Part I Shiitake

Chapter 4

Shiitake Bag Cultivation

WHEAT STRAW

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Traditional log cultivation of shiitake has been partly replaced by bag cultivation with sterilized sawdust due to this method's higher biological efficiency, and shorter production cycle. However, the sterilization process has a high initial installation cost, consumes more of energy, and is more susceptible to contamination (Kalberer, 1998). To solve these problems, pasteurized (65℃) wheat straw substrate has been adopted for shiitake cultivation in Europe (Delpech and Olivier, 1991) and in some countries of America (Mata *et al*., 2002).

Wheat Straw as an Alternative Substrate

Mushrooms such as shiitake are able to oxidize the lignin-polysaccharide complex of cereal straw without prior chemical or biological treatment.

Wheat straw substrate for shiitake cultivation has two considerable advantages over other agricultural by-products such as coffee pulp or sugarcane bagasse: a) wheat straw is stocked easily without problems of decomposition or fermentation; and b) contamination by moulds and bacteria is less frequent than other substrates due to the unique chemical composition of the wheat straw.

Straw being a crop residue, its chemical composition and degradation rate appear to be controlled by both genetic factors and cultural practices (Savoie *et al*., 1994). Though some differences exist according to species and how long they are stored, the nutrition of wheat straw, rice straw, and coffee pulp used for shiitake cultivation are generally analyzed as shown in Table 1.

Table 1. Nutrition of wheat straw, rice straw and coffee pulp (mg / 100g of dry substrate)

Composition	Wheat straw*	Rice straw**	Coffee pulp**
Protein	7.9	9.0	10.2
Carbon	52.5	N/A	N/A
C/N	41.0	N/A	N/A
Lignin	8.6	5.7	21.0
Cellulose	35.2	33.9	36.4
Hemicellulose	N/A	26.8	5.1

* Gaitán-Hernández and Mata 2004

** Vega *et al*., 2005

Figure 1. Cereal straw **A**: Wheat straw **B**: Rice straw

Shiitake Bag Cultivation with Wheat Straw

Substrate preparation

This process aims to prepare a substrate and bring it to a with favorable condition for the growth of shiitake mycelia. This is called the selectivity of substrates, and both the composition of the substrate materials and the heat treatments have a great effect on the substrate selectivity.

Wheat straw is shredded into pieces from 4 to 6cm in length for easy handling during pasteurization and bagging (Figs. 2). It is soaked in water for 6-12 hours at room temperature and mixed with 2 to 10% (dry weight) of gypsum. Several supplements containing materials that are lacking in wheat straw itself are added to provide sufficient nutrients for the shiitake mycelia. As a nitrogen and oligoelements source, soja flour added at 4kg per ton can increase the yield by 30%, but soja flour is more likely to lower substrate selectivity. Generally, substrate selectivity decreases by addition of components rich in nitrogen or oligoelements. Therefore, it is recommended to either add only a small quantity of them, or to sterilize the substrate if a large amount of the nitrogen rich components are added. Other supplements improve the competitiveness of shiitake against *Trichoderma* sp. Peat, sawdust or other water-soluble lignin derivatives not only have absorbing properties but also contain phenolic compounds that most competitors cannot degrade easily. Shiitake can degrade these soluble lignin with oxidative enzymes, so the shiitake mycelia have almost exclusive access to these supplements (Mata *et al*., 2001; Savoie *et al*., 2000). When 10% peat moss is added, the contamination rate by *Trichoderma* is decreased by 50% and the yield is increased by 30% (Mata *et al*., 1998).

Figure 2. Different machines for cutting wheat straw

Pasteurization

Wheat straw is pasteurized in order to kill possible competitor microorganisms as well as insects in the straw. Another goal of pasteurization is to propagate the thermophilic microorganisms that will improve the substrate selectivity by immobilizing the readily available nutrients to competitors and by producing toxic or inhibitory molecules to limit the rapid growth of competitors.

The substrate mixture is placed in containers for pasteurization with steam at 65 ℃ for 12-24 hours and then cooled to room temperature. Water content of the substrates after pasteurization is about 70% (Mata *et al*., 1998).

Spawning and incubation

The selection of genotypes appropriate for the chemical and structural properties of chosen substrates and thermal treatments are critically important to ensure a good production of fruiting bodies in the shortest time possible. Though the number of shiitake strains that are well adapted to pasteurized wheat straw is relatively low (Mata and Savoie, 1998), it is recommended that wheat straw using growers choose the shiitake strain with competitiveness in wheat straw.

After cooling, the pasteurized substrates are mixed with spawn in a clean environment. Aseptic conditions are not necessary because the substrate is not sterilized. In order to improve the competitiveness of shiitake during the first days after spawning, the mycelium in the spawn has to be vigorous, adapted to the components of the substrate and able to colonize all the particles of the substrate[1]. For these reasons, Delpech and Olivier (1991) recommended limiting the use of supplementation in wheat straw substrates to prevent the growth of bacteria and moulds. Spawn must be mixed with sterilized or pasteurized straw at 5-7% (w/w) and the mixture should be placed in plastic bags that are lightly perforated or equipped with a microporous filter (Figs. 3A and B). Incubation is one of the most important phases in the shiitake cultivation on alternative substrates because of the competition between shiitake and competitor moulds that occurs during the first weeks. The initial rate of substrate colonization by the antagonistic fungi is an important factor of the competitive interaction. If shiitake rejects the attack by the mould at this stage, no other problem is encountered afterward. Some strains of shiitake are able to reject the mould attacks under temperature and nutritive conditions favorable to them (Badham, 1991) and if their mycelium has colonized enough space before contacting competitor fungi (Savoie *et al*., 1998). Incubation must be carried out at 25 ±2 ℃ with a 12 hours light and 12 hours dark cycle, which is recommended at least for shiitake cultivation on wheat straw, for

Figure 3. Spawning shiitake on wheat straw **A** and **B**: Mixing spawn and substrate by hand **C**: Hand perforated plastic bags under incubation

1–2 months depending on the strain (Fig. 3C). At the end of incubation period, the entire surface of substrate turns brown, indicating that mycelium is ready for fructification (Przybylowicz and Donoghue, 1988; Donoghue and Dennison, 1996).

Obtained production on wheat straw

When incubation is completed, the plastic bags are removed and the substrate blocks are sprinkled with cold water. The room temperature is lowered to 17 ±1 ℃. A relative humidity of 90% and a cycle of 12 hours light/12 hours dark are necessary to encourage mushroom development. After the farm has harvested the first flush, the blocks can be rehydrated to induce a second flush by soaking them in water for 12 hours (Gaitán-Hernández and Mata, 2004) (Figs. 4). Under commer-

[1] For detailed information, *see* IMPROVEMENT OF SPAWN FOR CULTIVATION IN ALTERNATIVE SUBSTRATES in Chapter 2.

cial production conditions, when large blocks of supplemented and pasteurized straw are used (16kg), harvest can be performed for 12-16 weeks and biological efficiency reaches 50-100%. Table 2 provides some research results on biological efficiency obtained from wheat straw as well as other alternative substrates for shiitake. Though the same substrate is used, biological efficiency varies depending on the conditions of cultivation. Moreover, it should be kept in mind that conditions such as strains, temperature and humidity provided during cultivation are different in each experiment referred in Table 2. Wheat straw and sugarcane bagasse inoculated with supplemented spawn produce a high biological efficiency (Salmones *et al*., 1999; Savoie *et al*., 2000).

Table 2. Comparison of shiitake biological efficiencies obtained on different substrates

Substrate	Heat treatment	Spawn	Biological efficiency (%)	Reference
Wheat straw	Pasteurization with steam	Conventional	59.2	Mata and Savoie, 1998
		Conventional	15.9	Delpech and Olivier, 1991
		Supplemented	59	Mata *et al*., 1998
		Supplemented	116	Savoie *et al*., 2000
	Pasteurization in hot water	Supplemented	55.6	Gaitán-Hernández and Mata, 2004
Coffee residues	Sterilization	Conventional	88.6	Leifa *et al*., 1999
		Conventional	64.3	Mata and Gaitán-Hernández, 1994
Sugarcane bagasse	Sterilization	Conventional	133.4	Salmones *et al*., 1999
Sugarcane leaves	Sterilization	Conventional	97.8	Salmones *et al*., 1999
Bracts of pineapple	Sterilization	Conventional	37.5	Salmones *et al*., 1999

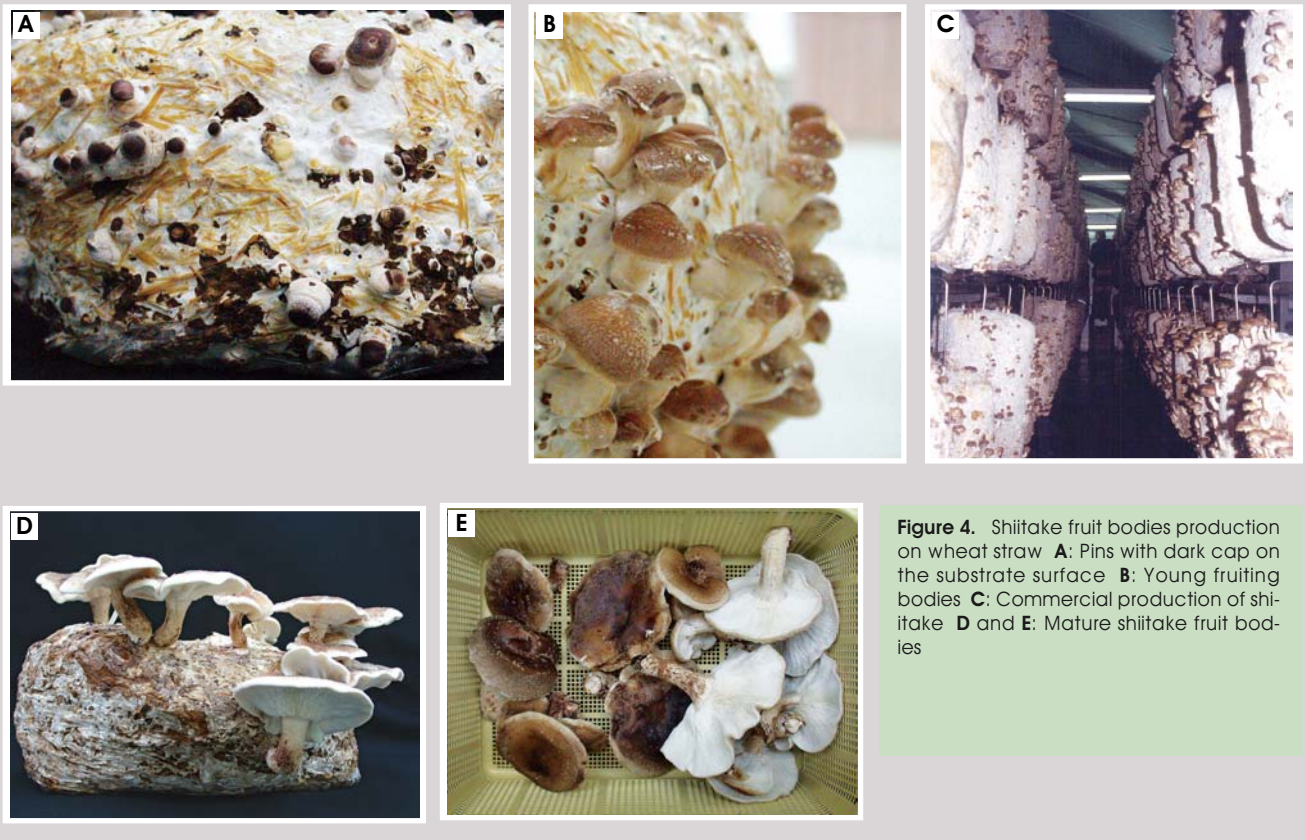

Figure 4. Shiitake fruit bodies production on wheat straw **A**: Pins with dark cap on the substrate surface **B**: Young fruiting bodies **C**: Commercial production of shiitake **D** and **E**: Mature shiitake fruit bodies

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