbury Press, Boston.

Pacumbaba, R.P. and R.O. Pacumbaba. 1999a. Shiitake mushroom growth on formulated culture media, production of spawn, and basidiocarp in the laboratory. HortTechnology 9(1):85–90.

Pacumbaba, R.P. and R.O. Pacumbaba. 1999b. Growing shiitake mushroom on hardwood sawdust in the greenhouse. HortTechnology 9(1):91–94.

Przybylowicz, P. and J. Donoghue. 1990. Shiitake cultivation on sawdust, p. 125–166. In: Shiitake growers handbook. The art and science of mushroom cultivation. Kendall/Hunt, Dubuque, Iowa.

Rinker, D.L. 1991. The influence of heat treatment, genotype and other cultural practices on the production of shiitake mushrooms on sawdust, p. 497–502. In: Maher (ed.). Science and cultivation of edible fungi. Balkema, Rotterdam, The Netherlands.

Sabota, L. 1994. The effects of shiitake mushroom strains and wood species on the yield of shiitake mushroom, p. 45–60. In: Proc. Natl. Shiitake Mushroom Symp. Ala. A&M Univ., Huntsville.

Song, C.H., K.Y. Cho, and N.G. Nair. 1987. A synthetic medium for the production of submerged cultures of *Lentinus edodes*. Mycologia 79:866–876.

Stamets, P. 1993. Growing gourmet and medicinal mushrooms. Ten Speed Press, Berkeley, Calif.

Suzuki, F., C. Suzuki, E. Shimomura, H. Maeda, T. Fuji., and N. Ishida. 1979. Antiviral and interferon-inducing activities of a new peptidomannan, KS-1, extracted from culture mycelia of *Lentinus edodes*. J. Antibiotiotics 32:1336–1345.

Zadrazil, F. 1985. Screening of fungi for lignin degradation and conversion of straw into feed. Angewandte Botanik 59:433–452.

Zadrazil, F. and R. Reinger. 1988. Treatments of lignocellulosics with white rot fungi. Elsevier Applied Science, London.