The Cultivation of *Flammulina velutipes* by Using Sunflower Residues as Mushroom Substrate

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***Abstract*—The present study was conducted to evaluate the feasibility of using sunflower residues, such as stalk (SS) and meal (SM), in substitution for the conventional materials, such as corn cob and rice bran, in the substrate for Enokitake mushroom *Flammulina velutipes*. A total of 9 substrates were prepared according to a 3 by 3 factorial design, where 3 rates (0, 50 and 100%) of replacement of rice bran with SM and 3 rates (0, 50 and 100%) of replacement of corn cob with SS were combined. The combination of 0% SM and 0% SS was used as a control group. The results revealed that the cultivation period, fruiting body yield and biological efficiency were affected by SS and SM significantly. The cultivation period in the SM50-SS0 group was much the same as that in the control group, but those in other groups were extended. Fruiting body yield and Biological Efficiency (BE) were greatest in SM50-SS0 group and smallest in SM100-SS100 significantly ($P<0.05$), and no statistical differences was found among other groups. The eating quality of fruiting bodies was affected neither by SM nor by SS significantly, but a significant interaction was found in umami intensity, umami preference, sweetness intensity and overall impression. In conclusion, the results revealed SM could replace rice bran up to 50% without negative effects on *F. velutipes* productivity, and suggested that a limited part of corn cob can be replaced with SS.**

***Index Terms*—corn cob, eating quality, *Flammulina velutipes*, rice bran, sunflower meal, sunflower stalk**

I. Introduction

Sunflower is one of the famous oilseed crops and the produced in many countries, such as Ukraine, Russia, Argentina, China and France, etc. After harvesting sunflower seed, Sunflower Stalk (SS) is left in the field, and after expressing oil, Sunflower Meal (SM), rich in fat and nitrogen is generated as a by-product [1]. Both of which are considered to be agro-residues: especially, the former has little economic value. Currently, the global efforts are being made to create innovative technologies for utilization of lignocellulosic residue as benefitable materials. In this connection, Reference [2] suggested that using such materials as materials for mushroom cultivation is one of the technologies.

In Japan, most materials used for mushroom substrates are dependent on imports: rice bran used as a nutritional material is domestic, but this is of extensive use, such as a bed for pickling and animal feed. Therefore, it may be important to develop substitute materials for securing of their stable supply. Consequently, it may be interesting to use the sunflower residues as materials for the mushroom substrate, because sunflower cultivation for oil production is getting popular in Japan. In addition, SS has good water absorbency and SM contains vegetable oil (rich in oleic acid), both of which are necessary for a mushroom substrates.

In the present study, *Flammulina velutipes* (*F. velutipes*) was used as an experimental species, because this is one of the most popular cultivated mushrooms in Japan. Generally, this is cultivated on substrates containing mainly imported corn cob as a base material and rice bran as a nutritional material, hence it is worth investigation to know whether corn cob and rice bran in the substitute can be replaced with SS and SM, respectively. We performed cultivation experiment of *F. velutipes* using substrates containing different levels of SS and SM, and evaluated its productivity and eating quality of fruiting bodies.

II. Materials and Methods

A. Substrate Preparation

SS was prepared as follows: the dried stalk of high oleic sunflower grew in Nagano, Japan was obtained and cut to the length of about 7 mm. This SS was used as a substitute for corn cob (CC), a base material. SM was obtained as the solid residue of mechanical oil-expression of the sunflower seeds and used as a substitute for rice bran (RB), a nutritional material. Carbon and nitrogen content as well as their ratio in CC, SS, RB and SM were presented in Table I. A total of 9 substrates were prepared

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according to a 3 by 3 factorial design, where 3 replacement rates (0, 50 and 100%, on DM basis) in the base material and 3 replacement rates (0, 50 and 100%, on DM basis) in the nutritional material. The combination of 0% and 0% was used as a control group (Table II).

TABLE I. THE CHEMICAL COMPOSITION OF INGRIDIENTS

Ingredients[1]	DM (%)	Carbon (C) (%)	Nitrogen (N) (%)	C/N ratio
CC	92.0	43.8	0.3	132.7
SS	90.0	38.5	0.5	82.0
RB	88.0	43.3	2.0	22.1
SM	96.1	48.1	3.3	14.6

[1]CC=corn cob, SS=sunflower stalk, RB=rice bran, SM=sunflower meal.

TABLE II. FORMULATION OF SUBSTRATE IN A BOTTLE[1]

Substrate (DM Basis, %)	Ingredients (g)[2]				C/N ratio
	RB	SM	CC	SS	
Control	63.4	0.0	67.3	0.0	33.1
SM0-SS50	63.4	0.0	33.7	33.2	31.7
SM0-SS100	63.4	0.0	0.0	66.5	30.4
SM50-SS0	31.7	34.6	67.3	0.0	28.2
SM50-SS50	31.7	34.6	33.7	33.2	27.1
SM50-SS100	31.7	34.6	0.0	66.5	26.1
SM100-SS0	0.0	69.2	67.3	0.0	24.8
SM100-SS50	0.0	69.2	33.7	33.2	23.9
SM100-SS100	0.0	69.2	0.0	66.5	23.1

[1]Each bottle contained 19.2g beat pulp, 9.6g cotton seed hull, 9.6g wheat bran, 9.6g sorghum powder, 7.3g oyster shell, and 5.8g dried tofu residue (DM basis).
[2]RB=rice bran, SM=sunflower meal, CC=corn cob, SS=sunflower stalk.

All substrates combination contained the common ingredients (beat pulp, cotton seed hull, wheat bran, sorghum powder, dried tofu residue, and oyster shell) around 61.2g on DM basis. C/N ratio of each substrate was measured using a CHNS/O Elemental Analyzer (2400 Series II, PerkinElmer). The substrates were moistened with water to be the final moisture content of 50-60%, mixed well, and then filled 548g of the substrate into mushroom culture bottles (850ml). The surface of the substrate was covered by 40g of the mixture of corn cob meal and rice bran at 3:1 ration to make it flat. All bottles were autoclaved at 121 ℃ for 2h, cooled to 17 ℃ and then inoculated with 10 g of spawn (*F. velutipes*).

B. Incubation and Cultivation of Mushroom

Mushroom cultivation was performed according to the standard method [3] (Fig. 1). A total of 81 bottles (9 bottles of each group) were incubated at 17 ℃ with relative humidity of 70-80% in dark condition for mycelial running. When 8 out of 9 bottles in each group were fully colonized, the surface mycelial layer was removed to arrange the shape of fruiting bodies, and the bottles were moved to a chamber set at 14 ℃ with a relative humidity of 95% in dark condition to stimulate the primordial formation. After that, the bottles were placed in a chamber kept at 7-9 ℃ with a relative humidity of 90% for acclimatization for three days, and then at 5-7 ℃ with a relative humidity of 95% for fruiting bodies formation. A plastic collar was fitted in the mouth of each bottle to elongate the mushroom stem.

At the harvesting day, they were weighted, and biological efficiency (BE) was calculated: BE is defined as the grams of fruiting body yield (on fresh basis) which produced per 100g of the mushroom substrate (on DM basis).

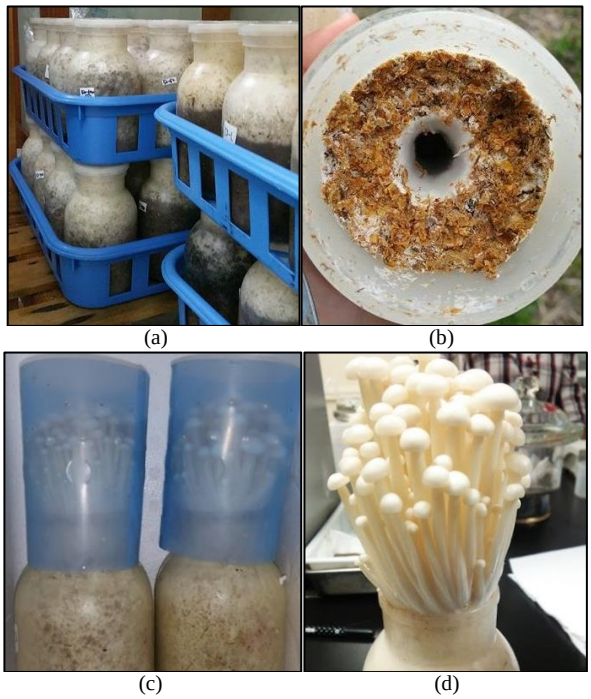

Figure 1. From incubation to harvesting stage of mushroom: a) incubation for running the spawn, b) removing surface mycelial layer, c) a plastic collar was fitted in the mouth of each bottles, d) harvesting stage of *Flammulina velutipes*.

C. Eating Quality of the Fruiting Body

F. velutipes obtained in the present study and the spawn-center of JA Green Nagano (Nagano, Japan) was used in the eating quality test, where latter one considered as standard. Eating quality test was performed according to the method described by Ref. [4] with a slight modification. Briefly, a total of 24 panelists (Faculty of Agriculture, Shinshu University, Japan) ate edible portion of fruiting bodies boiled with water for two minutes, and then examined the following parameters: 1) texture preference, 2) umami intensity, 3) umami preference, 4) bitterness intensity, 5) sweetness intensity, and 6) overall impression. The scoring range was from -2 to 2, where the score 0 meant no difference in the parameter from standard sample.

D. Statistical Analysis

Statistical significances among groups were determined by Tukey's multiple comparison tests at a significance level of 5% after one-way ANOVA. In addition, two-way ANOVA was done to test for main and interaction effects between SM and SS.

III. RESULTS AND DISCUSSION

A. Productivity of Mushroom

Cultivation period decreased in all SM50% groups and increased with increasing rate of SS, whereas fruiting body yield and BE increased in all SM50% groups and decreased with increasing rate of SS: significant

interactions were observed in all parameters (Table III). This suggesting that SM is more suitable nutritional material than rice bran for this mushroom.

The fat content of SM (20.2%) may be one of the factors which stimulate the growth of mushroom. Growth stimulating efficiency of lipid was reported by Ref [5] in the case of *Lentinus edodes*. In addition, Ref. [6] reported that oleic acid enhanced the mycelial growth of *Ganoderma lucidum* compared with linoleic acid, palmitic acid, and stearic acid. Similar result was reported by Ref. [5].This may be an another considerable factor in this study, because of the presence of high oleic acid (86.3% of total fatty acid) in SM.

It is, however, to be noted that all parameters showed deteriorated values in SM100% groups. There may be two reasons for this deterioration: firstly, providing that SM be more nutritious (high carbon and nitrogen contents) than rice bran, over-nutrition may be one of the explanation because it is well known that mushrooms cannot grow well in substrates containing excessive nutrition. In this connection, Ref. [7] found that higher nitrogen content in the substrate resulted in a lower yield of *P. ostreatus* and *P. sajor-caju* mushroom, whereas lower nitrogen content showed the highest yield. Similarly Ref. [8] found lower BE of *P. florida* when nitrogen-rich ingredients were used at higher levels as a substrate supplement. Increased substrate temperature due to the addition of nitrogen-rich supplements may be the reason for the lower yield of mushroom mycelium and basidiocarp [9], [10].

Moreover, the low C/N ratio in SM 100% group may be one of the reasons for the deterioration of mushroom growth. According to, Ref. [11] plant material with a low C/N ratio decayed more rapidly than those with a high ratio, indicating that mycelial growth rate is related to the bioavailability of nitrogen. Similar finding was reported by Ref. [12], who observed a positive correlation between fiurting body yield and C/N ratio in *P. eryngii* and *Agrocybe aegerita*, whereas, BE was negatively correlated with nitrogen content. In contrast, the opposite finding was reported by Ref [13] in this connection.

Secondly, there is a possibility that rice bran contains some specific nutrients necessary for mushroom growth, but SM. Further studies are needed to solve this question.

TABLE III. EFFECTS OF SUNFLOWER RESIDUES SUBSTRATE ON PRODUCTIVITY OF *FLAMMULINA VELUTIPRS*

Substrate	Cultivation period (days)[1]	Fruiting body yield (g)[1]	Biological efficiency (%)[1]
Control	57.22 ±0.15^{a}	212.00 ±4.57^{a}	110.50 ±2.38^{a}
SM0-SS50	57.63 ±0.17^{ab}	194.00 ±6.67^{a}	101.35 ±3.48^{a}
SM0-SS100	65.44 ±0.60^{e}	198.94 ±6.75^{a}	104.17 ±3.53^{a}
SM50-SS0	57.00 ±0.00^{a}	252.44 ±3.36^{b}	129.60 ±1.73^{b}
SM50-SS50	60.00 ±0.00^{cd}	207.33 ±7.82^{a}	106.69 ±4.02^{a}
SM50-SS100	58.00 ±0.41^{ab}	189.33 ±5.37^{a}	97.64 ±2.77^{a}
SM100-SS0	59.33 ±0.47^{bc}	197.44 ±7.02^{a}	99.87 ±3.55^{a}
SM100-SS50	58.78 ±0.40^{abc}	188.22 ±6.90^{a}	95.42 ±3.50^{a}
SM100-SS100	61.78 ±0.66^{d}	142.39 ±9.44^{c}	72.35 ±4.80^{c}
----------Probabilities----------			
SM[2]	**	**	**
SS[3]	**	**	**
SM × SS	**	**	**

[1]Values for each parameter represent a mean ±standard error (n=9).
[2]SM=sunflower meal.
[3]SS=sunflower stalk.
[a-e] Mean within the same column with different superscripts are significantly different (P<0.05).
**P<0.01.

On the other hand, SS affected all parameters adversely, although the effect was immaterial when the replacement rate was 50%. Similar finding was reported by Ref. [2], where the total friuting body yield and BE were decreased with increasing ratios of SS with paddy straw in the cultivation of *Calocybe indica*. Also, Ref [14] observed the longest cultivation period and the lower BE of *P. florida* and *P. djamor* were recorded in SS substrate, comparing with other substrates. In this context, SS has composed mainly of hard husks and spongy pith and has good physical characteristics, such as good water- and air-holding abilities [15], which are essential for a base material and decrease the mortality of young fruiting bodies due to water shortage. Therefore, the adverse effects of SS may probably result from the chemical materials. This finding coincided with the report of Ref. [16] who found lower BE of *P. ostreatus* when corn stem was used as substrate at a high ratio with cotton seed. In contrast, the opposite result was reported by Ref. [17] the using corn stalk as a substrate showed higher BE than corn cob in *P. ostreatus* cultivation.

Fruiting body yield and BE increased in the SM50-SS0 group and decreased in SM100-SS100 significantly, but no statistical difference was found among other groups. However, highest fruiting body yield and BE in the SM50-SS0 group compared with other substrate groups using SM and SS at different ratio may be positively correlated with the cultivation period. According to, Ref. [18] rapidity of spawn running is an indicator of the overall health of the substrate. The overall results of this study suggested that SM and SS could be used as substitutes for rice bran and corn cob, respectively, as long as the replacement rate was kept smaller than 100%.

Although, the reason why the yield and BE increased prominently in the SM50-SS0 group is obscure, but this result is very promising for future study on the use of sunflower residue for mushroom production. Further studies, such as chemical composition of SM and SS, are required.

B. Eating Quality of Fruiting Body

The eating quality of fruiting bodies was presented in Table IV. Neither SM nor SS affected all parameters measured in the present study, but a significant interaction was found in umami intensity, umami preference, sweetness intensity and overall impression. Unfortunately, there was no parameter which showed more improved values than control value, but it is noteworthy that bitterness intensity tended to decrease in most groups, comparing with the control group, because bitterness is the most unpleasant taste of mushroom. Therefore, for clarifying about the effect of SM and SS substrates on eating quality of *F. velutipes*, the analysis of the amino acid compositions are necessary.

TABLE IV. EATING QUALITY TEST OF *FLAMMULINA VELUTIPES*

Substrate	Eating quality parameters[1]					
	Texture preference	Umami intensity	Umami preference	Bitterness intensity	Sweetness intensity	Overall impression
Control	0.12±0.21	0.28±0.22^{a}	0.16±0.23^{abc}	-0.12±0.19	0.20±0.21^{ab}	0.36±0.22^{ab}
SM0-SS50	-0.04±0.19	0.20±0.21^{ab}	0.36±0.21^{ab}	-0.48±0.17	0.12±0.20^{ab}	0.20±0.22^{ab}
SM0-SS100	-0.20±0.13	-0.28±0.22^{ab}	-0.08±0.22^{abc}	-0.08±0.17	0.04±0.21^{ab}	-0.16±0.23^{ab}
SM50-SS0	-0.20±0.21	-0.12±0.20^{ab}	-0.52±0.20^{c}	-0.52±0.19	-0.52±0.20^{b}	-0.36±0.19^{b}
SM50-SS50	-0.08±0.18	0.04±0.20^{ab}	0.16±0.16^{abc}	-0.12±0.20	0.04±0.20^{ab}	0.32±0.18^{ab}
SM50-SS100	0.28±0.17	0.28±0.21^{a}	0.32±0.23^{abc}	-0.36±0.20	0.24±0.16^{ab}	0.40±0.21^{ab}
SM100-SS0	0.24±0.19	0.20±0.21^{ab}	0.44±0.15^{a}	-0.52±0.14	0.40±0.19^{a}	0.72±0.19^{a}
SM100-SS50	-0.12±0.25	-0.68±0.19^{b}	-0.48±0.16^{bc}	-0.44±0.20	-0.52±0.21^{b}	-0.40±0.23^{b}
SM100-SS100	-0.32±0.17	-0.16±0.17^{ab}	0.00±0.15^{abc}	-0.40±0.18	-0.08±0.17^{ab}	0.04±0.19^{ab}
----------Probabilities----------						
SM[2]	NS	NS	NS	NS	NS	NS
SS[3]	NS	NS	NS	NS	NS	NS
SM × SS	NS	*	**	NS	**	**

[1]Values for each parameter represent mean±standard error (n=24).
[2]SM=sunflower meal.
[3]SS=sunflower stalk.
$^{a-c}$ Mean within the same column with different superscripts are significantly different (P<0.05).
**P<0.01.
NS = Non-significant.

IV. CONCLUSION

The results obtained here suggest that SM can be used as a promising alternative of rice bran in the substrate for *F. velutipes* and its recommended replacement rate should be 50%, and the higher replacement rates led to decreased mushroom productivity. In addition, SS can be added to the substrate as an alternative of corn cob, but its replacement rate should be lower than 50%. Eating quality of the mushroom seems to be affected little by both of SM and SS.

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