Jour. Korean For. Soc. Vol. 94, No. 5, pp. 330~333 (2005)

JOURNAL OF KOREAN FOREST SOCIETY

Changes of Methyl *trans*-cinnamate Levels During Fruit-body Development in *Tricholoma matsutake*

Wi Young Lee[1], Youngki Park[1]*, Jin Kwon Ahn[1] and Kang Hyeon Ka[2]

[1]*Div. Biotechnology, Korea Forest Research Institute, Suwon 441-350, Korea*
[2]*Div. Wood Chemistry & Microbiology, Korea Forest Research Institute, Seoul 130-712, Korea*

Abstract : The relationship between the contents of methyl *trans*-cinnamate and the ratio of DP/DS (diameter of pileus / diameter of stalk) in the fruit-body of *Tricholoma matsutake* during its development was investigated. The stages of development were divided as follows: stage A is less than 1, stage B is from 1 to less than 2, stage C is from 2 to less than 3, and stage D is more than 3 of the values of DP/DS. The contents of methyl *trans*-cinnamate in pileus and stalk of pine mushroom ranged from 77 μg/g to 824 μg/g and from 7.6 μg/g to 22.4 μg/g, respectively during its development. In the part of pileus, there is no relevance of the methyl *trans*-cinnamate content of pine mushroom between the stage A and B, but there was significantly different among the stage of B, C and D. In the case of stalk, the relevance of the methyl *trans*-cinnamate content of pine mushroom between stage D and other stages showed a low difference. In addition, as pileus of pine mushroom developed the level of the aroma compound increased as well and showed higher correlation relationship (r^2=0.877) between the contents of methyl *trans*-cinnamate in the pileus and the ratio of DP/DS. From the results of this study, we can conclude that the aromatic component of pine mushroom can be deduced from the value of DP/DS, which indicates the stage of the development appearance.

Key words **:** *HPLC analysis,* Tricholoma matsutake, *aromatic compound, methyl trans-cinnamate, pileus*

Introduction

Tricholoma matsutake (called pine mushroom) is one of the most expensive edible mushrooms in Northeast Asia because Korean and Japanese tent to like the mushroom that has a peculiar perfume and chewing taste. It is well known as an ectomycorrhizal fungus. It has been known to produce useful substances, including anti-cancer components and thermostable enzymes (Kawagoe *et al.*, 1999). The host plant for *T. matsutake* are *Pinus densiflora* in Korea (Lee, 1983), *P. densiflora, P. yunnanesis* and *P. massoniana* in China (Wang *et al.*, 1997), and primarily *P. densiflora,* but additionally *Abies, Picea* and *Tsuga* trees in Japan (Ogawa, 1978; Murata *et al.*, 2001). The peculiar perfumes of the fungus are mainly 1-octene-3-ol, methyl cinnamate, 2-octanol and octyl alcohol, and these are make up 96.8% of total aroma components (Ahn and Lee, 1986). Among these compounds, methyl *trans*-cinnamate is a very significant flavor compound in *T. matsutake* (Yajima *et al.*, 1981; Takama *et al.*, 1984; Lee *et al.*, 2002). Generally, the analysis of methyl *trans*-cinnamate was conducted by GC or GC-MS involving complicated pretreatments likely to evaporate, separate or dehydrate procedures (El-Massry *et al.*, 2002; Wood *et al.*, 1996). Therefore, we tried to simplify the analysis of this compound using HPLC system (Lee *et al.*, 2003).

The aim of the present work was to examine the contents of methyl *trans*-cinnamate during the *T. matsutake* development. In this work, we divided the developmental process into four stages according to the ratios between pileus and stalk of the *T. matsutake* mushroom and compared the contents changes of methyl *trans*-cinnamate during fruit-body development by means of HPLC. This study also provides the basic data for the determination of the mushroom grade through the identification between the contents of methyl-trans cinnamate and the development stages of pine mushroom.

*Corresponding author
E-mail: wood0601@hanmail.net

Materials and Methods

1. Materials

The fruiting bodies of *T. matsutake* used in this study were collected from Hongchun in Kangwon province, Korea in October of 2003. All the samples were stored

Table 1. The characteristics of pine mushroom analyzed in the present study.

Stage	Fresh weight (g)	Length (cm)	Diameter of pileus (cm)	Diameter of stalk (cm)	Ratio (DP/DS)[1)]
A	23.3±8.3	6.9±0.9	2.4±0.4	2.5±0.6	0.95±0.1
B	78.3±22.1	10.0±0.6	4.5±0.8	3.0±0.6	1.51±0.1
C	88.0±12.5	12.1±1.0	6.8±0.7	2.8±0.2	2.44±0.4
D	58.0±22.3	11.5±3.1	8.2±0.7	2.0±0.4	4.26±0.8

[1)]DP/DS : diameter of pileus/diameter of stalk.
The values were obtained from triplicated samples (Mean ± SD).

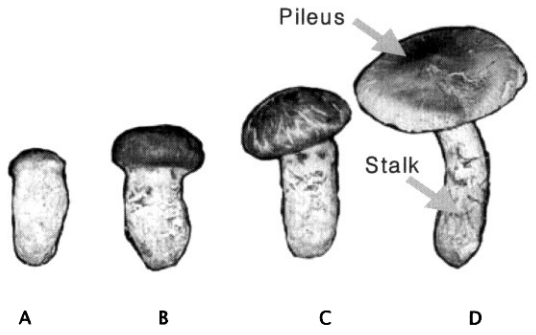

Figure 1. Classification of pine mushroom according to the ratio of the diameter of pileus and the diameter of stalk. The stages are as follows: stage A is less than 1, stage B is from 1 to less than 2, stage C is from 2 to less than 3, and stage D is more than 3 of the values of DP/DS.

in a freezer at -70°C prior to analysis. The samples were divided into four development stages (A, B, C and D) of the fruiting body by the criteria ratios of the diameter of pileus to stalk (DP/DS, Table 1). The stages are as follows: stage A (the diameter of pileus is smaller than that of stalk) is less than 1, stage B (the diameter of pileus is not 2 times higher than that of stalk) is from 1 to less than 2, stage C (the stalk was grew but the indusium was not open) is from 2 to less than 3, and stage D (the indusium was open) is more than 3 of the values of DP/DS. Table 1 and Figure 1 showed the characteristics of mushrooms used in this experiment. Fresh weight of pine mushroom at stage D was about 2.5 times higher than that of stage A. The length of pine mushroom at stage D was 1.6 times higher than that of stage A. These results means that as the fruit-body grew, the fresh weight, length and the diameter of pileus were increased except for the diameter of stalk.

2. Extraction of methyl *trans*-cinnamate

Methyl *trans*-cinnamate was purchased from Sigma Chemical Company (St. Louis, MO, USA) and the solvent used for the extraction of *T. matstake* was analytical grade methanol. The quantity of methyl *trans*-cinnamate were determined using the method of our previous study (Lee *et al.*, 2003). Approximately 0.3 g (dry weight) of the pileus including lamellae and stalk of pine mushroom powder which was crushed using pestle and mortar in the presence of liquid nitrogen was mixed with 10 mL of methanol, sealed with cap tube, then was subjected to sonicate for 1 hr and filtrated with 0.2 μm pore membrane filter.

3. HPLC analysis of methyl *trans*-cinnamate

The filtrates were analysed by HPLC (Thermo Separation Products) using 5 μm LiChrospher 100 RP-18 (26 × 8.0 mm) column. The UV spectra were recorded from 200 to 600 nm and the chromatograms were monitored at 300 nm by UV detector (TSP, spectrum system UV 3000HR). The elution consisted of 65% acetonitrile and 35% water for 15 min and the flow rate was 0.8 $mL \cdot min^{-1}$. Methyl trans-cinnamate from the samples was identified by comparing its retention time and UV spectra with that of authentic sample. The concentration of methyl *trans*-cinnamate was calculated on the basis of the peak area in the chromatogram.

Results and Discussion

1. Content of methyl *trans*-cinnamate

To determine the contents of methyl *trans*-cinnamate in pine mushroom, HPLC was used. Generally, the content of this compound were measured by using GC or GC-MS (Takama *et al.*, 1984; Wood *et al.*, 1996). However, this volatile compound can also be detected by HPLC, since its melting point is 36°C. The advantages of using HPLC for the analysis of this compound may be required of small size of samples, easy treatment for extraction and short analysis time (Lee *et al.*, 2003).

The contents of methyl *trans*-cinnamate of the pileus and stalk of pine mushroom which are at different stages of basidiocarp development were described in Table 2. As shown in Table 2, the content of methyl *trans*-cinnamate was much higher in the pileus than in the stalks. In *Hypsizygus marmoreus*, the contents of amino acids were also higher in the pileus (Harada *et al.*, 2003). In the stage A and B, which indicate that the ratio value of DP/DS is below 2, the contents of methyl *trans*-cinnamate in pileus of pine mushroom were 77 μg/g fresh

Table 2. Methyl *trans*-cinnamate content of pine mushroom in the pileus and stalk of pine mushroom.

Stage	Ratio of DP/ DS	Methyl *trans*-cinnamate (μg/g fresh weight)	
		Pileus	Stalk
A	1 >	77±10.5[1)] c[2)]	7.6±3.9 b[3)]
B	1-2	107±29.2 c	10.8±3.4 b
C	2-3	236±34.2 b	16.2±6.2 ab
D	3 <	824±91.9 a	22.4±5.9 a

[1)]: The values were obtained from triplicated samples (Mean±SD); [2)]: difference at the 1% significance level by Duncan's multiple range test; [3)]: difference at the 5% significance level by Duncan's multiple range test.

weight and 107 μg/g fresh weight, respectively. At stage D, methyl *trans*-cinnamte content of pileus and the stalk of pine mushroom were 10.7 and 2.9 times, respectively, higher than those of stage A. In the part of pileus, there is no relevance of the methyl *trans*-cinnamate content of pine mushroom between the stage A and B (see Table 2). However, the content of methyl *trans*-cinnamate of the pine mushroom in the stage of C and D have significantly different. In the case of stalk, the relevance of the methyl *trans*-cinnamate content of pine mushroom between stage D and other stages showed the difference.

From the results, we can be concluded that the content of methyl *trans*-cinnamate in the pine mushroom was increased during its development.

2. Corelation of methyl *tans*-cinnamate with the stage of development

The level of methyl-*trans* cinnamate increased in the pileus and stalks of pine mushroom during fruit-body development. Figure 2 showed the correlation between the quantity of methyl *trans*-cinnamate in the pileus and the ratio of the diameter of pileus to stalk. The results indicate a strong correlation between pileus and stalk (r^2 = 0.877), suggesting that this component is increased as

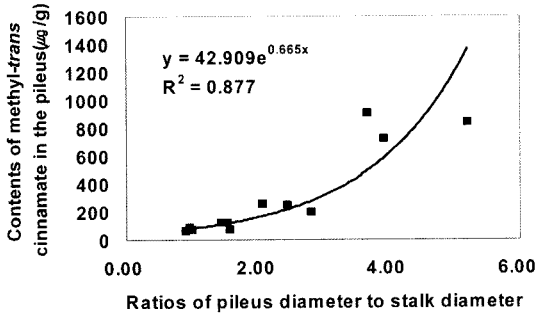

Figure 2. The correlation between the contents of methyl *trans*-cinnamate in the pileus and the ratio of the diameter of pileus to stalk of pine mushroom.

developing the pileus of pine mushroom. However, methyl *trans*-cinnamate level showed tendency not to increase when the ratio of the diameter of pileus to stalk become larger than 4 (in Figure 1). This result indicates that the changes in the level of this compound would contribute to a pleasant flavor of pine mushroom. The results agrees with that of Ohta (1983), who reported the methyl cinnamate level in *T. matsutake* increased with the growth of the fruit body and higher amount was observed in the lamellae and the spores than in the stipe.

Conclusion

In this work, we divided the developmental process into four stages (A, B, C and D) according to the ratios between pileus and stalk diameter of the *T. matsutake* mushroom and compared the contents changes of methyl *trans*-cinnamate during fruit-body development by means of HPLC. The content of methyl *trans*-cinnamate was much higher in the pileus than in the stalks. The level of methyl-*trans* cinnamate increased in the pileus and stalks of pine mushroom during fruit-body development. At the stage D, the pileus and stalks contained 10.7 and 2.9 times more methyl-*trans* cinnamate than in the stage A. The results indicate a strong correlation between pileus and stalk (r^2 = 0.877), suggesting that methyl *trans*-cinnamate is increased as developing the pileus of pine mushroom. In general, the grade of *T. matstake* mushroom has been judged by its morphological characteristics, not by chemical components. Therefore, the four development stages which defined in this paper would be useful for the judgement of the mushroom grade.

Literature Cited

1. Ahn, J.S. and Lee, K.H. 1986. Studies on the volatile components of edible mushroom (*Tricholoma matsutake*) of Korea. Journal of Korean Society of Food and Nutrition 15(3): 253-257.
2. El-Massry, K.A., El-Ghorab, H. and Farouk, A. 2002. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chemistry 79: 331-336.
3. Harada, A., Yoneyama, S., Doi, S. and Aoyama, M. 2003. Changes in contents of free amino acids and soluble carbohydrates during fruit-body development of *Hypsizygus marmoreus*. Food Chemistry 83: 343-347.
4. Kawagoe, M., Kawakami, K., Nakamura, Y., Naoe, K., Miki, K. and Noda, H. 1999. Submerged culture of *Tricholoma matsutake* mycelium in bubble column fermentors. Journal of Bioscience and Bioengineering 87: 116-118.
5. Lee, H.J., Choi, Y.J., Ka, K.H. and Park, W.C. 2002. Extractives of *Tricholoma matsutake* fruiting body. Pro-

ceedings of the Korean Society of Wood Science and Technology Annual Meeting. pp. 133-136.

6. Lee, T.S. 1983. Survey on the environmental conditions at the habitat of *Tricholoma matsutake* in Korea. Mokchae Konghak 11: 37-44.
7. Lee, W.Y., Park, Y.K., Ahn, J.K. and Ka, K.H. 2003. HPLC analysis of methyl trans-ciinamate contents in *Tricholoma matsutake* mushroom in Korea. Journal of Korean Forestry Society 92: 470-474.
8. Murata, T, Takahashi, Y., Horahira, K. and Adachi, Y. 2001. Productivity of matsutake in a natural forest of Todo-fir and environmental improvement for its occurence. Bulletin of the Hokkaido Foresty Research Institute. 38: 1-22.
9. Ogawa, M. 1978. Biology of Matsutake mushroom. Tsukiji Shokan Co., Ltd. pp. 326.
10. Ohta, A. 1983. Quantative analysis of odorous compounds in the fruit bodies of *Tricholoma matsutake.* Trans. Mycol. Soc. Japan 24: 185-190.
11. Takama, F., Ishii, H. and Muraki, S. 1984. Flavor components in Japanese and Korean matsutake-*Tricholoma matsutake* (S. Ito et Imai) Sing. and their change during storage. Japan Society of Food Science and Technology 31: 14-18.
12. Wang, Y., Hall, I.R. and Evans, L.A. 1997. Ectomycorrhizal fungi with edible fruiting bodies 1. *Tricholoma matsutake* and related fungi. Economic Botany 51: 311-327.
13. Wood, W.F., Lancaster, W.C., Fisher, C.O. and Stotler, R.E. 1996. Trans-methyl cinnamate: The major volatile from some populations of the liverwort, *Conoceohalum conicum*. Phytochemistry 42(1): 241-242.
14. Yajima, I., Yanai, T., Nakamura, M., Sakakibara, H. and Hayashi, K. 1981. Volatile flavor compounds of Matsutake -*Tricholoma matsutake* (Ito et Imai) Sing-. Agriculture and Biological Chemistry 45: 373-377.

(Received July 20, 2005; Accepted August 31, 2005)