Mycelial Yield of *Pleurotus ostreatus* Using Thinned Apple, Pear, and Peach on Submerged Culture

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Abstract The effect of thinned fruits, apple, pear and peach, on the mycelial growth of mushrooms was investigated. The growth of mycelia with the addition of thinned fruit was clearly better than that in the control for all the tested mushrooms. The growth rate of *Pleurotus ostreatus* was faster than any other mushroom. The optimal concentrations of thinned apple, pear, and peach in a solid culture were 1.0%, 1.0%, and 3%, respectively, while in a liquid culture the optimal concentrations were 5,0%, 3.0%, and 5.0%, respectively. When *Pleurotus ostreatus* was incubated in a 20-L pilot scale fermenter with 10 L of a liquid medium containing 3% thinned fruit at 25°C and 6 vvm for 10 days, the mass-production of mycelia was 74.2 g/10 L (apple), 96.2 g/10 L (pear), and 86.3 g/10 L (peach). The mycelial yield of *Pleurotus ostreatus* in a medium containing thinned fruit was 2 ~ 3 times higher than that in the control.

Keywords: thinned fruit, mushroom, *Pleurotus ostreatus*, submerged culture

INTRODUCTION

Mushrooms are an alkali food with a high nutritious value [1,2] and multifunctional food with medical value [3-5], including anti-cancer action and the prevention of geriatric diseases.

A good quality spawn is essential for the stable production of mushrooms. Oyster mushrooms are usually cultivated using sawdust spawn added to rice bran. However, sawdust spawn forms a cake, unlike grain spawn, thus it needs to be broken into small and uniform pieces before being inoculated. To solve this problem, many researchers have investigated the use of liquid spawn, some of which have already been practically applied in mushroom cultivation. Since the mycelial vitality of liquid spawn is stronger than that of sawdust spawn or grain spawn, liquid spawn provides several advantages, including shortening the duration of mycelial growth and pinhead formation, while also decreasing the contamination rate. As a result, mushroom production is increased. Therefore, more research on the physical factors, cheaper media, growth promotion materials and cultivation devices is needed to produce better liquid spawn [6-11].

In an orchard, in order to produce a high quality harvest, many fruits are thinned out before maturation and discarded on the ground. However, the thinned fruits can also become a significant source of secondary infections, such as brown rot [12]. Meanwhile, the thinned fruits contain nutrients, such as protein, carbohydrate, organic acid, and inorganic ions, plus physiological factors, such as polyphenols, hormones, vitamins, and enzymes.

Accordingly, the current study was carried out to investigate the effect of thinned apple, pear and peach on the mycelial growth of *Pleurotus ostreatus* in a submerged culture and examine a cultivation method for liquid spawn.

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MATERIALS AND METHODS

Materials

Pleurotus ostreatus, Flammulia velutipes, Pleurotus eryngii, and *Grifola frondosa* kept in Jeollabukdo A.R.E.S. were used in the current study and maintained at 4°C on potato dextrose agar (PDA) slants with 3 month transfers. The cultivars of apple, peach, and pear used in this experiment were Fuzi, Youmeong, and Niitaka, respectively. The average weight of the first thinned apple, peach, and pear was 2.9 ± 0.5 g, 4.1 ± 0.6 g, and 2.5 ± 0.5 g, respectively, while the average weight of the second thinned apple, peach, and pear was 6.3 ± 1.1 g, 13.9 ± 2.1 g, and 5.2 ± 1.0 g, respectively.

Proximate Composition Analysis

The proximate composition of the thinned fruits was analyzed according to the AOAC method [13]. The moisture content was quantified by the drying method at 105°C, the crude protein by the semi-micro kjeldhal method, the crude fat by the soxhlet extraction method, and the ash by the ashing method at 550 °C. The amount of carbohydrate was then determined by subtracting the amount of moisture, crude fat, crude protein, and ash from 100.

Table 1. Proximate composition of thinned apple, pear, and peach

(%)

Thinned fruits	Thinning Period	Moisture	Crude protein	Crude fat	Ash	Carbohydrate
Apple	First	86.8	1.51	0.79	0.66	10.2
	Second	88.2	1.28	0.66	0.61	9.2
Pear	First	80.3	2.50	0.36	0.98	15.8
	Second	79.9	2.04	0.32	0.96	16.7
Peach	First	87.8	1.75	0.32	0.72	9.4
	Second	88.7	1.54	0.20	0.51	8.9

Solid Culture

Prior to the experiment, the thinned fruit was cut into small peaces, about 5 × 5 × 5 mm for all treatments. To test the effect of the thinned fruits on the mycelial growth of four mushrooms, 10 g/L of thinned fruit was added to a basal solid medium (g/L), consisting of glucose 10, peptone 2, KH_2PO_4 1, $MgSO_4$ 0.2, and agar 15, then homogenized. For the concentration test, 5 to 50 g/L of thinned fruit was added to the basal solid medium and homogenized. Next, 50 mL of the medium was dispensed into a 250 mL flask and autoclaved at 121°C for 15 min, then each flask was inoculated with an 6 mm disc of seed mycelia and incubated in the dark at 25°C for 7 days [14-16]. The seed cultures of the strains were grown in a potato dextrose agar at 25°C.

Liquid Culture

The composition of the shaking culture medium for the mycelial growth of *Pleurotus ostreatus* was as follows (g/L): glucose 10, peptone 2, KH_2PO_4 1, $MgSO_4$ 0.2, and thinned fruit 10~70. 100 mL of this medium was dispensed into a 500-mL flask and autoclaved at 121°C for 15 min, then each flask was inoculated with 3 mL of homogenized mycelia and incubated on a rotary shaker (150 rpm) at 25°C for 10 days. For the pilot culture, the medium consisted of 10 g glucose, 2 g peptone, 1 g KH_2PO_4, 0.2 g $MgSO_4$, and 30 g thinned fruit per liter of distilled water. 10 L of this medium was dispensed into a 20-L jar and autoclaved at 121°C for 15 min, then the jar was inoculated with 300 mL of homogenized mycelia and incubated with aeration (6 vvm) at 25°C for 10 days [14, 17]. The seed culture of *Pleurotus ostreatus* was inoculated into a 500-mL flask containing 100 mL of medium (PDB). After 7 days of cultivation, the medium including the mycelia was homogenized aseptically in an omni-mixer for 3 min.

Measuring Mycelia Growth

The mycelial growth in the solid culture was measured based on the diameter of the colony on the plate. The mycelia in the shaking and pilot culture were collected using filter paper (No. 2) and a sieve (100 mesh), dried at 80°C for 24 h, and measured based on the dry weight (DW) [15].

RESULTS AND DISCUSSION

Proximate Composition of Thinned Fruits

The proximate composition of the thinned apple, pear, and peach used in the current experiment was analyzed, as shown in Table 1. The thinned fruits contained 79.9 ~ 88.7% moisture, 1.3 ~ 2.5% crude protein, 0.2 ~ 0.8% crude fat, 0.5 ~ 1.0% ash, and 8.9 ~ 16.7% carbohydrate. The thinned pear exhibited a higher content of crude protein, ash and carbohydrate compared to the thinned apple and peach. The content of crude fat in the thinned apple was higher than that in the thinned pear and peach. The first thinned fruits contained a higher crude protein, crude fat, ash, and carbohydrate content compared to the second thinned fruits. When the proximate compositions of the thinned fruits were compared to those of the ripened fruits, the crude protein, crude fat, and ash contents in the thinned fruits were all higher than those in the ripened fruits for all three kinds of fruit. However, the carbohydrate content in the thinned fruits was lower than that in the ripened fruits(data not shown) [18].

Solid Culture

To examine the effect of thinned fruits on myceilial growth, *Pleurotus ostreatus, Flammulia velutipes, Pleurotus eryngii,* and *Grifola frondosa* were cultivated in a solid medium containing 1% (w/v) thinned apple, pear, and peach.

Table 1 shows the mycelial growth of the four kinds of mushroom with the thinned fruits. The growth of mycelia with the thinned fruit was clearly better for all the tested mushrooms than in the control. Among the tested mushrooms, the mycelial growth of *Pleurotus ostreatus* and *Flammulia velutipes* was promoted regardless of the kind of thinned fruit. In particular, the thinned apple had a significant effect on the mycelial growth of *Pleurotus ostreatus*, while the thinned pear and peach had a significant effect on the mycelial growth of *Flammulia velutipes*. The growth rate of mycelia was determined based on the

Table 2. Effect of thinned fruits on mycelial growth of mushrooms at 25°C for 7 days

Thinned fruits	Colony diameter(cm/7 days)			
	Pleurotus ostreatus	*Flammulina velutipes*	*Pleurotus eryngii*	*Grifola frondosa*
Apple	7.8[1)]	7.7	5.9	4.3
Pear	7.2	7.5	5.6	3.5
Peach	7.2	7.7	5.4	4.7
Control	4.7	6.5	5.2	3.2

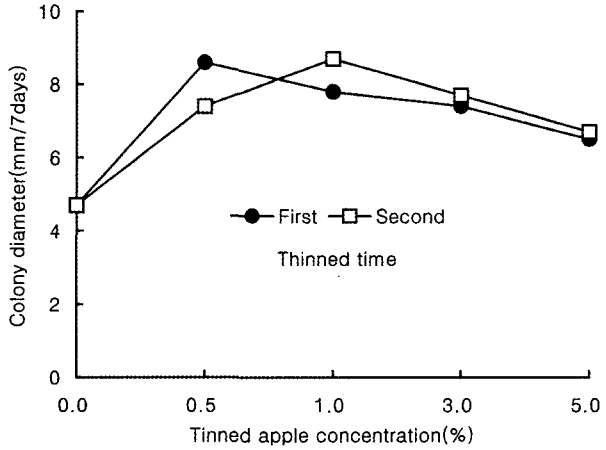

Fig. 1. Effect of thinned apple on mycelial growth of *Pleurotus ostreatus* in solid medium at 25°C for 7 days.

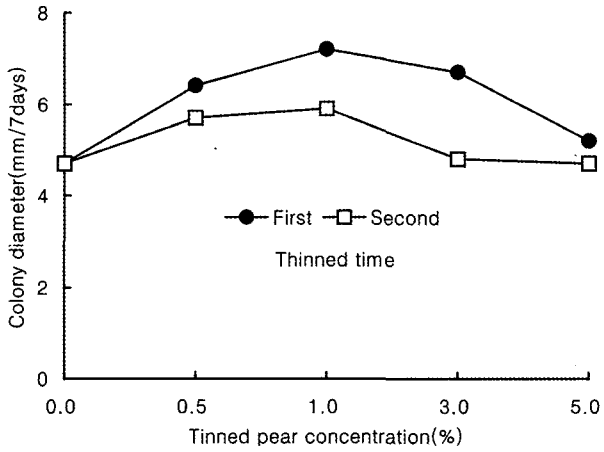

Fig. 2. Effect of thinned pear on mycelial growth of *Pleurotus ostreatus* in solid medium at 25°C for 7 days.

colony diameter and compared with the control. Since the fruit-added media had a more outstanding effect on *Pleurotus ostreatus* (1.5 times) than on *Flammulia velutipes* (1.2 times), the remaining experiments were carried out using *Pleurotus ostreatus*.

To determine the optimal concentration of thinned fruit for mycelial growth, *Pleurotus ostreatus* was cultivated in a solid medium containing 0.5 ~ 5.0% thinned fruits at 25°C for 7 days (Figs. 1-3).

The mycelial growth was better in the medium with the thinned apple than in the control. The optimal concentration of thinned apple was 0.5% with the first thinned fruits and 1.0% with the second thinned fruits. When the thinned apple content was increased, the mycelial growth exhibited a tendency to decrease gradually. The mycelial growth in the medium with the thinned pear was outstanding compared to the control, and the effect was better with the first thinned fruits than with the second. The optimal concentration appeared to be 1.0%, regardless of the thinning period. When the thinned pear content was increased, the mycelial growth also decreased gradually. The mycelial growth in the medium with the thinned peach was better with the first thinned fruits than with the second. The optimal concentration of thinned peach appeared to 1.0% ~ 3.0% with the first thinned fruit and 3% with the second.

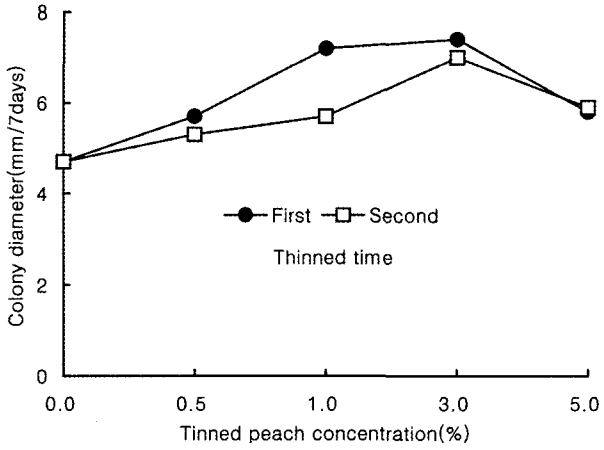

Fig. 3. Effect of thinned peach on mycelial growth of *Pleurotus ostreatus* in solid medium at 25°C for 7 days.

Liquid Culture

To investigate the effects of a shaking culture on the mycelial yield of *Pleurotus ostreatus*, the first and second thinned fruits were mixed with an equal amount of dis-

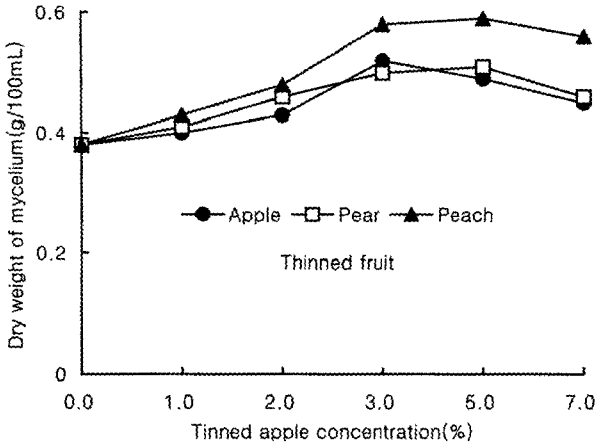

Fig. 4. Effect of thinned fruits on mycelial growth of *Pleurotus ostreatus* in liquid medium at 25°C, 150 rpm for 10 days.

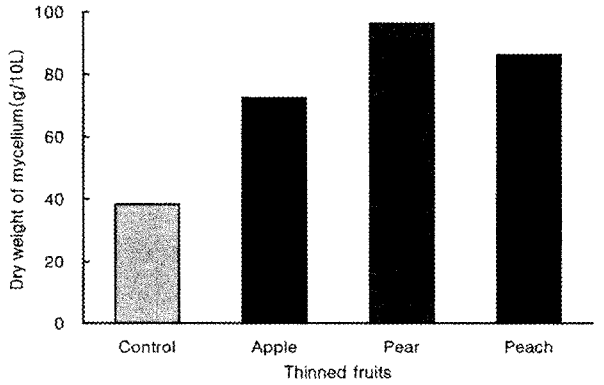

Fig. 5. Mycelial yield of *Pleurotus ostreatus* on pilot scale at 25°C and 6 vvm for 10 days.

tilled water (ratio of 1:1), then the mixture, ranging from 0.5 to 5.0%, was added to the medium and incubated for 7 days in a shaking incubator at 150 rpm.

As shown in Fig. 4, the mycelial yield of *Pleurotus ostreatus* increased when the thinned fruit concentration was increased up to 5.0%, then gradually decreased thereafter. Among the three kinds of thinned fruit, the peach produced the highest dry weight of mycelia. The optimal levels of thinned peach, apple, and pear for a liquid culture of *P. ostreatus* were 5.0%, 3.0%, and 5.0%, respectively, implying that a slightly higher concentration of thinned fruits was required in a shaking culture than in a solid culture for mycelium production.

Jung *et al.* [14] reported that the addition of IAA increased the mycelial growth of *Ganoderma lucidum* 1.2 times, while the addition of GA increased the mycelial growth of *Grifola frondasa* 1.3 times. It has also been reported that the addition of 2,4- dichlorophenoxyacetic acid to *Lentinus edodes* increases the mycelial growth 1.1 times [6]. Consequently, the results with the thinned fruits in the current study are equivalent to those achieved with the above plant growth regulators.

For the mass-production of liquid spawn, *Pleurotus ostreatus* was incubated in a 20-L pilot scale fermenter with 10 L of a liquid medium containing 3% thinned fruit at 25°C and 6 vvm for 10 days. As shown in Fig. 5, the mycelial yield of *Pleurotus ostreatus* in the medium containing thinned fruit was 2 ~ 3 times higher than that in the control. Plus, the pilot culture exhibited much better mycelial growth than the shaking culture, presumably because agitation provided better conditions for oxygen supply than aeration. This result was similar to a submerged mycelial culture of *Lentinus edodes* [6], submerged culture of *Naematoloma sublateritium* mycelia [17], and the growth conditions of a liquid culture of *Pleurotus ostreatus* [15].

The maximum production of *Pleurotus ostreatus* mycelia was achieved with the thinned pear at 96.2 g/10 L, followed by the thinned peach (86.3 g/10 L) and thinned apple (74.2 g/10 L). Furthermore, the current study found that the pilot scale culture of liquid spawn was optimized based on a 3% inoculum, 6 vvm aeration rate, and incubation at 25°C for 10 days.

REFERENCES

[1] Chang, S. T., J. A. Buswel, and S. W. Chiu (1993) *Mushroom Biology and Mushroom Products.* The Chinese University Press, China

[2] Chang, S. T. and W. A. Hayes (1978) *The Biology and Cultivation of Edible Mushroom.* pp. 137-168. Academic Press, NY, USA.

[3] Chang, S. T. and P. G. Miles (1989) *Edible Mushrooms and Their Cultivation.* pp. 27-38. CRC Press, Inc., Florida, USA.

[4] Mori, K., T. Toyomasu, H. Nanba, and H. Kuroda (1989) Anti tumor action of fruit bodies of edible mush rooms orally administered to mice. *Mushroom Sci.* 12: 653-660.

[5] Sung, J. M., H. W. Moon, and D. S. Park (1999) Growth condition of liquid culture by *Pleurotus ostreatus. Kor. J. Mycol.* 27: 1-9.

[6] Lee, B. W., G. H. Im, D. W. Kim, K. M. Park, S. H. Son, and T. H. Shon (1993) Cultural characteristics and pilot scale fermentation for the submerged mycelial culture of *Lentinus edodes. Kor. J. Appl. Microbiol. Biotechnol.* 21: 606-614.

[7] Genshiro, K., K. Hiroe, and O. Katsumich (1996) Effect of liquid mycelial culture used as a spawn on sawdust cultivation of shiitake (*Lentinula edodes*). *Mycoscience* 37: 201-207

[8] Ha, T. M., J. H. Chi, Y. H. Kim, and J. U. Eum (1998) The characteristic of crude extracts from *Phellinus linteus* submerged culture broth with food by-product. *RDA. J. Indus. Crop Sci.* 40: 70-76.

[9] Lee, T. S., N. S. Cho, and D. S. Min (1998) Effect of sawdust culture on oak mushroom, *Lentinula edodes* (Berk.) Pegler by inoculation of the liquid spawn. *Mokchae Konghak* 26: 19-28.

[10] Yang, B. K., Y. J. Jeon, Y. J. Jeong, D. H. Kim, J. Y. Ha, J. W. Yun, D. H., Shon, G. I. Go, and C. H. Song (1999) Hepatoprotective effect of exo-polysaccharide produced

from submerged mycelial culture of *Ganogerma lucidum* WK-003 by using industrial grade medium. *Kor. J. Mycol.* 27: 82-86.

[11] Lee, W. Y., J. K. Ahn, O. W. Kwon, K. H. Ka, and Y. J. Kwon (2002) Submerged culture of *Tricholoma matsutake* mycelium by balloon type air bubble bioreactor. *J. Kor. For. Soc.* 91: 260-267.

[12] Hong, C., B. A. Holtz, D. P. Morgan, and T. J. Michailides (1997) Significance of thinned fruit as a source of the secondary inoculum of *Monilinia fructicola* in California nectarine orchards. *Plant Disease* 81: 519-524

[13] AOAC (1995) *Offical Methods of Analysis,* 16th ed., Association of Official Analytical Chemists, Washington, D.C., USA.

[14] Jung, G. S. (1991) Submerged culture of *Ganoderma lucidum* and *Grifola frondasa* using cheese whey. *Bull. Food Technol.* 4: 23-27.

[15] Sung, J. M., H. W. Moon, and D. S. Park (1999) Growth condition of liquid culture by *Pleurotus ostreatus*. *Kor. J. Mycol.* 27: 1-9.

[16] Kang, M. S., T. S. Kang, A. S. Kang, H. R. Shon, and J. M. Sung (2000) Studies on mycelial growth and artificial cultivation of *Pleurotus eryngii*. *Kor J. Mycol.* 28: 73-80.

[17] Kang, A. S., T. S. Kang, S. M. Cho, and S. H. Yu (2001) Studies on submerged culture and mycelial components of *Naematoloma sublateritium* mycelia. *Kor J. Mycol.* 29: 22-27.

[18] Joung, G. J. (2001) *Food Composition Table*. 6th ed., pp.168-171, R. D. A. National Rural Science Institute, Suwon, Korea.

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