

GOVP1200101761

635.8

L2934

최 종  
연구보고서

병버섯 폐배지를 이용한  
팽이 및 표고버섯 생산기술 개발

Reutilization of enokitake cultural waste as  
cultivating substrates for production of enokitake,  
*Flammulina velutipes* and shiitake, *Lentinus edodes*.

전 남 대 학 교

농 립 부

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2000. 11. .

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18%

pH 5.8

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20%

10-40%

20%

가

가

0.17-0.23(g/cc)

Ca(OH)<sub>2</sub> 0.1%

20 30%

20 , 10 40%

가 가

40%

가 가

가

가 . , 110  
 . 90 1  
 2 95  
 1 2,3 .

가 가 가  
 100 110 가

가 가 가  
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3.

가.  
 가 10%  
 20 40% .  
 10%  
 20%  
 M50 0.21 (g/cc) TK 0.17 0.23  
 (g/cc)  
 Ca(OH)2 0.1%

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10% 가  
20% .

· 2 가  
가 2  
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## SUMMARY

### . Title of Research

Reutilization of enokitake cultural waste as cultivating substrates for production of shiitake, *Lentinus edodes* and enokitake, *Flammulina velutipes*

### . The Objective and Importance of Research

Mushroom production is one of the few large-scale commercial applications of microbial technology for bioconversion of agricultural and forestry waste materials to valuable foods.

Softwood sawdust (*Cryptomeria japonica*, *Pseudotsuga menziesii* and *Pinus* spp. are widely used for commercial cultivation) has been largely used as a substrate for *F. velutipes*. There is so much cultural waste of enokitake in Korea as enokitake bottle culture has been fully commercialized. The cultural waste produced by the mushroom farms are mostly discarded but are partly recycled for making compost.

*F. velutipes* colonizes the substrate so fast and fruits. These growth characteristics imply 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. If the cultural waste can be recycled and reutilized for cultivating many other mushrooms, the effective use of wood resources will be made and lower cost mushrooms will be available.

The purpose of this research is to recycle and reutilize the enokitake cultural waste as cultivating substrates for shiitake and enokitake production.

## . The Contents and Category of Research

1. Degradation characteristics of softwood sawdusts cultivated with enokitake.

A. Physiochemical characteristics of enokitake cultural waste

B. Microscopical characteristics of enokitake cultural waste

2. Reutilization of enokitake cultural waste as shiitake cultivation substrate.

A. Mycelial growth characteristics of *L. edodes* on enokitake cultural waste

B. Fruiting characteristics of *L. edodes* on enokitake cultural waste.

3. Recycling of enokitake cultural waste and the potentiality of 2nd flush for enokitake production.

A. Mycelial growth characteristics of *F. velutipes* on enokitake cultural waste

B. Fruiting characteristics of *F. velutipes* on enokitake cultural waste

C. Potentiality of 2nd flush of enokitake

## . Results of Research

1. Degradation characteristics of softwood sawdusts cultivated with enokitake.

A. Physiochemical characteristics of enokitake cultural waste showed that the cultural waste were a little degraded and suggested the probability that most component lost and consumed by *F. velutipes* after harvesting could be rice bran.

B. Most of wood speices (mill-waste) were in *Pinus* spp., used for enokitake production.

C. Distribution of enokitake hyphae was restricted to ray parenchymas and tracheids exposed to fungi and degree of cell wall

degradation was slight.

D. The thinning of secondary cell wall in some tracheids was shown by light microscopy.

E. The loss of birefringence was observed only in a few latewood tracheids under polarized microscopy.

F. All the middle lamella remained intact.

The results in present sections showed clearly that the cultural waste held enough cell wall materials for other mushroom cultivation.

2. Reutilization of enokitake cultural waste as shiitake cultivation substrate.

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 on waste were accelerated when supplemented by rice bran to the percent of 40 (w/w) but decreased or suppressed at above ratios (30 and 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.

Oak sawdust was supplemented and the effect on the quality of fruit-bodies was determined. The diameter and thickness was examined as indexes for fruit-body quality because commercial value of shiitake depends largely on the shape of fruitbody (thickness and diameter of pileus). The diameter and thickness of pileus tend to

decrease and the quality was lowered, may be owing to the increase of fruiting numbers.

The effects of rice bran supplementation and incubation time on the yield and quality of fruit-bodies on enokitake cultural waste based culture was investigated on a commercial scale. The total yield of fruiting bodies increased as the incubation time increased from 70 to 110 days although decreased above. However, there were outbreaks of the first flush when incubated from 90 to 120 days, which showed a significant difference ( $P=0.05$ ) for 70-day-old culture. Numerous fruiting bodies were produced with longer incubation time, especially in the first flush, but prolonged incubation tended to cause the fruiting bodies miniaturized and smaller.

3. Recycling of enokitake cultural waste and the potentiality of 2nd flush for enokitake production.

The recycling method of enokitake cultural waste and the potentiality of second flush for enokitake were determined, because this fungus is not as prolific as the more commonly cultivated white rot fungi in the conversion of sawdust components to mycelial mass.

The mycelial growth of *F. velutipes* on several substrates, variously treated with rice bran was most promoted at ratios of 10-20% (w/w) on all substrates, but suppressed at above ratios, although some difference was there. The mycelial densities generally increased correlated to the supplementation contents of rice bran. It could be concluded that *F. velutipes* preferred mild acidic to acidic

conditions for mycelial growth, considering that the mycelial growth rate was highest on waste of pH 6.01, treated with 0.1% Ca(OH)<sub>2</sub> and on Populus mixed waste of pH 6.02, non treated. The ranges of substrate bulk densities, which was pertinent for mycelial linear growth were from B. D. 0.17 to 0.23 (g/cc) on waste and Populus mixed waste all. The pertinent contents of rice bran supplementation in bottle cultivation was from 20 to 30% on waste and 20% on Populus mixed waste, considering the required duration for pinheading and fruiting yields. Standard bulk density for filling and utilizing the waste and Populus mixed waste for commercial *F. velutipes* cultivation were B. D. 0.19 0.23 and 0.23 0.25 (g/cc), which could be conversed to 510 540g/900ml and 520 570g/900ml, respectively. The second flush of *F. velutipes* was tried and the re-inoculation by sawdust and liquid spawn showed somewhat good results, indicating the potentiality of second crop and suggesting further research for it.



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§ 1.

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§ 3.            2            가

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*(Flammulina velutipes)*

(Agaricales)

(Tricholomataceae)

A. D.

800

가

(Chang, 1993).

1928

Mbrimoto (森本)

(Tonomura, 1978)

(Shiio ,

1974).

1992

가

가

(

, 1998).

(Ohga , 1993;

, 1998).

. Nakajima

(1980)

Kawachi

(1991)

(ferruginol, thymol, -isopropylphenol )

가

Ohga (1993)

가 가

(1999)

(2000)

가

(Mata & Savoie, 1998). Park (1992)

Coffee waste

가

Terashima (1994) *Pasania edulis*

*Fagus crenata*

. Chai

(1999)

(*Robinia pseudoacacia*)

가

(*Quercus serrata*)

가

(Park , 1994)

(fatty acid)

(Ohga , 1977; Kawachi , 1991). , Ohga (1990)

Takabatak (1994)

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(Douglas-fir)

(

, 1999).

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(Ohga , 1985).

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(*Flammulina velutipes*) (Agari-  
 cal es) (Tricholomataceae) ,  
 , ,  
 .  
 A. D.  
 800 가 (Chang, 1993).  
 1928 Morimoto (森本)  
 (Tonomura, 1978)  
 (Shio  
 , 1974). 1992  
 가 가 (  
 , 1998).  
 (Ohga , 1993;  
 , 1998).

. Nakajima (1980) Kawachi (1991)  
(ferruginol, thymol, -isopropylphenol )

가

Ohga (1993)

가 가

(1999)

(2000)

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, 90

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(Douglas-fir)

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, 1999).

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1.

가. Water Content, Weight Losses, pH

( : M50) ( ) , pH

1) Ash

2g 가 (Crucible) 575 ± 25 (TAPPI) 3  
(W)

2) Hot-water Extracts

2g 100ml 가 250ml  
3 Glass Filter  
(1G3) (105 ± 3 )

3) Cold-water Extracts

2g 300ml 가 500ml 가  
25 ± 5 48 Glass Filter(1G3)

4) 1% NaOH

2g 1% NaOH 100ml 가 250ml  
1 . Glass  
Filter(1G3) 300ml, 10% Acetic Acid 50ml,  
300ml .

5) Organic Solvents Extracts

2g Soxhlet Ethanol - Benzene  
(1:2, v/v) 150ml 가 70 80 6  
. (105 )  
.

6) Lignin Contents

Klason Lignin Lignin .

7) Holocellulose Contents

Wise Holocellulose .

2.

가. ( ; Mixed Sawdust)

가  
Glycerin 1:1 105 20  
Slide microtome 10 20 $\mu$ m .



### 3

1. ( )

Table 1.1. Physiochemical characteristics of cultural substrate degraded 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
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	28.4
Holocellulose (%)	62.3	70.64	67.17

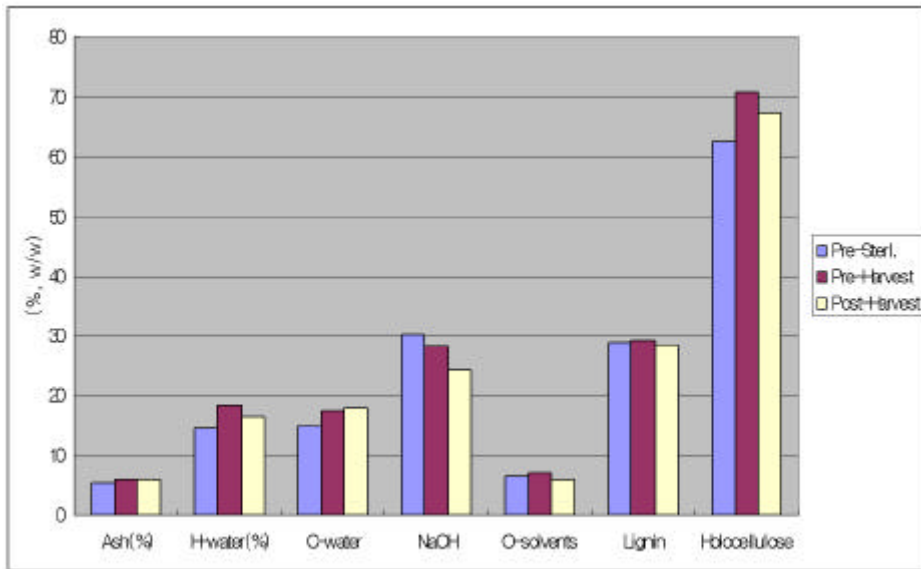


Fig 1.1. Chemical characteristics of cultural substrate degraded by *F. velutipes*.

Notes. H- and C-water mean extracts of hot and cold water, and NaOH and O-solvents mean extracts of NaOH and Organic solvents(alcohol-benzene), respectively

(Sawdust of *Pinus* spp./Rice bran = 75 80/25 20)  
 (water contents) 66%, 58.6%  
 (weight losses) 4.5%,  
 18% , pH 6.4  
 , pH 6.5 , pH 5.8  
 가 .

Table 1.1. ( , 5.49%) ( ,  
 , 5.88%, 5.96%) (Ash) 가 ,  
 . ,



( ) 5.49%

(cotton-waste)

(rice-straw)

(outdoor fermentation)

(Iiyama , 1995).

가(Ohga , 1993; Mun ,

1999)

가

가

가

가

가

(Lignin)

(Holocellulose)

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, 가

NIR (Resink Hurk, 2000)

(Determin-

ation of Rice Bran Contents by NIR Analysis)

가

2.

가.

(*Pinus spp.*) . , 1-A  
가  
가 . ( 1-B) 가  
가 가 ,  
. ( 1-C).  
(Hard Pine)  
90% .  
. , ( 2-A) 가 ,  
가  
. (Epithelial Cell) 厚壁  
. 가 가 . 가  
가 . 가 1 2  
. ( 2-B).  
, 가 가  
( 2-C). 가  
.

1  
Safranine 가 ( 3). Safranine  
3-A B  
Safranine 가

Astra Blue  
3-B 4-A  
, 가 ( 4-B). , 가  
가  
( 4-C).

가 ( 5-A, B).  
가 (Eaton & Hale, 1993)  
5-C

Erosion  
가  
가 , 가

Cellulose, Hemicellulose, Lignin

Lignin Cellulose, Hemicellulose

(Eriksson etc.,

1990).

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Guaiacyl  
Syringyl

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Guaiacyl  
Syringyl

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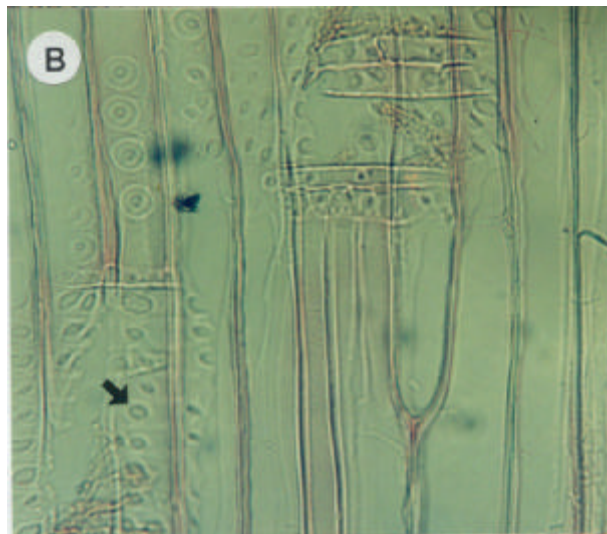
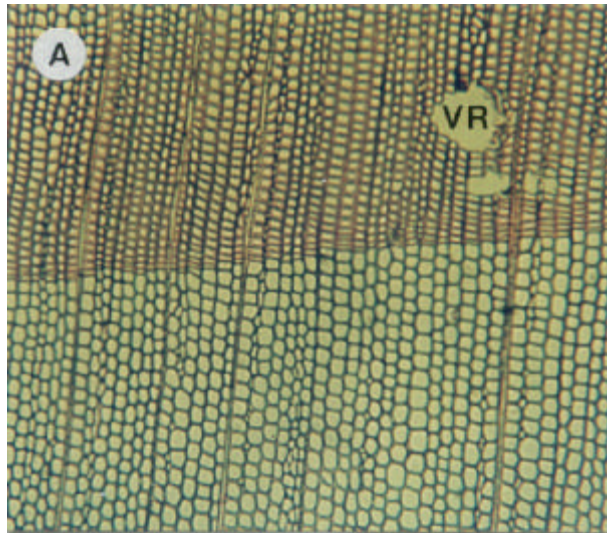
, Guaiacyl

Guaiacyl

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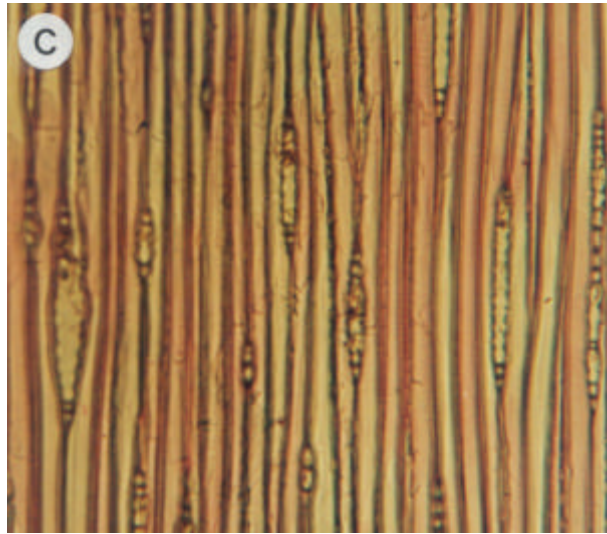
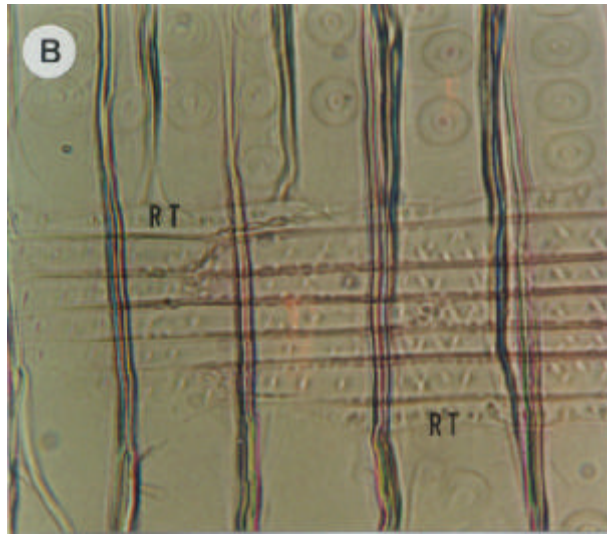
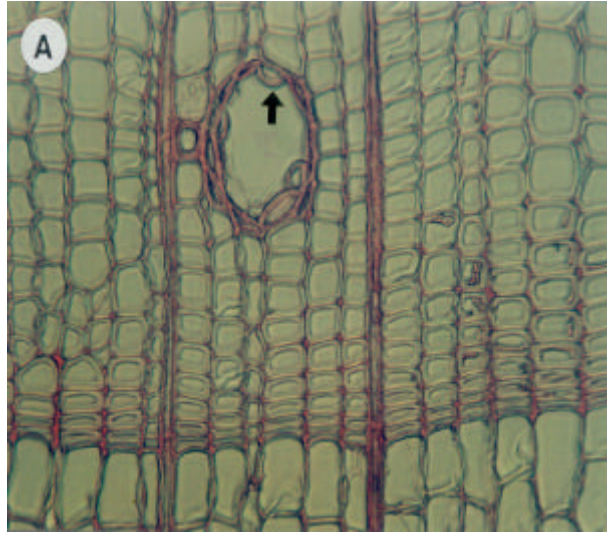


Fig. 1. Anatomical characteristics of sawdusts identified as in *Finus* spp.

A: Abrupt transition from earlywood to latewood and vertical resin canal (VR) (transverse section); B: Dentate ray tracheids and window-like pit pairs in cross field type (radial section); C: Fusiform ray with longitudinal resin canal (tangential section).



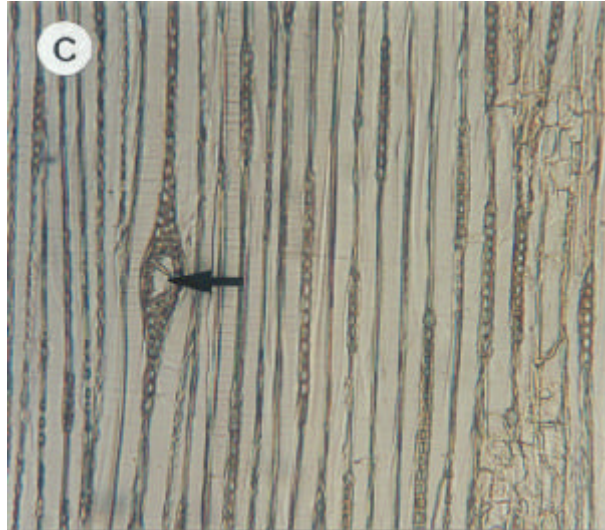


Fig. 2. Anatomical characteristics of sawdust identified as Douglas-fir (*Pseudotsuga menziesii*).

A: Abrupt transition from earlywood to latewood and thick-walled epithelial cells (arrow) in vertical resin canal (transverse section); B: Nondentate ray tracheids (RT) and piceoid type pit pairs in cross field (radial section); C: Vertical resin canal (arrow). Note the helical thickenings in earlywood tracheids and tylosoid (tangential section).



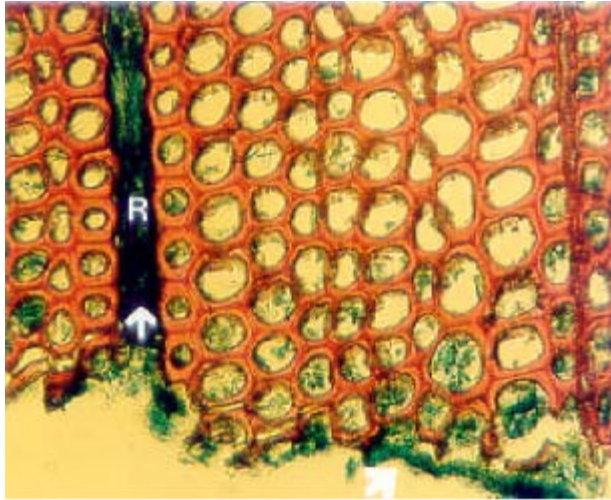


Fig. 3. Distribution of mycelia in hard pine. Distribution of fungal hyphae was restricted to ray parenchyma (R: arrow) and the tracheids exposed to hyphae.

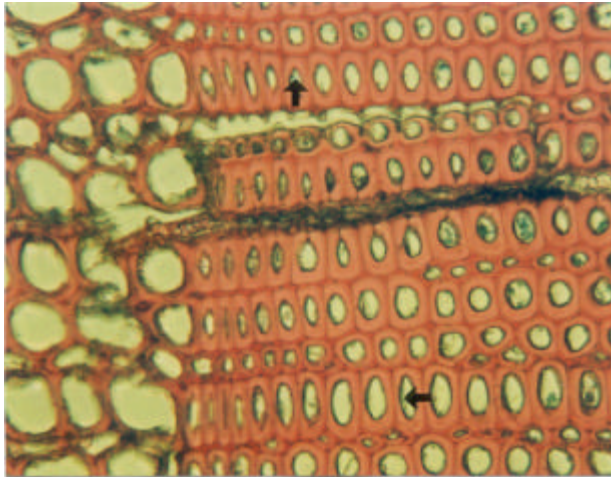


Fig. 4. Thinning of cell walls (arrow) of pine tracheids by enokitake fungi in latewood tracheids.

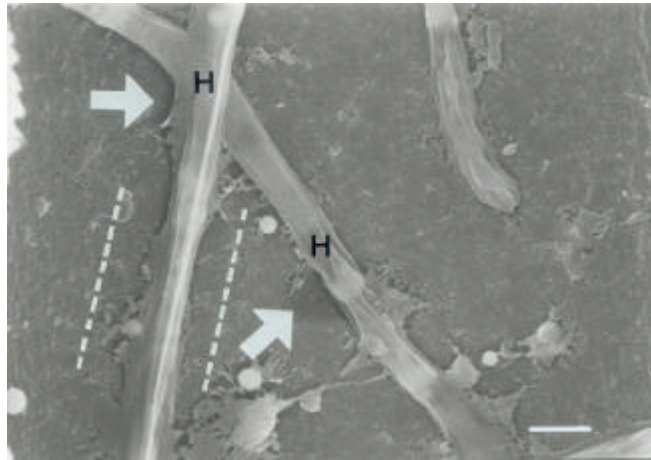


Fig. 5. Fungal slime (dotted line) and erosion trough (arrow) by enoki take hyphae. bar= $2\mu\text{m}$

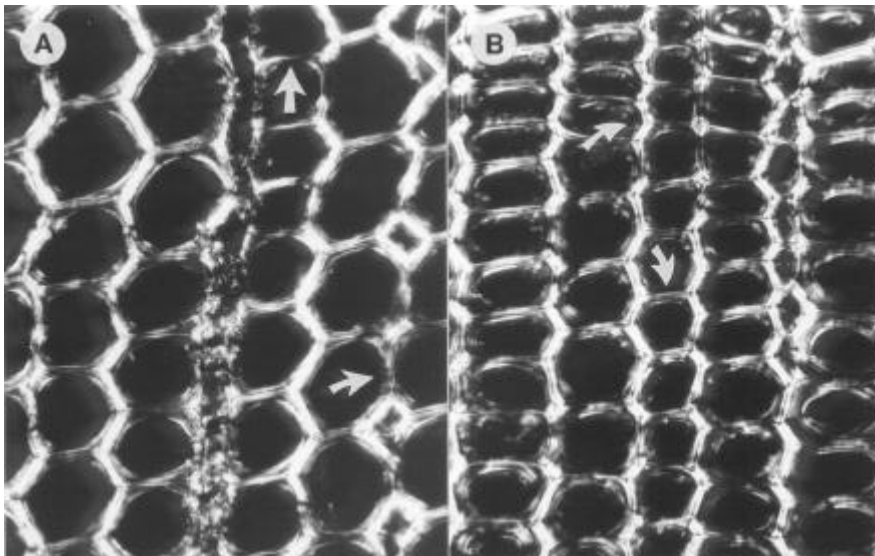


Fig. 6. Loss of birefringence (arrow) in some tracheids under polarized light microscopical observation: Earlywood (A) and latewood (B).

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가. 1 18% pH  
5.8 .

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가 ,

(Nata & Savoie, 1998). Park (1992)

Coffee waste 가

Terashina (1994) *Fasaria edulis*

*Fagus crenata* , ,

. Chai

(1999)

(*Robinia pseudacacia*)

가

(*Quercus serrata*)

가

(Park , 1994)

(fatty acid)

(Ohga , 1977; Kawachi , 1991). , Ohga (1990)

Takabatak (1994)

2

가  
가,

가

가

가

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1.

,  
가 , , pH

가. 가

(10mesh ) 0 40%(w/w)

65-67% . Test-tube

(D24mm × L20mm) 62g(Wet Weight) (B. D. , 0.21)

Cotton-plug Autoclavation (121 , 30min) .

(15 ) Petri-dish (Sanjo

No. 5, 6, N603 ) (Cork borer No. 5) . 5 2

(Linear Growth Rate, cm/24h) .

( Aging *Finus* spp. ), 1

(Enokidake cultural waste; Waste), 30 40

(Aging

waste),

(*Quercus serrata*)

10: 2(w/w)

65-67%

Test-tube(D24mm × L20mm) 62g(Wet weight)

(B. D. , 0. 21)

Cotton-plug

Autoclavation (121 , 30min)

(1

5 ) Petri-dish

(Sanjo No. 5, 6, N603 )

(Cork borer No. 5)

5

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(Linear growth rate, cm/24h)

(B. D. 0. 17-0. 27, g/cc)

Test-tube(D24mm × L20mm)

Cotton-plug

Autoclavation(121 , 30min)

(15 ) Petri-dish

(San 5, 6, N603 )

(Cork Borer No. 5)

5

2

(Linear Growth Rate, cm/24h)

가

(10mesh )

10: 2(w/w)

0, 5, 10, 20%(w/w),

65-67%

Test-tube(D24mm × L20mm) 62g(Wet weight)

(B. D. , 0. 21)

Cotton-plug

Autoclavation

(121 °C, 30min) . (15 ) Petri-  
dish (Sanjo No. 5, 6, N603 ) (Cork borer  
No. 5) . 5 2 (Linear  
growth rate, cm/24h) .

. pH

Ca(OH)<sub>2</sub>

0 0.5%

.

.

(Sanjo No. 5)

(*G. serrata*)

10: 2(w/w)

65 67%

Autoclavation

(Sanjo No. 5) .



2.

가. 가

(10mesh ) 0 40%(w/w)  
65-67%  
Autoclavable polypropylene vynil(5kg) (B. D. 0.21 0.23)  
Air-filter가 Screwcap  
Autoclavation(121 , 60nin) (15 )

, , ,  
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.

(*Q. serrata*) 0, 20, 40, 60, 80, 100%(w/w)  
가 10:2(w/w) 65 67%  
. Autoclavation(121 , 60nin)  
70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120

가 Fig. 2. 1

(a) (b)

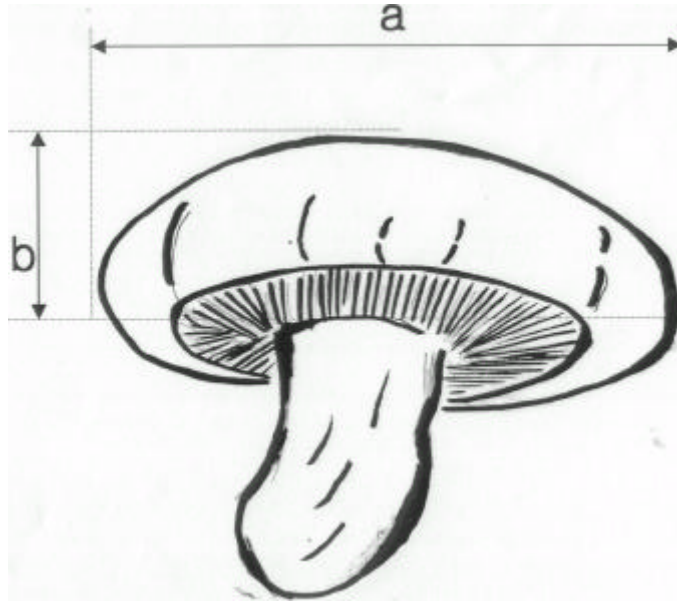


Fig. 3. 2. 1. Parts for determining the quality of *L. edodes* fruitbodies.

Note. a : pileus diameter, b: pileus thickness

20% 가 (Sanjo No. 5)

70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120

20% 가 (Sanj o No. 5)  
70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120



Fig. 3. 2. 2. Preparation of sawdust block before sterilization.

Note. This work was undertaken at the experimental forestry station, Chonnam Nat. Univ. High-pressure retort (left) and steam boiler (right) for sterilization of sawdust substrate were shown.



Fig. 3. 2. 3. Incubation of sawdust blocks for vegetative growth and ripening in temperature and light controlled room.

Note. This work was undertaken at the experimental forestry station, Chonnam Nat. Univ.

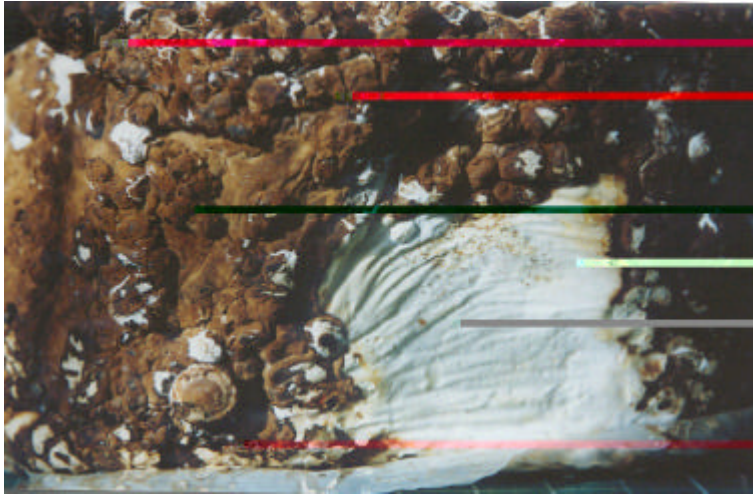


Fig. 3. 2. 4. Shiitake mycelial block ready to fruit.

Note. Shiitake mycelium formed lumps (so called, pop-corning phenonena) on brown-coated area reacting to the light and oxygen and did not on white mycelial area not exposed to the light or oxygen.



Fig. 3. 2. 5. Small fruitings through protective brown skin on sawdust substrate.



Fig. 3. 2. 6. Mature shiitake just before harvesting.

Note. Large and small plates were 10 and 5.5cm, respectively.



Fig. 3. 2. 7. Harvesting of mature shiitake

Note. Large and small plates were 10 and 5.5cm, respectively.

### 3

1.

가.

가

가 0 40%(w/w) (enokitake cultural waste) (Linear growth rate)

Table 3. 2. 1.

Table 3. 3. 1. Linear growth rate of shiitake fungus on enokitake cultural waste supplemented by rice bran.

Rice Bran (%)	Fungi		
	Sanjo No. 5	Sanjo No. 6	N603
0	0. 26 ± 0. 06c	0. 30 ± 0. 05c	0. 21 ± 0. 04d
10	0. 37 ± 0. 07b	0. 39 ± 0. 09b	0. 39 ± 0. 07c
20	0. 42 ± 0. 06a	0. 46 ± 0. 07a	0. 42 ± 0. 05c
30	0. 41 ± 0. 07a	0. 43 ± 0. 07b	0. 45 ± 0. 09b
40	0. 41 ± 0. 04a	0. 42 ± 0. 04b	0. 48 ± 0. 06a

Notes. Growth rate is expressed by the average and standard deviation of 10 replicates and values followed the same letter in the same column mean no significant difference (p=0. 05).

(0%) 20% 30 40% 20%

(Significant difference) . , Sanjo

No. 5, Sanjo No. 6 20% , 가

. N603 가 가 가 가

가 가 .

(Aging *Pinus* spp.),

1 (Fresh eonkidake cultural waste), 30-40

(Aging waste)

(*Quercus serrata*) 가

(10: 2, w/w) 5 2

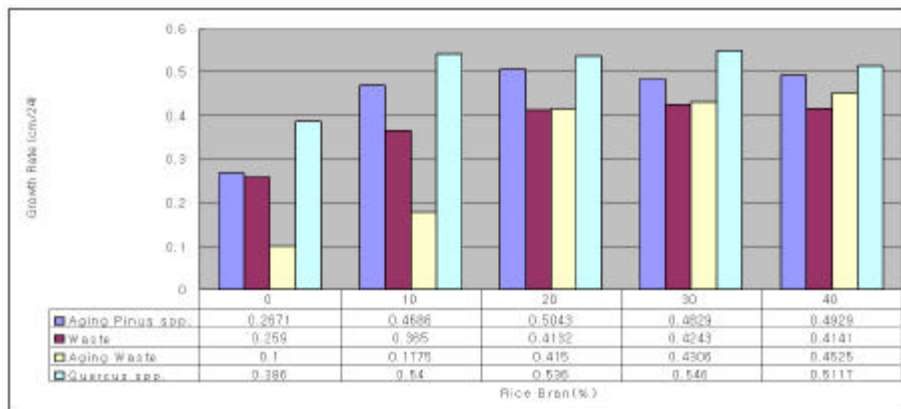


Fig. 3. 3. 1. Linear growth rate of *L. edodes* (Sanjo No. 5) on various sawdust substrates.



Sanjo No. 5 (Quercus serrata) 가 (Aging Pinus spp.) 10 (Waste) (Aging Waste) 20 40% , 20% 가 , (Fig. 3. 3. 1. ).

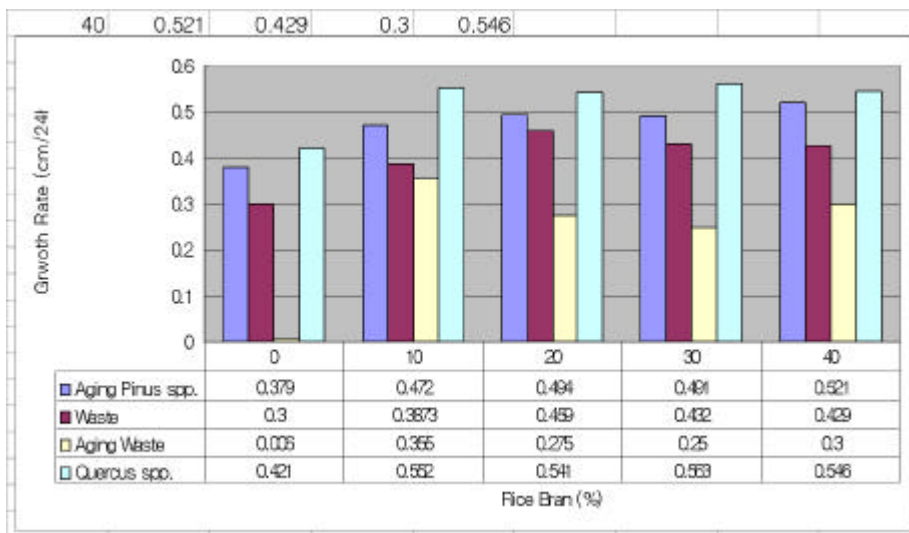


Fig. 3. 3. 2. Linear growth rate of *L. edodes* (Sanjo No. 6) on various sawdust substrates.

Fig. 3. 3. 2. Sanjo No. 6 10 40% 가 가 가 가 . sanjo No. 6 10% 가

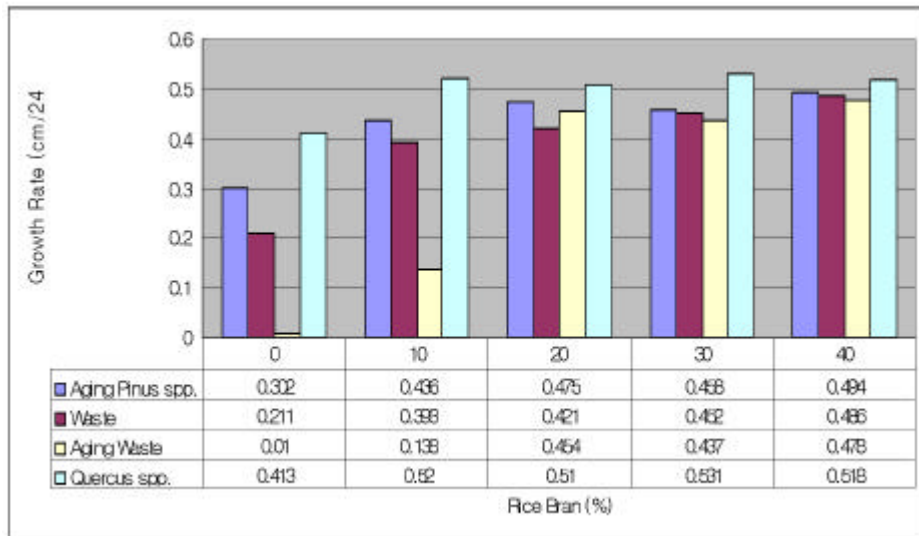


Fig. 3. 3. 3. Linear growth rate of *L. edodes* (N603) on various sawdust substrates.

N603

10%

가

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0 10%

20%

(Fig. 3. 3. 3).

, , 가

가

20%

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(g/cc)

Table 3.3.2.

Table 3.3.2. Effects of bulk densities of enokitake cultural waste on the linear growth of *L. edodes*.

Fungi Bulk Density(g/cc)	Growth Rate (cm/24h)		
	Sanjo No. 5	Sanjo No. 6	N 603
0.17	0.36 ± 0.06b	0.38 ± 0.03	0.44 ± 0.09
0.19	0.40 ± 0.05a	0.36 ± 0.04	0.39 ± 0.08
0.21	0.37 ± 0.03a	0.34 ± 0.04	0.40 ± 0.05
0.23	0.37 ± 0.04a	0.32 ± 0.06	0.33 ± 0.02
0.25	0.30 ± 0.05	0.31 ± 0.04	0.32 ± 0.02
0.27	0.33 ± 0.05	0.29 ± 0.05	0.29 ± 0.04

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

가

Sanjo No. 5

Bulk density 0.17 (g/cc)

가

0.21 (g/cc)

Sanjo No. 5



pH

Ca(OH)<sub>2</sub> 0% 0.5%

Table 3.3.4. Effects of Ca(OH)<sub>2</sub> addition to the enokitake cultural waste on the linear growth of *L. edodes*.

Fungi Ca(OH) <sub>2</sub> (%)	Growth Rate (cm/24h)			pH
	Sanjo No. 5	Sanjo No. 6	N603	
0	0.44 ± 0.06c	0.43 ± 0.09c	0.45 ± 0.10	5.91
0.1	0.47 ± 0.08c	0.28 ± 0.06	0.52 ± 0.06c	6.01
0.2	0.37 ± 0.06	0.32 ± 0.10	0.40 ± 0.14	6.04
0.3	0.37 ± 0.04	0.33 ± 0.10	0.41 ± 0.17	6.15
0.4	0.35 ± 0.04	0.34 ± 0.13	0.37 ± 0.12	6.21
0.5	0.35 ± 0.07	0.32 ± 0.09	0.43 ± 0.13	6.23

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

Ca(OH)<sub>2</sub>

pH 가

가

가

(Table 3.3.4).

Sanjo No. 5

Ca(OH)<sub>2</sub> 0%, 0.1%

가 가

Sanjo No. 6

0%, N603

0.1%

가

pH가 5 6 가

2.

가. 가가

(w/w) 가 ,

Table 3.3.5. Effects of rice bran addition to the fruting patterns and incubatin periods (Sanjo No. 5)

	Sanjo No. 5				
(%)		(g)	( )	( )	( )
0	18.32	105.32	60	60	5.4
10	20.02	220.44	45	50	8.7
20	15.76	436.65	40	40	10.6
30	17.55	421.32	40	40	9.6
40	26.33	532.12	40	40	6.4

Note. Weight of Medium was 2.5kg, first flush.







90 95 . 20%, 105  
 600g/2.5kg 95 110  
 10% 110 590g/2.5kg  
 110 40%  
 560g/2.5kg . ,  
 가 30%  
 100  
 가 . 20% 105

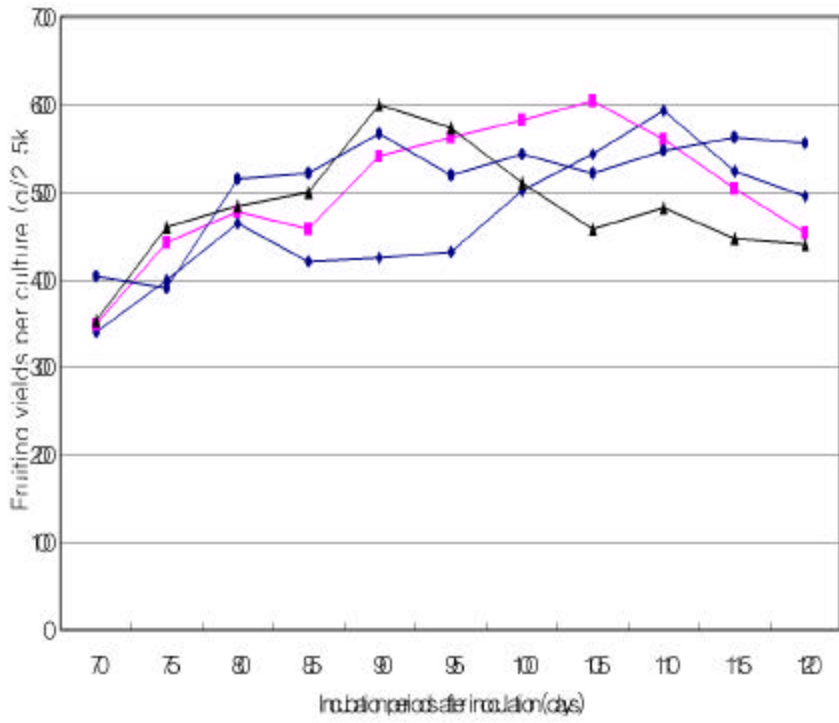
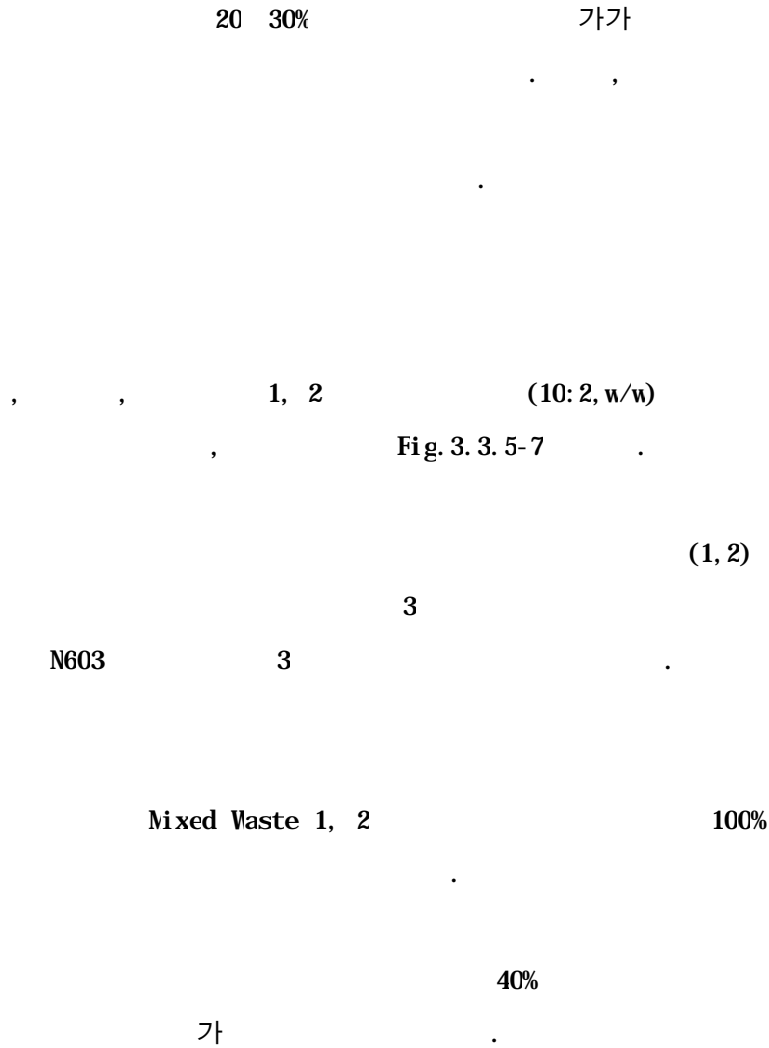


Fig. 3. 3. 4. Effect of rice bran contents on fruiting yields and

incubation periods of *L. edodes* on enokitake cultural waste.

Notes. Rice bran was supplemented in ratios of 10% ( ), 20% ( ), 30% ( ) and 40% ( ) to the dried weight of sawdust.



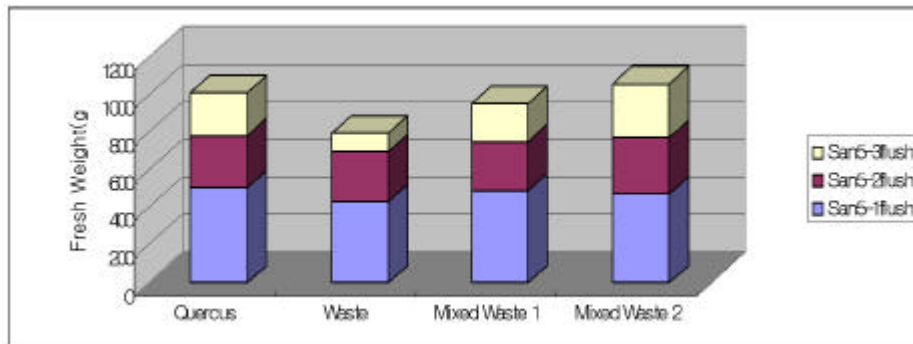


Fig. 3.3.5

(San 5)

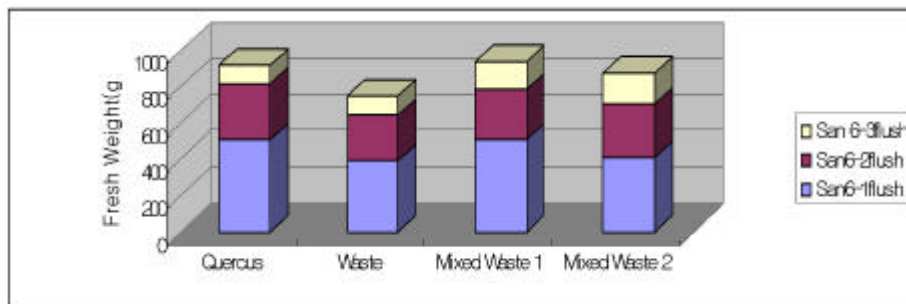


Fig. 3.3.6

(San 6)

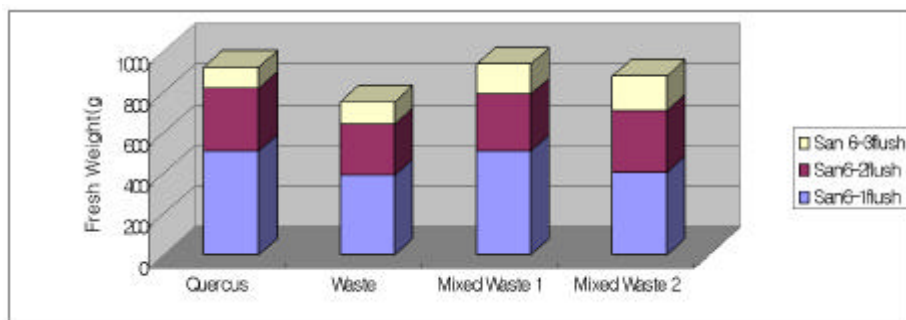


Fig. 3.3.7

(N603)

가가

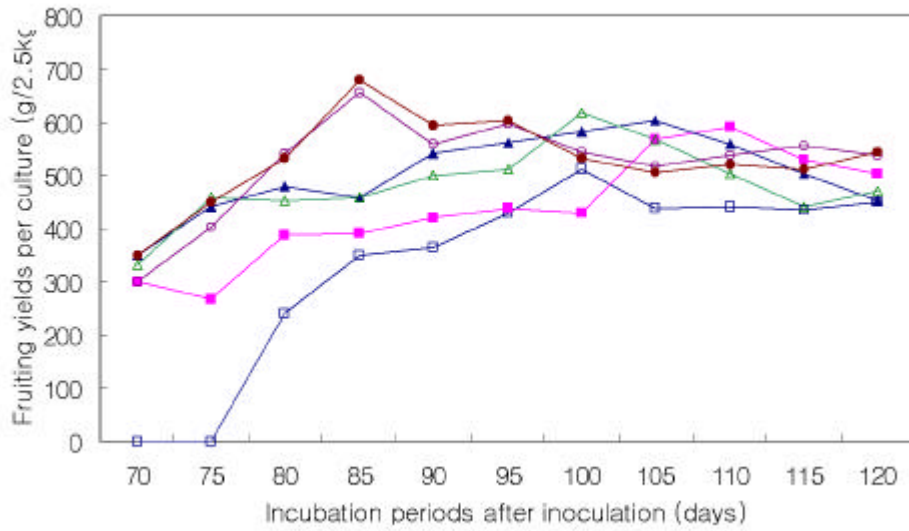


Fig. 3. 3. 8. Effect of oak sawdust contents on fruiting yields and incubation periods of *L. edodes* on enokitake cultural waste.

Notes. Oak sawdust was supplemented in ratios of 0%(□), 20%(■), 40%(▲), 60%(△), 80%(○) and 100%(●) to the dried weight of waste and rice bran was added in ratio of 20 % to the mixtures of oak and waste sawdust.

0, 20, 40, 60, 80, 100% (w/w)

Fig. 3. 3. 8

100

70 , 75

20% 105  
 40% 90 115  
 80 100% 85  
 650 680g/2.5kg

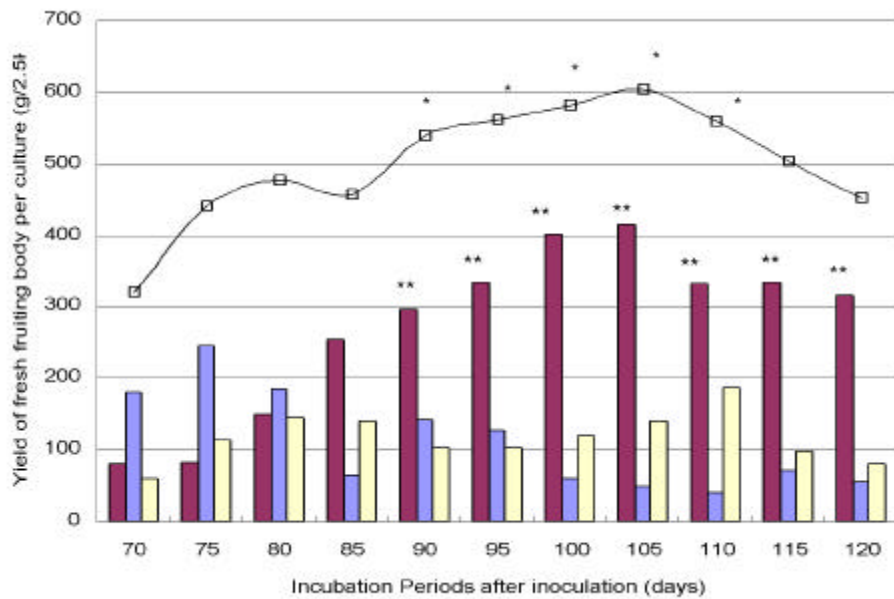


Fig. 3. 3. 9 Effect of incubation periods on fruiting yields and flushing patterns of *L. edodes* on enokitake cultural waste supplemented by rice bran (20%, w/w).

Notes. (■) first flush; (■) second flush; (■) third flush; (—□—) total yield. Each of asterisks (total yields) and double asterisks (first flush) mean significance of correlation coefficients for the 70-day-old culture at the P=0.05 level according to Duncan's new multiple range test.

70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120

Fig. 3.3.9 .

Fig Fig  
가 . , 110 120  
70 90 2 1  
. , 95  
1 2 , 3  
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Fig. 3.3.12 .

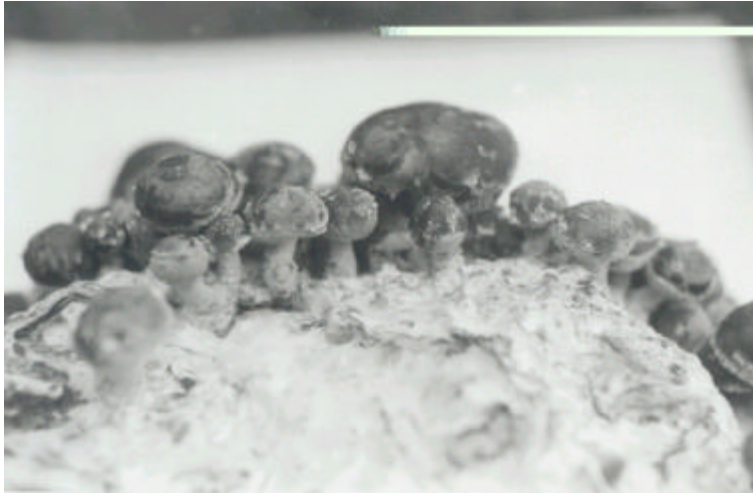


Fig. 3.3.10. Numerous fruit-bodies, miniaturized and densely fruited owing to prolonged incubation (120 days) on waste based culture.

Note. 156 fruit-bodies were harvested at 1st flush on the culture in this photograph.

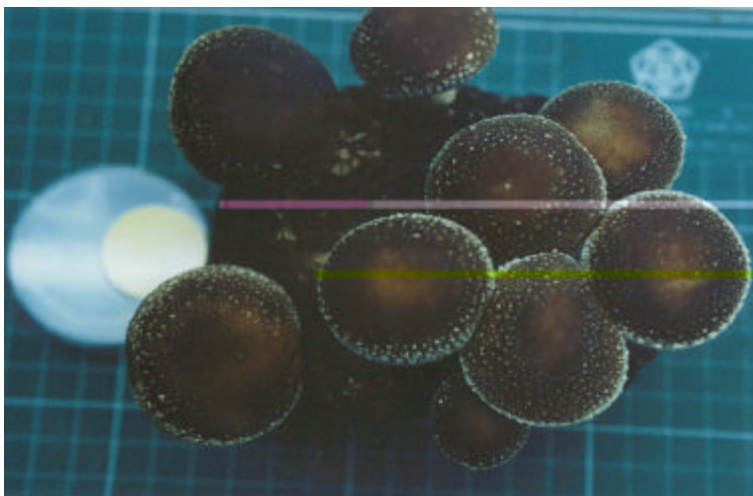


Fig. 3.3.11. Several larger fruit-bodies on waste based culture incubated for 105 days.





40% (w/w)

Fig. 3. 3. 14, 15

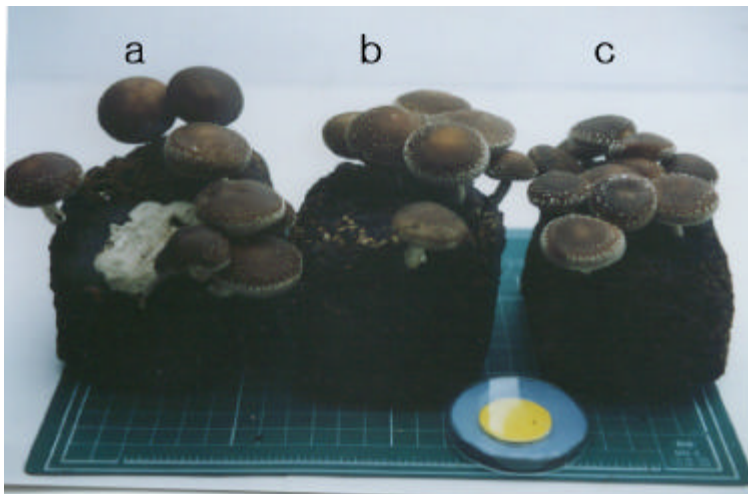


Fig. 3. 3. 13. Effect of oak sawdust supplementation on the pileus diameter of *L. edodes* on enokitake cultural waste.

Note. a: waste; b: Oak mixed waste; c: Oak sawdust. Supplementation of oak sawdust to the waste caused the size and thickness of the pileus smaller and thinner.

					4
14cm	60%	7	11cm	23%	가
10cm					2
13cm	60%	7	9cm	23%	가

8cm . 가  
 5 13cm 60% 8 10cm 23% 가  
 9cm .  
 가 가 가 가 가

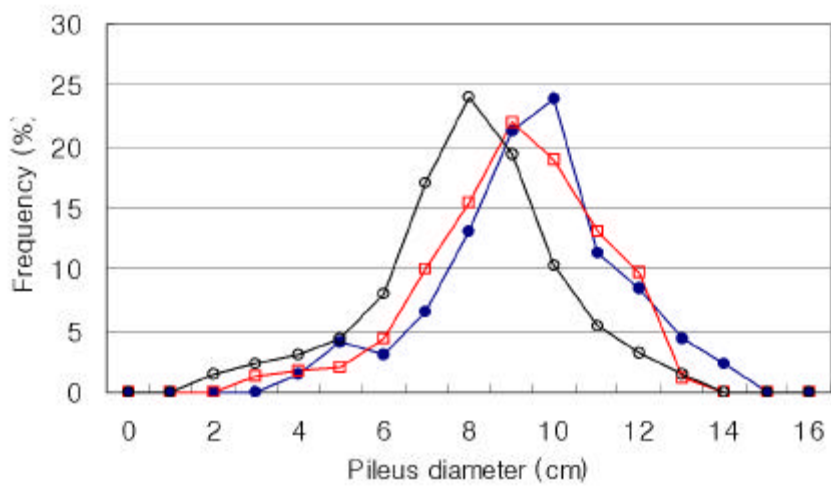


Fig. 3.3.14. Effect of oak sawdust supplementation on the pileus diameter of *L. edodes* on enokitake cultural waste.

Note. (●) waste (□) Oak mixed waste (○) Oak sawdust.

0.6 2.9cm  
 30% 1.8 2.0cm 18% 가 2.0cm  
 가 0.2 2.4cm  
 30% 1.6 1.8cm 18% 가 1.8cm

가 0.2  
 2.8cm 30% 1.8 2.0cm 22%  
 가 1.8cm

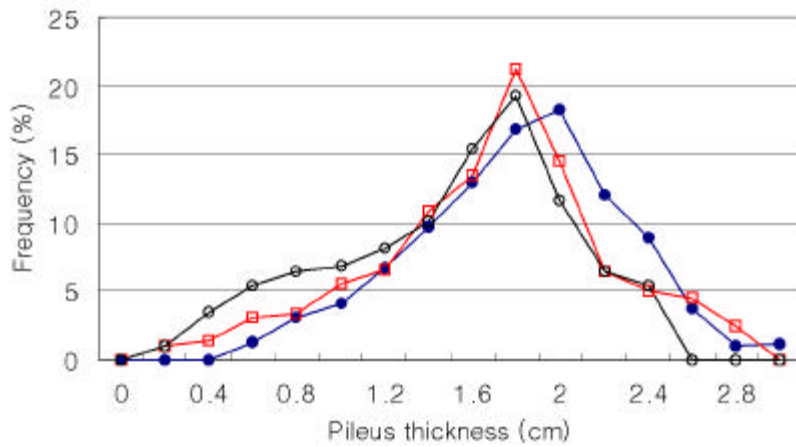


Fig. 3.3.15. Effect of oak sawdust supplementation on the pileus thickness of *L. edodes* on enokitake cultural waste.

Note. (●) waste (□) Oak mixed waste (○) Oak sawdust.

4

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가.

가

20%

30 40%

20%

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10 40%

20%

가

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가

0.17 0.23 (g/cc)

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Ca(OH)<sub>2</sub>

0.1%

.



가

가 가

4

1

*(Flanmlulina velutipes)*

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가

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1950

1926

1987

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1989

가

10

가

1992

( , 1998).

( , , , )

)

( , )

가

(Stanetes, 1993).

가

가

가가

(Zadrazil, 1985)

(White rot)

Syringyl type

Guaiacyl type

*Tranetes versicolor*

(Highley, 1982).

(cell wall thinning)

(erosion troughs)

(Eriksson, 1990).

가

가 .

middle

lanella

(2001; unpublished data)

1

가

가 . ,

2

가

가



2

1.

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가 , , pH

가. 가

(10mesh ) 0 40%(w/w)

65- 67% Test-tube

(D24mm × I20mm) 62g(Wet weight) (B. D. , 0.21)

Cotton-plug Autoclavation (121 , 30min)

(15 ) Petri-dish (TK,

N50) (Cork borer No.5) . 5 2

(Linear growth rate, cm/24h) .

( Aging *Finus* spp.), 1

(Enokidake cultural waste; Waste), 30 40

(Aging

waste),

(*Quercus serrata*)

10: 2(w/w)

65 67%

Test-tube(D24mm x L20mm) 62g(Vet weight)

(B. D. , 0. 21)

Cotton-plug

Autoclavation (121 , 30min)

(1

5 ) Petri-dish

(TK, M50)

(Cork borer

No. 5) . 5 2

(Linear

growth rate, cm/24h)

(B. D. 0. 17 0. 27, g/cc)

Test-tube(D24mm x L20mm)

Cotton-plug

Autoclavation(121 , 30min)

(15 ) Petri-dish

(TK, M50)

(Cork borer

No. 5) . 5 2

(Linear

growth rate, cm/24h)

가

(10mesh )

10: 2(w/w)

0, 5, 10, 20%(w/w),

65-67%

Test-tube(D24mm x L20mm) 62g(Vet weight)

(B. D. , 0. 21)

Cotton-plug

Autoclavation

(121 , 30min)

(15 ) Petri-

dish (TK, M50) (Cork borer No. 5) .  
5 2 (Linear growth rate,  
cm/24h) .

pH

Ca(OH)<sub>2</sub>

0 0.5%

2.

가. 가

(10mesh ) 0 40%(w/w)

65-67%

Autoclavable polypropylene bottle(900ml) (B. D. 0.21 0.23)

Air-filter가 Screwcap

Autoclavation(121 , 60min) . (15 )

. 90%

10 12

80 85%

가 가

5 8

가 2 3cm

가 2cm ,

(Waste), (Aging waste), (Aging *Finus* spp. ,  
 2(P: V=6: 4), 1(P: V=4: 6),  
 65 67% 10: 2(w/w)  
 (N50, TK) Autoclavation(121 , 60min)

(10mesh ) 0 40%(w/w)  
 65- 67%  
 Autoclavable polypropylene bottle(900ml) 0.17 0.29  
 Air-filter가 Screwcap  
 Autoclavation(121 , 60min)  
 (15 )  
 . 90% , ,

3. 2

가. 2

1 ( )

(Renains)

(Dextrin, Dextrose; 0, 0.1, 0.5, 1%, w/v),  
acid; 0.01, 0.1, 1%, w/v), ( )  
water)

(Peptone, Glutamic  
(Distilled  
.

. 2

1 ( )

(Renains)

(10 20 )

.

### 3

1.

가. 가

(Fresh enoki dake cultural waste) (w/w)  
 (TK, N50) 5 2  
 (Growth rate, cm/24h) .

Table 1. 1. Effects of rice bran on the linear mycelial growth of *F. velutipes* on enokitake cultural waste.

Strains Rice Bran (%)	N50	TK
0	0.30 ± 0.05b	0.36 ± 0.12b
10	0.34 ± 0.08	0.44 ± 0.08
20	0.34 ± 0.10	0.43 ± 0.09
30	0.36 ± 0.10	0.44 ± 0.08
40	0.39 ± 0.05	0.41 ± 0.04

Notes. Growth rate is expressed by the average and standard deviation of 9 replicates and b means significant difference among the growth ratio in five media differently treated.

가 0 40%(w/w) (enoki take cultural waste) (M50, TK) (Linear growth rate) Table 1. 1. 가 가 M50 (0%) 가 가 TK , 10% 가 40% 가 10 40% 가 TK 가 10%가 .

(Aging *Finus* spp.), 1 (Fresh enokidake cultural waste), 30-40 (Aging waste), (*Quercus serrata*) 가(10: 2, w/w) 5

2 TK 10% 가 . 10% 20% (Fig. 5. 2. 4-1). M50 가 .

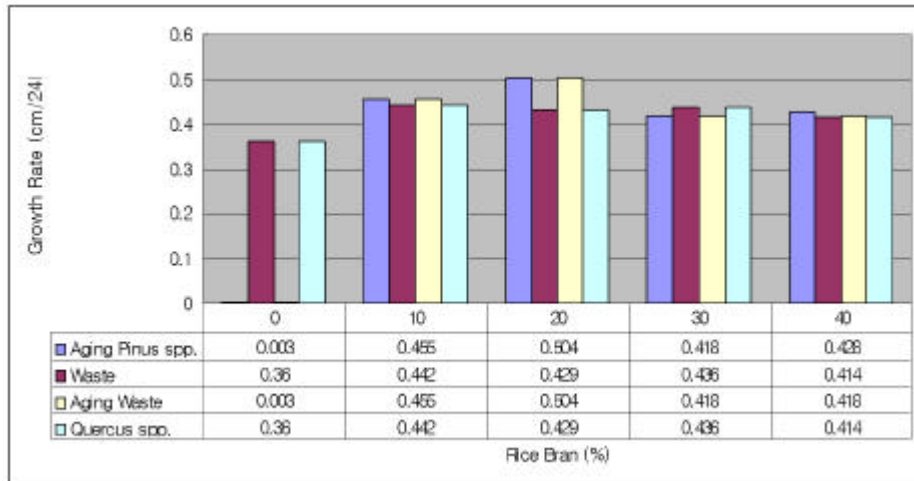


Fig. 1.1. Linear growth rate of *F. velutipes*(TK) on variously treated sawdust substrates.

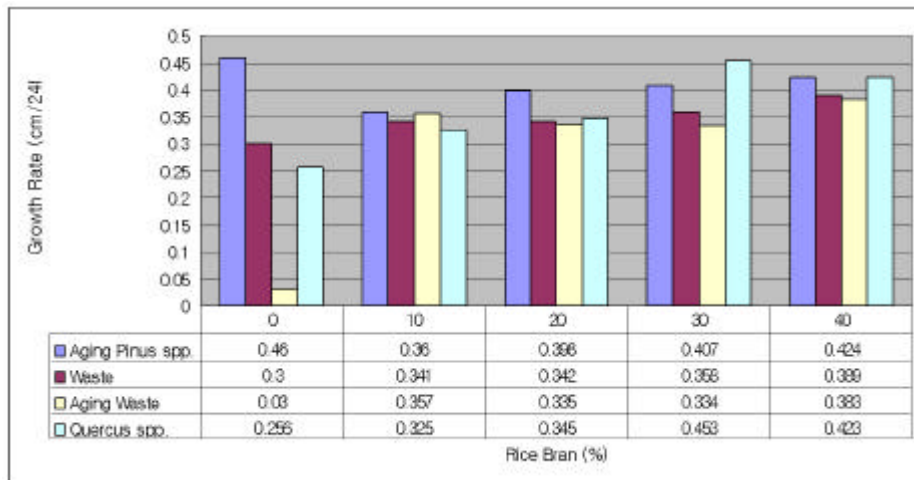


Fig. 1.2. Linear growth rate of *F. velutipes*(M50) on variously treated sawdust substrates.



(g/cc)

Table 1. 2.

Table 1. 2. Effects of bulk densities of enoki dake cultural waste on the linear growth of *F. velutipes*.

Strains Bulk Density (g/cc)	Growth Rate (cm/24h)	
	M50	TK
0. 17	0. 34 ± 0. 03	0. 40 ± 0. 02
0. 19	0. 35 ± 0. 06	0. 39 ± 0. 02
0. 21	0. 37 ± 0. 02	0. 41 ± 0. 05
0. 23	0. 31 ± 0. 05	0. 39 ± 0. 04
0. 25	0. 29 ± 0. 03	0. 35 ± 0. 02
0. 27	0. 29 ± 0. 04	0. 33 ± 0. 01

Notes. a and b mean the average and standard deviation of 9 replicates and c means significant difference among the growth ratio in five media differently treated.

M50 TK 가 가 .  
M50 0. 21 (g/cc) , 0. 21  
TK 0. 17 0. 23 (g/cc)

Fig5. 2. 5 Linear Regression

Model . San 5  
 (RE1) 가 San 6, N603  
 (RE2, 3) . (M50, TK)  
 .

- Y = 0.104+3.09X-0.86X<sup>2</sup> ----- [Regression Equation 1]
- Y = 0.509-0.79X ----- [Regression Equation 2]
- Y = 0.668-1.38X ----- [Regression Equation 3]
- Y = 0.042+3.47X-9.65X<sup>2</sup> ----- [Regression Equation 4]
- Y = -0.151+5.64X-14.33X<sup>2</sup> ----- [Regression Equation 5]

Fig. 5. 2. 5 가  
 가 (Negative Correlation)  
 N603 San 6 San 5 M50  
 TK TK 가  
 (M50, TK)  
 BD 0.19 0.23 .

가

(w/w)

Table 1.3.

Table 1.3. Effects of rice hull supplementation to the enokidake cultural waste on the linear growth of *F. velutipes*.

Strains Bulk Density (g/cc)	Growth Rate (cm/24h)	
	M50	TK
0	0.43 ± 0.13	0.45 ± 0.08
5	0.30 ± 0.07c	0.35 ± 0.11c
10	0.29 ± 0.08c	0.35 ± 0.11c
15	0.31 ± 0.08c	0.34 ± 0.02c
20	0.33 ± 0.11c	0.33 ± 0.09c

Notes. a and b mean the average and standard deviation of 10 replicates and c means significant difference (p=0.05) in their growth rates with that of control.

pH

Table 1.4. Effects of Ca(OH)<sub>2</sub> addition to the enokitake cultural waste on the linear growth of *F. velutipes*.

Strains Ca(OH) <sub>2</sub> (%)	Growth Rate (cm/24h)		pH
	N50	TK	
0	0.40 ± 0.14	0.45 ± 0.11	5.90
0.1	0.40 ± 0.06	0.52 ± 0.06 <sup>c</sup>	6.01
0.2	0.34 ± 0.19	0.40 ± 0.14	6.10
0.3	0.36 ± 0.12	0.41 ± 0.17	6.15
0.4	0.36 ± 0.11	0.37 ± 0.12	6.21
0.5	0.35 ± 0.08	0.43 ± 0.13	6.23

Notes. a and b mean the average and standard deviation of 10 replicates and c means significant difference (p=0.05) in their growth rates with that of control.

, Ca(OH)<sub>2</sub> 0%, 0.1% 가  
 , TK 0.1% 가  
 .  
 , (Enoki dake  
 Cultural Waste) pH가 pH  
 , 0.1% .





2.

가.

Table 2. 1.

( )

Rice Bran(%)	M50		TK	
	Periods for Pinhead- initiation(day)	*Yields(g)	Periods for Pinhead- initiation(day)	Yields(g)
0	55.3a	87.02a	50.1a	90.65a
10	44.6	130.41a	43.4	119.89a
20	45.1	163.46	39.9	156.44
30	42.9	159.80	41.8	160.97
40	40.6	162.78	43.4	155.49

Notes.

\* means the fresh weight of fruitbodies per bottle (900cc) which is average of 160 replications (10Box).

a indicate the significant difference (p=0.05) among the treated media.

0%)  
TK  
10 40%  
M50

20 40%  
10% 가  
10% 가  
20% 가 30, 40%  
가 20%

(Aging Waste), (Aging Pinus spp.), (Fresh Waste),  
(Mixe Waste1, 2)

M50, 1, 2  
42 43 47  
167.34g





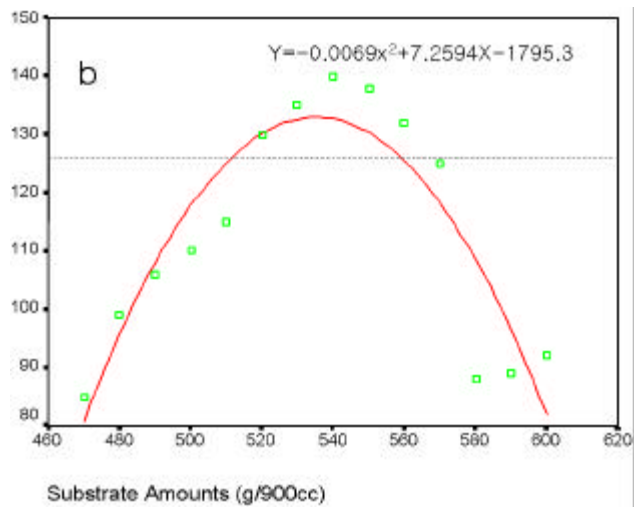
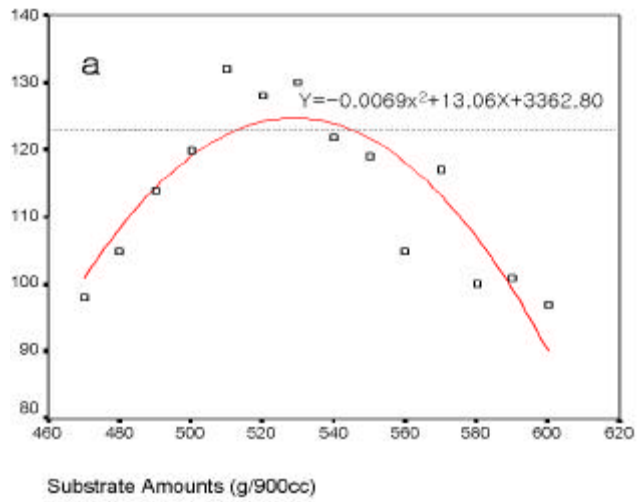


Fig. 1.1. Effect of substrate amount on the fruiting yields of *F. velutipes*.

Note. Each a and b show the regression curve and equation of the fruiting yields according to the amounts of substrate, enokitake cultural waste and *Populus* mixed waste.

3. 2

가. (M50) 2

Table 3. 1.

Additives	Concentration	Yields (g)	%
Distilled Water (nl)	0	32.90	100.0
	20	48.02	146.0
	40	68.28	207.5
	60	68.90	209.4
	80	40.08	121.8
	100	36.02	109.5

1 가 2 40  
60nl 68.28 68.90g 가  
(0nl) 200 210% .

. 2

(40 60nl) 가 5.2.2  
. Dextrin, Carrot Extracts, Scallian Extracts, Onion  
Extracts, Rice Bran Extracts 가 .

Table 2.1. Second flushes of *F. velutipes* on waste substrate, treated with several carbon, nitrogen sources and natural extracts.

Additives	Concentration (%)	Yields (g)
Distilled Water	-	68.55
Dextrin	0.1	54.33
	0.5	57.24
	1	80.35
Dextrose	0.1	68.34
	0.5	48.78
	1	61.76
Peptone	0.01	67.88
	0.1	48.90
	1	30.22
Glutamic Acid	0.01	64.33
	0.1	63.31
	1	38.93
Rice Bran Extracts	0.1	60.55
	1	92.06
	3	63.01

Dextrin 0.5%  
 1% 117.15%  
 Rice Bran 1% 0.1%, 3%



4

1.

가. 가 10%

20 40%

10%

20%

M50

0.21 (g/cc)

TK

0.17 0.23 (g/cc)

Ca(OH)<sub>2</sub> 0.1%

2.

가. 10% 가

20%



5

1

1.

가.

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2.

가.

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3.

가.

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2

가



1.

가.

1) , , , , 2001. 1

(*Flammulina velutipes*)

( )

2) Chai J.K., K.-M.Wi, S.J.Lee and Y.J.Kin, 2000. Fruiting patterns of *Lentinus edodes* on enokitake cultural waste depending on rice bran supplenation and incubation time. Res. Bullet. Forests. Chonnam Nat. Univ. No.16

3) Chai J.K., S.J. Lee, K.-M.Wi and Y.J.Kin, 2000. Reutilization of enokitake cultural waste as *Lentinus edodes* cultivation substrate. Plant Resources 3(2).

4) Chai, J.K., S.J. Lee, Y.J.Kin, Y.-I Kim and N.H.Kin, 2000. The recycling of enokitake cultural waste and the potentiality of 2nd flush for enokitake production.

1) , , . 1999.

- .

6

2) , , . 1999.

- . 6 .

3) , , . 1999. 2 .

6 .

4) Chai, J. K., S. J. Lee, Y. S. Kim and K. H. Lee, 1999. Biodegradation of mill-waste substrate by *Flammulina velutipes*. 6

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## 6

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