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# Reutilization of Enokitake Cultural Waste as *Lentinus edodes* Cultivation Substrate

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## ABSTRACTS

**The availability of enokitake cultural waste for *Lentinus edodes* cultivation was investigated, although hardwood sawdust has traditionally been used as a substrate for this fungus. Firstly, physiochemical characteristics of cultural waste were analysed. Secondly, mycelial growth characteristics and fruiting yields of *L. edodes* on waste treated in some methods were determined.**

**Physiochemical characteristics of enokitake cultural waste showed that the millwaste complex was a little degraded by enokitake fungus and suggested the probability that most component lost by enokitake could be rice bran. Mycelia of *L. edodes* grew and fruited well on waste supplemented by fresh rice bran and *Quercus* sawdust although didn't on waste only. Mycelial growths of these fungi on waste were accelerated when supplemented by rice bran to the percent of 40(w/w) but decreased or suppressed at above ratios(30, 40%, w/w). Supplementations of oak sawdust at above 40%(w/w) of the waste and rice bran at 20%(w/w) of the sawdust allowed such a good mycelial growth as to be selected as a pertinent mixing ratio for fruiting medium. A fruiting yield on enokitake cultural waste supplemented by oak sawdust (at 40% of the waste, w/w) and rice bran (at 20% of the sawdust, w/w) was not inferior to that on oak sawdust supplemented by rice bran only (at 20% of the sawdust, w/w). These results indicated strongly the potentiality of enokitake cultural waste as raw materials for shiitake cultivating substrates.**

***Key Words*** **:** *Cultural waste, Degradation characteristics, Fruiting yield, Mill waste, Oak sawdust, Rice bran.*

## INTRODUCTION

There is a growing interest about mushrooms in Europe and the Americas as well as in Asia. As the higher consumptions are, the more supplies will be there. To satisfy the consumptions, much more lignocellulosic materials will be necessary. However, the rapid expansion of mushroom cultivation and the protection of the ecological equilibrium of forest resources have thrown a serious problem to the mushroom industries. These phenomena made several researchers to introduce alternatives for cultivation (Park *et al*, 1992, 1994; Ohga *et al*, 1993; Takabatak,

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1994; Terashima, 1994; Togashi, 1995; Mata & Savoie, 1998; Chai *et al.*, 1999).

Shiitake grows on the wood of dead deciduous trees. Suitable species for bed log are Fagaceae, namely oaks (*Quercus* spp.), shii (*Castanopsis* spp.), chestnut (*Castanea* spp.), hornbeams (*Carpinus* spp.) and more rarely other genera. As commercialized and worldwided, several tree species of sawdust, as alternatives, were employed for sawdust cultivation. Terashima (1994) reported from the results of decomposing two kinds of sawdust, *P. edulis* and *F. crenata* that there were not any significant difference in mycelial growth, frutibody yields, and changes in sawdust components. Poplar supplemented with coffee waste and tannic acid was also adequate for shiitake (Park *et al*, 1992). Chai *et al.* (1999) indicated strongly the potentiality of black locust (*R. pseudoacacia*) as raw materials for edible and medicinal mushrooms including shiitake. Mata and Savoie (1998) addressed the question by measuring enzyme activities produced by six strains of *L. edodes* that were cultivated on wheat straw and that were able to produce sporophores. Whey permeate, a dairy by-product, was studied as a growth medium and mycelial growth and phenoloxidase production appeared to be highly stimulated when the fungi were grown on the permeate instead of synthetic medium (Dilena & Sermanni, 1994). Softwood sawdust had been tried (Park *et al.*, 1994; Kalberer, 1989; Kawachi *et al*, 1991). Ohga (1977) found that fatty acids and phenolics of red pine (*Pinus densiflora*) and yamamomo (*Myrica rubra*) respectively exhibited strongly harmful effects on the growth of shiitake-fungus. Kawachi *et al.* (1991) found and assumed that ferruginol and thymol in *Cryptomeria japonica* significantly inhibited shiitake mycelial growth. However, Ohga (1990) suggested a potential use of the waste liquor-component from pulping made from softwood to produce a large amount of fruit bodies with much shorter periods of cultivation time. In 1994, Takabatake *et al.* found that KWE (water extracts from Siberian larch wood (*Larix* sp.) increased the mycelial growth slightly and mycelial dry weight twice that of control.

Softwood sawdust can be available cheaply as the softwood are mostly used. Softwood sawdust (*Cryptomeria japonica, C. obtusa, Pseudotsuga menziesii* and *Pinus* spp.) has been largely used as a substrate for *Flammulina velutipes* (Chang and Miles, 1978; Ohga, 1993). There is so much cultural waste of enokitake in Korea as enokitake bottle culture has been fully commercialized (Kim, 1997). *F. velutipes* colonizes the substrate so fast and fruits. These growth characteristics imply that there are much lignocellulosic materials not degraded by this fungi, especially supplemented with rice bran. If so, the cultural waste could be useful for cultivation of several edible and medicinal mushrooms. Hence, the present work was undertaken to examine the feasibility of the enokitake cultural waste as cultivating substrate for shiitake production.

## MATERIALS and METHODS

### 1. Organisms

Sawdust spawn of commercial strains (Sanjo No. 5,6) of *Lentinus edodes* were employed as inoculants.

### 2. Preparation of Cultivating Substrates

Cultural waste after enokitake harvesting was employed as a major cultivating substrate, supplemented with oak sawdust and rice bran in several ratios. Cultivating substrates were mixed and watered (M.C. 65%). These mixtures were filled into the test tube and polypropylene bag (P.P. Bag) allowing air supply through a filter. After capped, the media were sterilised at 121℃ for 30 min. in case of tubes and for 90 min. in bags. After inoculation by *L. edodes*, they were placed in room of constant air temperature (25℃)

and humidity (75%) and incubated in darkness. Sawdust blocks in P.P. bag, completely colonized by shiitake mycelia, were allowed to be exposed to the white light (ca. 200lux) for the surface mycelia to be coated brown.

### 3. Degradation Characteristics of Mill waste by *Flammulina velutipes*

#### A. pHs, Weight Losses and Moisture Contents

The variations of the pH, weight loss and moisture content of substrate were examined for monitoring the physiochemical characteristics of enokitake cultural waste before and after harvesting of *Flammulina velutipes.*

#### B. Chemical Characteristics

#### Low-molecular-weight Substances

The organic part of sawdust substrate was analyzed as ash by incineration of the organic material at 575± 25℃ for 3 hours. The inorganic matter were obtained as extractives with hot, cold water, alcohol-benzen and 1% NaOH for 48, 3, 6 and 1 hours, respectively.

#### Macromolecular Substances

Lignin and holocellulose were analyzed by Klason lignin and Wise method, respectively.

### 4. Growth Characteristics of *L. edodes* on Enokitake Cultural Waste

As a additive, rice bran was supplemented in ratios of 0～40% and the growth rates (cm/24h) were calculated to select the pertinent content of rice bran. Several substrates (enokitake cultural waste, waste + oak sawdust in ratios of 2:8, 4:6, 6:4 and 8:2, oak sawdust were employed as substrates for shiitake fungi and the growth rates were caculated.

### 5. Fruitbody Yields of *L. edodes* on Enokitake Cultural Waste

Sawdust blocks, completely brown coated were transferred on the growing shelf and over-sprayed with foggy mist for fruiting. After misting, the blocks were exposed to lower temp.(15±2℃), higher humid.(over 90%) and brighter light(ca. 200lux) than incubated. The harvesting room was kept at 15±2℃(temp.) and 75-80%(humid.) just after pinheading and the tiny fruit bodies were cultivated and harvested. Each fresh fruiting bodies were counted and biological efficiencies were caculated by the following equation : Y = 100A/B, where Y is the biological efficiency (%), A is the yield of fresh shiitake, and B is the quantity of dry substrate.

## RESULTS and CONSIDERATIONS

### 1. Physiochemical Characteristics of Mill-Waste Degraded by *F. velutipes*

#### The pHs, Weight Losses and Moisture Contents

The variations of the pH, weight loss and moisture content of substrate were examined for monitoring the degradation characteristics of enokitake cultural waste before and after harvesting of *F. velutipes* (Table 1).

The pHs of the substrates before inoculation (as control) were 6.4, however, before and after harvesting

**Table 1.** Changes of Water contents, Weight losses and pH in Cultural Substrate Cultivated by *F. velutipes*

| After Steril. | Pre-harvest | Post-harvest | |
|---|---|---|---|
| Water Content (%) | 65 | 66 | 58.6 |
| Weight Loss (%) | 0 | 4.5 | 18 |
| pH | 6.4 | 6.5 | 5.8 |

the fruitbodies, the values were 6.5 and 5.8, respectively. Dry matter decreased continuously until the initiation of fruitbody while moisture content did not show much change. After harvesting, the dry matter loss was about 18% of original dry-weight (control). Moisture content of the substrates was about 58.6%.

Chai *et al.*(1999) indicated that the pHs of the substrates colonized by several fungi decreased continuously until the pre-harvest and thereafter it remained constant, and addressed that most of the medicinal and edible fungi kept the pHs of the media dropped somewhat much during fungal degradation process. It has been widely known that organic acids and water in the media could be accumulated during the bioconversion process by mycelial mass and these changes varied with individual species, strain and even isolate and media (Kalberer, 1995; Jablonsky, 1981). The results of our study, however, showed somewhat difference which could be considered as a result of different sawdust and fungal species used. A little decrease in the pHs of the media would might be a indicator of a little degradation of media components, in comformity with a little loss in dry weight of substrates.

**Chemical Characteristics**

As commonly known, as degradation is advanced, the content of NaOH extractives increases and that of lignocellulosics decrease. Therefore, these could be the indicator of the degradation degree. As degraded, the extractives content increase generally in case of the bedlog for *Lentinus edodes* (Ohga *et al.*, 1993; Mun *et. al.*, 1999). The content of NaOH soluble matter of *L. edodes* bedlog increased 58 percentage to the control (Mun *et. al.*, 1999). However, the contents of cold and hot water extractives increased a little or not in this study with *F. velutipes* sawdust substrate (Table 2).

In case of lignocellulosics, the contents were increased a little or somewhat much, lignin or holocellulose respectively, before harvesting. However, the contents were again decreased after harvesting (Table 2).

Considering that the enokitake cultural substrate is consisted with sawdust and rice bran in the ratio of about 8 : 2 (w/w), the above phenomenon could be resulted. May be, it is supposed that rice bran was exhausted much more than sawdust was during the incubation period. And this relative increase in content of lignocellulosics remained after degradation allowed the content of NaOH extractives to decrease.

It could be suggested, considering the above results, that the biomass remained and physicochemical characteristics of enokitake cultural waste were sufficient to be a candidate for the cultural substrates of the white rot fungi, having a aggressive rotting ability, especially like *Lentinus, Pleurotus, Ganoderma* spp. etc..

**2. Growth Characteristics of *Lentinus edodes***

**Table 2.** Chemical Characteristics of Cultural Substrate Degraded by *F. velutipes*

| | After Stril. | Pre-harvest | Post-harvest |
|---|---|---|---|
| Ash(%) | 5.49 | 5.88 | 5.96 |
| Hot-water Extracts (%) | 14.54 | 18.39 | 16.28 |
| Cold-water Extracts (%) | 15.03 | 17.25 | 17.95 |
| 1% NaOH Extracts (%) | 30.02 | 28.24 | 24.23 |
| Organic Solvents Extracts | 6.32 | 7.04 | 5.84 |
| Lignin (%) | 28.79 | 29.11 | 28.40 |
| Holocellulose (%) | 62.30 | 70.64 | 67.17 |

**Table 3.** Linear Growth Rate of Shiitake fungus on Enokitake Cultural Waste Supplemented by Rice Bran

| Rice Bran (%) \ Employed Fungi. | Sanjo No.5 | Sanjo No.6 |
|---|---|---|
| 0 | $0.26 \pm 0.06^{c}$ | $0.30 \pm 0.05^{c}$ |
| 10 | $0.37 \pm 0.07^{b}$ | $0.39 \pm 0.09^{b}$ |
| 20 | $0.42 \pm 0.06^{a}$ | $0.46 \pm 0.07^{a}$ |
| 30 | $0.41 \pm 0.07^{a}$ | $0.43 \pm 0.07^{b}$ |
| 40 | $0.40 \pm 0.04^{a}$ | $0.42 \pm 0.04^{a}$ |

**Notes.** Growth rate is expressed by the average and standard deviation of 10 replicates and values followed by the same letter in the same column means no significant difference (p=0.05)

**Table 4.** Linear Growth Rate of Shiitake fungus on enokitake cultural waste mixed with oak sawdust.

| Sawdust \ Employed Fungi. | Sanjo No.5 | Sanjo No.6 |
|---|---|---|
| Enokitake Cultural Waste | $0.42 \pm 0.06^{b}$ | $0.41 \pm 0.04^{b}$ |
| Cultural Waste + Oak (2:8) | $0.44 \pm 0.07^{b}$ | $0.43 \pm 0.07^{b}$ |
| Cultural Waste + Oak (4:6) | $0.51 \pm 0.07^{a}$ | $0.53 \pm 0.06^{a}$ |
| Cultural Waste + Oak (6:4) | $0.52 \pm 0.07^{a}$ | $0.55 \pm 0.06^{a}$ |
| Cultural Waste + Oak (8:2) | $0.51 \pm 0.06^{a}$ | $0.54 \pm 0.04^{a}$ |
| Oak Sawdust (*Q.serrata*) | $0.52 \pm 0.05^{a}$ | $0.55 \pm 0.05^{a}$ |

**Notes.** Growth rate is expressed by the average and standard deviation of 10 replicates and values followed by the same letter in the same column means no significant difference (p=0.05)

**Linear Growth Rate of Shiitake fungus on Enokitake Cultural Waste Supplemented by Rice Bran**

With addition of 20% rice bran, mycelial growth rate (cm/24h) of both strains were mostly promoted. At above this ratio, the promoting effects were decreased without significant difference with 20% treatments. This result proposed that enokitake cultural waste can be used as a substrate for the shiitake fungi, when supplemented with rice bran (about 20%) (Table 3).

**Linear Growth Rate of Shiitake fungus on Several Substrates Supplemented by Oak Sawdust**

Mycelial growths on substrates at several mix ratios of enokitake cultural waste and oak sawdust (*Q. serrata*) showed that oak sawdust supplementations at above 40% (w/w) allowed good mycelial growth in both strains without any significant difference among treatments. This results indicated that enokitake cultural waste could be the candidate for shiitake cultivation substrate, with addition of 40% oak sawdust (Table 4).

**3. Fruitbody Formation of Shiitake fungus on enokitake cultural waste mixed with oak sawdust**

Fig. 1. illustrates the biological efficiency, which shows the effects of oak sawdust supplementations. At above 40% supplementation by oak sawdust, fruiting yields were obviously increased and no differences among the treatments of 40～100% were detected. Present work suggested strongly that the enokitake

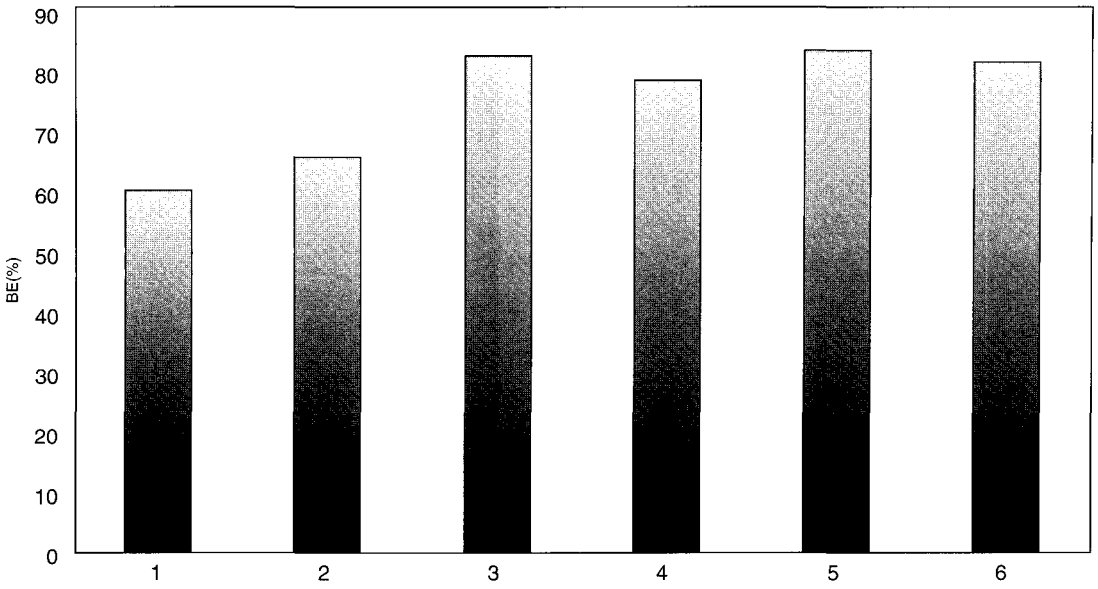


**Fig. 1.** Biological efficiencies on the enokitake cultural waste mixed with oak sawdust.

**Notes.** 1: Waste, 2: Waste + Oak Sawdust 20%, 3: Waste + Oak Sawdust 40%, 4: Waste + Oak Sawdust 60%, 5: Waste + Oak Sawdust 80%, 6: Oak Sawdust

cultural waste can be converted efficiently into value-added products.

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