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Influence of Microbial Inoculants on Feeding Value of Spent *Lentinula edodes* Substrate

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ABSTRACT

Sawdust-based Spent *Lentinula Edodes* Substrate (SLES) is an important agricultural waste resource for its' huge production amount, on the other hand, it is hard to recycling because of the low digestibility. For the purpose of recycling the SLES, a study was conducted to improve the feeding values of SLES via microbial inoculation. The SLES was ensiled with 0.5% (v/w) Lactic Acid Bacteria (LAB, *Lactobacillus plantarum*) or 0.5% (v/w) yeast (*Saccharomyces cerevisiae*) for 15 days. Four treatments were made included 100% SLES (control), 99% SLES +0.5% LAB (T1), 99% SLES +0.5% yeast (T2) and 99% SLES +0.5% LAB +0.5% yeast (T3). Compared with the raw SLES (not fermentation), 100% SLES (control) after ensiling showed higher ($p<0.05$) pH (5.47) and lower lactic acid production. The addition of microbe to the SLES improved most of the physical parameters, fermentation parameters and microbial populations compared to the control experiments. On the other hand, microbial-blending to SLES decreased most of the chemical parameters except for the Crude Protein (CP). Compared to the raw, ensile fermentation would increase the amino acids and microbial inoculants to the SLES could increase the total amount of amino acids further and the most abundant component of essential-amino acid and non-essential amino acid were valine and glutamate, respectively. Among the four ensile treatments, the impact of the addition of 0.5% LAB and 0.5% yeast (T3) on the SLES storage and feeding value was the greatest one ($p<0.05$). In conclusion: Microbial inoculation improved ensiling and feeding values of SLES.

Keywords: Spent *Lentinula Edodes* Substrate, Feeding Value, Microbe Inoculation, Ensiling

1. INTRODUCTION

Lentinula edodes (*Berk.*) *Sing.* was first cultivated 800 years ago in China. It ranks second next to the button mushroom in the world production of mushrooms. Since the late 1980's, China has become the largest producer of *L. edodes* (1.68×10^{10} kg of dried product in 2007). About 5 kg of Spent Mushroom Substrate (SMS) is produced for each kilogram of mushrooms (Williams *et al*., 2001), so the SMS production in China is huge. Spent *Lentinula Edodes* Substrate (SLES), an important agricultural waste and major subgroup of SMS, composed of a substrate mixture of sawdust, wheat bran, corn flour, calcium phosphate and residues of inorganic nutrients and pesticides. The total production of SLES was approximately 8.40×10^{10} kg in 2007. Recently, there has been increasing public concern on the effects of SMS disposal on the environment.

According to recycling methods, SMS might be used for animal feed because of nutritional values in it. SMS contains several bioactive compounds such as polysaccharides and proteins formed during mushroom growth, therefore it could be a potentially value-added product. Previous researches have proved that SMS could be used as animal feed resource (Zhang *et al*., 1995; Suzuki *et al*., 1995; Adamovic *et al*., 1998; Kakkar and Dhanda, 1998; Bae *et al*., 2006; Kim *et al*., 2007). However, as the digestibility of sawdust-based SMS like SLES is much lower than that of cotton/straw based SMS, the sawdust-based SMS should be further processed and improved nutritionally before feeding. Furthermore, SMS is hard to store due to it is wet and putrefactive.

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Biotransformation during microbial ensiling processes can prevent the SMS from putrefaction and also preserve and convert it into economically useful feedstuffs. Direct-fed microbial including yeast and LAB in animal diets have been proved to be beneficial to animal performance (Krehbiel *et al*., 2003) and yeast may have positive effect on the growth of LAB because of facultative anaerobic (Yang *et al*., 2006). Microbial inoculation was also proved efficacious in improving ensiling characteristics of straws (Gao *et al*., 2008), yeast and LAB were also widely used in bioconversion of agricultural organic wastes like cotton waste, straws and corn cobs (Xu *et al*., 2007; 2010; Chu *et al*., 2012), while little is known about the impact of yeast and LAB on ensiling characteristics of SLES. Therefore, the objective of this study was to evaluate the impacts of yeast and LAB inoculants on the ensiling and feeding values of sawdust-based SLES.

2. MATERIALS AND METHODS

2.1. Fermentation of the SLES

Spent *L. Edodes* substrate (SLES) was collected from a local *L. edodes* farmland in Ya′an city, Sichuan province, China. The original mushroom substrate was composed of sawdust (80%), wheat bran (17%), sugar (0.55 %), $CaCO_3$ (1 %), plaster powder (1.45%) on a dry basis. The SLES was air-dried and ground to pass through a 1 mm screen and water was added to make the total humidity be 65%. And then the mixture was treated as following: CK: control, no microbial inoculants (100% SLES); T1: 99.5% SLES + 0.5% (v/w) LAB; T2: 99.5% SLES + 0.5% (v/w) yeast inoculums; T3: 99% SLES + 0.5% (v/w) LAB + 0.5% (v/w) yeast inoculums. Each SMS treatment (approximately 2.5 kg) was sealed in a polyethylene bag (1600×1200×0.1 mm, Tuntian, Beijing, China) which were placed in 2 kg airtight plastic containers and ensiled at room temperature for 15d.

For the microbial inoculants, the yeast (*Sacchromyces cerevisiae*) was grown on YM (Invitrogen) broth at 25°C for 48 h and LAB (*Lactobacillus plantarum*) was cultured on MRS (Invitrogen) broth at 37°C for 24 h. After the treatments (three replicates for each treatment) were ensiled for 15 d, samples were taken from the center of the containers and stored at-20°C for further analysis.

2.2. Physical and Fermentation Parameters

Physical properties of the ferments were observed for fungal growth and for fermentative odor and acidic odor. Five lab-trained evaluators observed the ferments subjectively by a casual observation method. Fungal growth was determined by and acidic odor was based on 5-point scales as follows: 1 = very bad, 2 = bad, 3 = moderate, 4 = good and 5 = very good. The pH was measured using a compound electrode (E-201-C, Shanghai Shengguang Instrument. Co. Ltd. Shanghai, China). Lactic acid was monitored following the method of Shirazinejad and Ismail (2010).

2.3. Microbial Parameters

Microbial analyses of the samples (10 g of sample size) were conducted according to the methods of Horwitz (2005) as follows, total bacterial count was determined on Plate Count Agar (PCA, Invitrogen Corporation, USA) incubated at 30°C for 48 h. LAB were determined on MRS agar (Invitrogen Corporation, USA) incubated at 37°C for 24 h. Yeasts were determined on Yeast-Malt (YM) extract agar (Invitrogen Corporation, USA) incubated at 37°C for 48 h.

2.4. Chemical Analysis

Dry matter was analyzed by drying samples at 65°C for 48 h to constant weight. Crude ash was determined by heating samples at 600°C for 3 h. Ash free Neutral Detergent Fiber (NDF), acid detergent fiber ADF were determined according to the method of Vorlaphim *et al*. (2011). Hemicellulose was calculated as NDF-ADF. Water Soluble Carbohydrates (WSC) was analyzed by the method of Amini (2005). Ether Extract (EE) was determined by the AOAC method using petroleum ether for distillation instead of diethyl ether. Crude protein (CP, N×6.25) was determined by Horwitz (2005) methods and NH_3-N by the method of Fan *et al*. (2008). Amino acid was monitored by an automatic amino acid analyzer (Biochrom 30^+, DKSH and England).

2.5. Statistical Analyses

All the statistical analyses were made by One-Way Analysis of Variance (ANOVA). General contrasts of means among treatments were raw vs. control; control vs. T1, T2 and T3; T1 vs. T2 and T3; T2 vs. T3. Significant differences were detected at $p<0.05$. All the data were analyzed by using the SPSS statistical software version 12.0 (SPSS Inc., Chicago).

3. RESULTS

3.1. Physical and Fermentation Parameters

The SLES was anaerobic fermented with yeast and LAB. Physical parameters of the ferments were analyzed subjectively by five trained evaluators and presented in **Table 1**. After 15 days of ensiling, SLES fermented with both yeast and LAB (T3) had a better fermentative odor score than the raw SLES and other three ferments and

the acidic odor score in T3 was also the highest one among all the treatments. As a result, all the treatments had undergone a desirable fermentation, which was evident by favorable fermentative odor and acidic odor.

Different fermentation parameters changes were caused by inoculated different microbial to the SLES (**Table 1**). As different microbial was added to SLES, pH decreased while the lactic acid increased. And these parameters were most significantly affected by both yeast and LAB inoculation. Among all the treatments, the treatment of T3 gained the lowest pH value and the highest lactic acid content.

3.2. Microbial Parameters

Populations of total bacteria, LAB, yeast and mould in the SLES with different microbial inoculants are shown in **Table 2**. Ferment SLES with single microbial (e.g., yeast, LAB) increased populations of total bacteria, LAB and yeast but decreased the population of mould present. All microbial populations except mould were the highest in T3 treatment which showed the most favorable microbial parameters.

3.3. Chemical Analyses

The chemical compositions of SLES with different fermentation treatments are presented in **Table 3**. Compared with the raw SLES, most of the ensiling treatment increased the amount of Water Soluble Carbohydrates (WSC) except of T3 (which content was 2.58%). Compared to the CK, SLES fermented with yeast and LAB resulted in lower fiber content (NDF, ADF and Hemicellulose) but higher crude protein. The lowest content of crude protein is only 7.43% which emerged in single microorganism fermentation and the content of crude protein in CK slightly higher than single microorganism, while the highest one is 11.2% in the treatment with both yeast and LAB fermentation. The contents of dry matter, crude ash and ether extract were not significantly different among all the four treatments.

The composition and amount of amino acids varied among different fermentation treatments (**Table 4**). Compared to the raw (not fermentation) SLES, any ensile fermentation treatments would increase the amino acids and microbial inoculants to the SLES could increase the total amount of amino acids further. Compared to the CK, microbial inoculants influenced the amino acids composition and amount variously. The total amount of amino acids in T1 was 5.08 g 100^{-1} g of dry SLES, which were 4.71 and 6.09 in T2 and T3 treatments, respectively. The most abundant component of essential-amino acid and non-essential amino acid were valine and glutamate in all treatments. Treatment T3 contained 0.52 g valine 100^{-1} g of dry SLES, while treatment CK, T1 and T2 contained 0.45, 0.47 and 0.47 g valine 100^{-1} g of SLES, respectively. The highest amount of glutamate appeared in T3 treatment was 0.82 g 10$\tilde{0}$1 g of dry SLES, while the least one was in T2 in which 0.71 g 100^{-1} g of dry SLES was detected. The essential-amino acid methionine in CK and non-essential amino acid tyrosine in T1 had the lowest amount of 0.17 and 0.01 g 100^{-1} g of dry SLES, respectively. Additionally, another major component of non-essential amino acid, aspartic, ranged from 0.59 g to 0.79 g/100 g of dry SLES.

Table 1. Physical, fermentation parameters of SLES after fermentation [a]

Items	Raw	CK[b]	T1[b]	T2[b]	T3[b]	SE[b]
Fermentation odor	2.920	3.850	4.170	4.260	4.650	0.24 [f]
Acidic odor	2.520	2.510	2.800	3.100	3.500	0.07 [c,d,e,f]
pH	5.570	5.420	4.800	4.600	4.100	0.12 [c,e,f]
Lactic acid (%)	0.032	0.038	0.211	0.181	0.375	0.01 [d,f]

[a] Based on 5-point scales, 5: very good, 1: Very bad. [b] CK= control, 100% SLES, T1 = 99.5% SLES + 0.5% (v/w) LAB, T2 = 99.5% SLES + 0.5% (v/w) yeast inoculums, T3 = 99% SLES + 0.5% (v/w) LAB + 0.5% (v/w) yeast. SE = Standard error. [c] Raw differs from control ($p < 0.05$). [d] Control differs from T1, T2 and T3 ($p<0.05$). [e] T1 differs from T2 and T3 ($p<0.05$). [f] T2 differs from T3 ($p<0.05$)

Table 2. Microbial populations of the SLES under different fermentation treatments[a]

Items	Raw[b]	CK[b,c]	T1[b,c]	T2[b,c]	T3[b,c]	SE[c]
Lactobacillus	2.36	5.74	7.53	5.66	8.39	0.08 [d,f,g]
Yeast	2.33	5.55	5.58	7.51	8.56	0.05 [d,f,g]
Mould	3.76	5.92	3.71	3.32	2.73	0.04 [d,e,f,g]
Bacteria	4.66	7.38	8.81	7.54	8.72	0.06 [d,e,g]

[a] Wet basis. [b] $\log_{10}$ cfu/g: Colony-forming unit per gram of wet samples. [c] CK= control, 100% SLES, T1= 99.5 % SLES + 0.5% (v/w) LAB, T2 = 99.5% SLES + 0.5% (v/w) yeast inoculums, T3 = 99% SLES + 0.5% (v/w) LAB + 0.5% (v/w) yeast. SE = Standard error. [d] Raw differs from control ($p < 0.05$). [e] Control differs from T1, T2 and T3 ($p< 0.05$). [f] T1 differs from T2 and T3 ($p<0.05$). [g] T2 differs from T3 ($p<0.05$)

Table 3. Chemical composition in SLES with different fermentation treatments[a]

Items	Raw	CK[b]	T1[b]	T2[b]	T3[b]	SE[b]
Neutral detergent fiber (%)	65.6	65.1	63.90	63.40	60.8	1.30
Acid detergent fiber (%)	62.2	62.4	61.50	61.80	59.3	1.05
Hemicellulose (%)	3.4	2.76	2.43	1.64	1.50	0.48 [c]
Dry matter (%)	37.5	37.3	37.20	37.40	37.4	0.41
Crude ash (%)	10.7	10.6	10.40	10.30	10.3	0.22
Crude protein (%)	6.84	8.96	7.43	7.43	11.2	0. 29 [d,f]
Water soluble carbohydrate (%)	3.14	5.27	4.31	4.45	2.58	0.27 [d,f]
Ether extract (%)	1.64	1.63	1.61	1.61	1.62	0.03
NH_3-N (ppm)	234	442	452.00	451.00	398	9.37 [c,f]

[a] Dry basis. [b] CK = Control, 100% SLES, T1 = 99.5% SLES + 0.5% (v/w) LAB, T2 = 99.5% SLES + 0.5% (v/w) yeast inoculums, T3 = 99% SLES + 0.5 % (v/w) LAB + 0.5% (v/w) yeast. SE = Standard error. [c] Raw differs from control ($p<0.05$). [d] Control differs from T1, T2 and T3 ($p<0.05$). [e] T1 differs from T2 and T3 ($p<0.05$). [f] T2 differs from T3 ($p<0.05$)

Table 4. The amino-acid concentration of the SLES under different treatments (g 100^{-1} g DM[a])

Amino acid	Raw	CK[b]	T1[b]	T2[b]	T3[b]	SE[b]
Threonine	0.242	0.295	0.302	0.295	0.364	0.03
Valine	0.390	0.454	0.473	0.478	0.522	0.02
Methionine	0.141	0.182	0.184	0.175	0.234	0.01 [c,d,e,f]
Isoleucine	0.150	0.183	0.195	0.172	0.405	0.01 [d,e,f]
Leucine	0.230	0.293	0.306	0.284	0.236	0.02
Phenylalanine	0.170	0.174	0.172	0.184	0.242	0.01 [d,e,f]
Lysine	0.173	0.194	0.215	0.195	0.324	0.02 [e,f,g]
Total essential amino acids	1.490	1.780	1.850	1.780	2.330	
Aspartic	0.502	0.610	0.635	0.596	0.794	0.02 [c,d,e,f]
Glutamate	0.670	0.711	0.774	0.722	0.825	0.02 [d,f]
Alanine	0.280	0.295	0.343	0.316	0.406	0.01 [d,e,f]
Glycine	0.264	0.314	0.375	0.314	0.425	0.01 [d,e,f]
Serine	0.282	0.324	0.342	0.334	0.403	0.02
Prolinamide	0.241	0.252	0.294	0.264	0.325	0.02
Tyrosine	0.091	0.094	0.099	0.104	0.146	0.01 [d,e,f]
Arginine	0.220	0.245	0.235	0.247	0.245	0.02 [c,d,e,f]
Histidine	0.060	0.088	0.146	0.084	0.172	0.00
L-cysteine	0.030	0.059	0.053	0.050	0.095	0.00 [c,d,e,f]
Total non-essential amino acids	2.640	2.990	3.300	3.030	3.840	
Total amino acids	4.130	4.770	5.150	4.810	6.170	

[a] Dry matter [b] CK = control, 100% SLES, T1 = 99.5% SLES + 0.5% (v/w) LAB, T2 = 99.5% SLES + 0.5% (v/w) yeast inoculums, T3 = 99% SLES + 0.5% (v/w) LAB + 0.5% (v/w) yeast. SE = Standard error. [c] Raw differs from control ($p<0.05$). [d] Control differs from T1, T2 and T3 ($p<0.05$). [e] T1 differs from T2 and T3 ($p<0.05$). [f] T2 differs from T3 ($p<0.05$)

4. DISCUSSION

Biotransformation is a possible way in recycling SMS. Studies showed microbial inoculants would contribute to the SMS digestibility and the well-preserved TMR silage (Kim *et al*., 2011). Winsen *et al*. (2001) reported that after feed fermentation, the important difference between the feed and fermented feed was found in low pH (pH<4.5). Low pH prevents overgrowth of putrefying contaminants. Similar to the previous studies, in our study, the addition of microbe to the SLES changed its physical and fermentation parameters differently. Fermentation of SLES with both yeast and LAB resulted in the lowest pH and highest scores of fermentation odor and acidic odor ($p<0.05$), which indicated desirable fermentation parameters.

Chemical compositions of SMS were one of the main limiting factors for its digestibility. The component of SMS varied significantly among different sources (Adamovic *et al*., 1998; Okano *et al*., 2004; 2006), study the chemical composition of different SMS would contribute to better understand the digestion process of SMS. Okano *et al*. (2004; 2006) reported that NDF, hemicellulose, cellulose and lignin contents for sawdust or corncob meal-based SMS were in the ranges of 64.4-75.2%, 16.6-28.4%, 39.2-44.4% and 4.6-7.9% on Dry Matter (DM) basis. In this study, the levels of fiber components in SLES were similar to those in sawdust

based spent king oyster mushroom substrate (Kwak *et al*., 2008), but higher than those in spent rice straw or spent wheat straw (Adamovic *et al*., 1998; Bisaria *et al*., 1997). Zhang *et al*. (1995) reported that after fermentation by a yeast and a mold, the CP content of *Pleurotus osteratus* spent compost increased significantly from 24.1 to 32.3%. *In vitro* digestibility of the CP was also improved to 70% after the fermentation. Kwak showed that the simultaneous LAB and yeast inoculants and addition of molasses to the SMS improved silage quality (Kwak *et al*., 2008). In present study, similar results were observed, when the SLES was fermented with yeast and LAB, the chemical composition changed variously and the silage quality of SLES was also improved.

Amino acids (lysine) especially the essential-amino acids content have the greatest impact on the rate of protein and fat deposition, so they are the primary considerations when formulating diets for animals. To our knowledge, this might be the first report about the impact of microbial inoculants on SLES amino acid profiles and amounts. The result showed microbial inoculants especially the mixture of yeast and LAB to the SLES significantly affected the amino-acid amount. This was probable due to that yeast were facultative anaerobic, they might have beneficial effects on the growth of LAB by utilizing lactic and organic acids (Yang *et al*., 2006). In the present study, different amino acid profiles were gained under different fermentation treatments. Valine and glutamate were the most abundant component of essential-amino acid and non-essential amino acid in SLES, respectively. Previous study showed that valine and glutamate were also the most abundant amino acid in the *L. edodes* fruiting body (Wang *et al*., 2004), which showed the abundant amino acid in the SLES was very similar to that in the fruiting body of *L. edodes*.

5. CONCLUSION

Impact of microbial inoculants on storage and feeding values of spent *Lentinula edodes* substrate were studied and the physical characteristic, fermentation parameters, microbial populations and chemical components of SLES were significantly affected via microbial inoculants. Amino acid amounts of SLES were improved through microbial inoculation especially with both yeast and LAB and the most abundant component of essential-amino acid and non-essential amino acid in all four ensiling treatments were valine and glutamate, respectively. Microbial inoculants increased the feed values which provided a prospect of utilizing the SLES and further studies should pay much attention to the impact of fermented SLES on animal performance.

6. ACKNOWLEDGEMENT

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