Ekoloji 27(106): 713-720 (2018)

Ecological Research on Flos Sophorae Dregs Fermentation for Animal Feed

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Abstract

In this ecological study, we produce biodiverse animal feed from Flos Sophorae dregs using solid state fermentation process with Rhizopus oryzae and Aspergillus niger. By the orthogonal design method, we analyze the content of crude fiber, crude protein, crude ash from the product, and by multiple regression analysis, we get the result that when the solid state fermentation process with Rhizopus oryzae, the ratio of Flos Sophorae dregs and bran is 2:1 with temperature at 28℃ for 72 hours and the inoculation quantity of Rhizopus oryzae is 15 %, the content of crude fiber is 11.31%, the content of crude protein is 42.7%, the content of crude ash is 5.62%, has reached the third-class standard feed; when the solid state fermentation process with Aspergillus niger, the ratio of Flos Sophorae dregs and bran is 2:1 with temperature at 30℃ for 96 hours and the inoculation quantity of Rhizopus oryzae is 15 %, the content of crude fiber is 9.77%, the content of crude protein is 34.6%, the content of crude ash is 5.21%, has reachedthe secondary standard feed.

Keywords: ecology, Flos Sophorae, Chinese medicine dregs, Rhizopus oryzae, Aspergillus niger

Wu X, Li T, Tao F, Zhao Z, Wang Z, Zhang Q, Zhou T, Cui J, Liu M, Huo Q (2018) Ecological Research on Flos Sophorae Dregs Fermentation for Animal Feed. Ekoloji 27(106): 713-720.

INTRODUCTION

As the ecological industry of traditional Chinese medicine expands, the applications of Chinese herbal medicine and Chinese patent medicine become popular. Large amount of abiotic dregs are created in the process of medicine productions environment, among which Chinese patent medicine produces 70% of the waste (Wu et al. 2015). In China, the emission of botanical dregs is over 600 thousand tons.

These residues usually contain lots of water and some nutritional components which make them easily rotten and cause pollution. Nowadays, the dregs are landfill, incineration or piling up in fixed are, etc. (Shi et al. 2012, Zhang 2013). This massive emission is both pollution and a waste created in environment. Therefore, many researchers are devoted to utilize these ecological dregs in an environmental-friendly way and enter them in food chain of animals.

Flos Sophorae is a dry flower buds of Sophora japonicum L. from the Fabaceae family, One of the main chemical components is rutin, which has anti-inflammatory, anti-viral effect (Ma and Wang 2007). At present, there are over 600 manufacturers producing rutin-containing drug products in China, which emit about 1.6×10^7kg of Flos Sophorae dregs each year, as shown in **Table 1**.

Traditional Chinese medicine dregs contain at least 30% of biotic ingredients, due to the limitations of extraction methods (Guo et al. 2015). At a low cost, microorganism can degrade cellulose, break the cell wall, so that the active and biotic substances remaining in the dregs are released. Because of the natural medical component in animal feed, the disease incidence can be decreased significantly without using antibiotic. In addition, the side products from microbial fermentation are beneficial bacteria which could increase protein (Fan et al. 2010); also, the microbial ecology process of these bacteria produces organic acid and enzymes affecting immune system (He et al. 2014, Li et al. 2017, Wang et al. 2007). From a wider point of view, using Chinese medicine dregs as a sources of animal feed is a way of saving food, can remit the problem of the increase of population and the decrease of land and the shortage of feed-protein Therefore, Chinese medicine dregs is a potential source of animal feed with many ecological advantages.

 Received: 11 May 2017 / Accepted: 10 Sep 2018

Table 1. The annual emissions of Flos Sophorae dregs situation in China

Rutin preparation	Number of enterprises	Annual output of Flos Sophorae dregs × 10^3 kg
Compound Rutin Tablets	69	2000
Troxerutin Injection	46	1500
Troxerutin Tablets	245	5700
Troxerutin oral liquid	118	3200
Rutin Tablets	66	1800
Troxerutin and Glucose Injection	76	2300

Table 2. Factors and levels of orthogonal experimental design

Levels	Factors			
	A Substrate ratio FSD:WB	B Temperature ℃	C Time h	D Inoculation quantity %
1	1:1	28	48	10
2	3:2	29	72	15
3	2:1	30	96	20

In this paper, we will produce animal feed by Flos Sophorae dregs using solid state fermentation. The production process is designed and optimized by the orthogonal design method. Fiber, protein and fat will be weighted and decomposed to accessible nutrition for animals.

EXPERIMENTAL

Materials

Analytical methods

Microorganisms and Culture Conditions

Rhizopus oryzae accession number 3.819 and Aspergillus niger accession number 3.879 were obtained from the China General Microbiological Culture Collection Center (CGMCC). The primary culture for Rhizopus oryzae (Aspergillus niger) was prepared by inoculating the Rhizopus oryzae (Aspergillus niger) strain to Potato Dextrose Agar (PDA) solid medium and culturing it at 28℃ for 5-7 days. PDA solid medium was composed of potato 200g, dextrose 20g, agar 15~20g, and water 1000mL, and pH=6.8-7.0. The spore suspension was prepared by gentle pipetting under strict sterile conditions and the number of shed spores was determined. The concentration of the Rhizopus oryzae (Aspergillus niger) spore suspension was adjusted to (0.25-1.00)×10^8 spores/mL (Yuan and Yan 2017).

Biomass Materials and Pretreatment

Wheat bran (1-3 mm in size) was collected in its original state from local pet markets at Yuquan Road, Beijing. Flos Sophorae dregs (FSD) were obtained by students from the Biochemical Engineering College of Beijing Union University via the preparation of rutin from Flos Sophorae. Flos Sophorae were boiled for 30 minutes in water that was 2-3 times the Flos Sophorae' weight and then boiled two additional times, each time for 15 minutes. The filtrate contained a large amount of rutin, the filter residue was Flos Sophorae dregs. Then the dregs were filtered while they were still hot and the solids were rinsed twice in hot tap water, filtrated until they were dry, and finally dregs placed in the oven for dry, the temperature 105 ± 2℃, dried for 3 hours.

Animal Feed Production by Solid State Fermentation

Animal feed was produced through solid-state fermentation (SSF) in a 250mL Erlenmeyer flask containing the Flos Sophorae dregs medium, which was prepared by adding 30g of a mixture of wheat bran (WB) and the pretreated Flos Sophorae dregs (FSD), with FSD:WB = 1:1 - 2:1, 0.6% corn flour, 0.6% ammonium chloride, 0.4% potassium dihydrogen phosphate, and sterile water (in an amount that was equal to the mixture). The Flos Sophorae dregs medium had a natural pH level and was autoclaved at 121℃ for 30min. The Rhizopus oryzae (Aspergillus niger) primary cell culture was added to the Flos Sophorae dregs medium at a volume of 10~20mL culture / 100g Flos Sophorae dregs, fermented at 25~30℃ for 48~96h, and shaken uniformly every 12h. The contents of crude fiber, crude protein and crude fat were determined respectively after the fermentation was completed (Feng et al. 2017).

The process of Flos Sophorae dregs animal feed preparation is illustrated in **Fig. 1**. The process parameters for dregs fermentation were optimized based on the orthogonal experiment design as shown in **Table 2**.

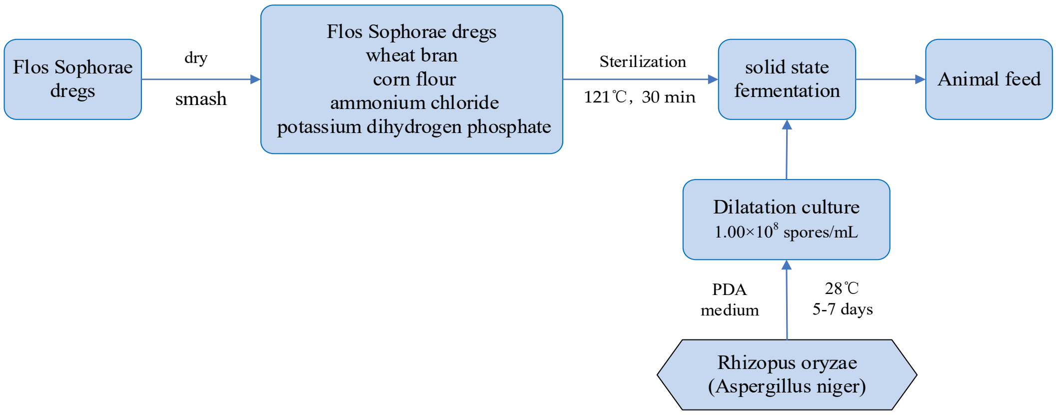

Fig. 1. Manufacturing process of Flos Sophorae dregs fermentation for animal feed

Crude Fiber Determination

Crude fiber content (%) was detected by Tupu Yunnong Crude Fiber Detector (F800, China, Shanghai), the principle of the device was based on using acid and alkali digestion samples, then removed the soluble substances with ethanol; after high temperature burning, deducted the amount of minerals, then obtained the crude fiber content of the sample.

$$Crude\ fiber\ content\ (\%) = \frac{m_1 - m_2}{m} \times 100 \qquad (1)$$

where: m_1 ----- after 130°C drying 4 hours, the crucible and sample residue weight;

m_2 ----- after 500°C burning 2 hours, the crucible and the sample residue weight;

m ------ the sample (not degreasing) weight.

Crude Protein Determination

Crude protein content (%) was determined by Kjeldahl-method, the equipment was HUAYE - SLO-6, China, Shanghai. Kjeldahl-method was based on the principle of measuring the content of nitrogen in the sample to calculate the protein content, the percentage crude protein content was calculated as follows:

$$Crude\ protein\ content\ (\%) = \frac{(V - V_O)C_B \times 0.0140 \times 6.25}{m} \times 100 \qquad (2)$$

where: V ----- volume of HCl consumed in sample, mL;

V_O ----- volume of HCl consumed in blank titration, mL;

C_B ----- normality of HCl, mol/L;

m ----- weight of sample, g;

0.0140 - The molar mass of nitrogen, kg/mol; Crude protein is the amount of nitrogen multiplied by the factor 6.25.

Crude Ash Determination

The percentage of crude ash content was determined by using furnace apparatus. The crucibles with samples were placed in muffle furnace and waited till the temperature reached 550± 5°C for 5hr, till all the organic matter was oxidized and lost as CO_2, the residual represented inorganic constituents of total ash while the loss in weight was taken as the organic matter. the percentage crude ash content was calculated as follows:

$$Crude\ ash\ content\ (\%) = \frac{m_2 - m_0}{m_1 - m_0} \times 100 \qquad (3)$$

where: m_0 ----- weight of empty crucible;

m_1 ----- weight of crucible plus sample;

m_2 ----- weight of crucible plus sample after ash.

RESULTS AND DISCUSSION

The Properties of Flos Sophorae Dregs

Flos Sophorae is a commonly used traditional Chinese medicine that is derived from the Fabaceae family (Dong and Li 2001). The active ingredient is rutin, triterpenoid saponins, betulin, sophoradiol and glucuronic acid. Every 100 grams of fresh Flos Sophorae contain water 78g, protein 3.1g, fat 0.7g, carbohydrates15g, calcium 8.3mg, phosphorus 69mg, iron 3.6mg, carotene 0.04 mg, vitamin B_1 0.04mg, vitamin B_2 0.18 mg, niacin 6.6mg, vitamin C 66mg. Because of its rich nutrition content, Flos Sophorae is not only used as a raw material for extracting rutin in the field of medicine, but also often used in people's diet. In the face of a large number of Flos Sophorae Dregs, we hope that these solid waste can be reused again. GB 6432-6439 "Quality indicators and grading standards for rice bran feed" See **Table 3**. Every 100 grams of Flos Sophorae Dregs contain crude fiber 22.76g, crude protein 20.7g, crude ash 7.40g.

Table 3. Quality indicators and grading standards for rice bran feed

	First-class	Second-class	Third class
Crude Protein (%)	≥13.0	≥12.0	≥11.0
Crude Fiber (%)	<8.0	<10.0	<12.0
Crude ash (%)	<8.0	<9.0	<10.0

Table 4. The crude fiber result of orthogonal design for optimization of Rhizopus oryzae fermentation process

Level	Factors				Crude fiber %
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	17.967
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	17.599
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	18.737
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	12.570
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	18.237
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	11.434
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	12.783
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	12.641
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	11.767
K1	54.303	43.320	42.042	47.971	
K2	42.241	48.477	41.936	41.816	
K3	37.191	41.938	49.757	43.948	
Range	17.112	6.539	7.821	6.155	

Table 5. The crude protein result of orthogonal design for optimization of Rhizopus oryzae fermentation process

Level	Factors				Crude protein %
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	22.4
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	29.8
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	20.8
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	21.3
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	24.8
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	21.5
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	26.2
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	41.1
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	34.3
K1	73.0	69.9	85.0	81.5	
K2	67.6	95.7	85.4	77.5	
K3	101.6	76.6	71.8	83.2	
Range	34.0	25.8	13.6	5.7	

Table 6. The crude ash result of orthogonal design for optimization of Rhizopus oryzae fermentation process

Level	Factors				Crude ash%
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	9.394
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	10.573
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	14.135
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	9.022
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	8.520
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	7.170
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	7.818
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	6.063
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	6.611
K1	34.102	26.234	22.627	24.525	
K2	24.712	25.156	26.206	25.561	
K3	20.492	27.916	30.473	29.220	
Range	13.610	2.760	7.846	4.695	

Fermentation of Flos Sophorae Dregs by Rhizopus Oryzae to Production Animal Feed

Orthogonal experiment design is one way to qualitatively analyze the correlations among the relevant variables at different levels through designing an orthogonal table and statistical analysis (Liu et al. 2010). It can be utilized for the optimization of various process such as substrate ratio of FSD:WB, fermentation temperature, fermentation time and inoculum quantity (Gong et al. 2008). According to the crude fiber, crude protein and crude ash, the results were obtained as shown in **Table 4-6**, we quantitatively analyzed the relationship between the parameters and levels of the fermentation process by Rhizopus oryzae.

Table 7. The crude fiber result of orthogonal design for optimization of Aspergillus niger fermentation process

Level	Factors				Crude fiber%
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	13.092
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	10.621
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	10.094
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	12.165
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	14.273
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	11.259
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	10.967
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	11.420
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	11.079
K1	33.807	36.224	35.771	38.444	
K2	37.697	36.314	33.865	32.847	
K3	33.466	32.432	35.334	33.679	
Range	4.231	3.882	1.906	5.597	

Table 8. The crude protein result of orthogonal design for optimization of Aspergillus niger fermentation process

Level	Factors				Crude protein %
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	24.2
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	28.7
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	26.0
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	24.4
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	24.6
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	19.8
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	33.1
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	28.2
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	29.7
K1	78.900	81.700	72.200	78.500	
K2	68.800	81.500	82.800	81.600	
K3	91.000	75.500	83.700	78.600	
Range	12.1	6.2	11.5	3.1	

On the basis of crude fiber result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: A>C>B>D. The optimum process parameter was $A_3B_3C_2D_2$, which means substrate ratio of FSD:WB = 2:1, temperature 30°C, time 72h and inoculum quantity 15%.

On the basis of crude protein result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: A> B > C >D. The optimum process parameter was $A_3B_2C_2D_3$, which means substrate ratio of FSD:WB = 2:1, temperature 28°C, time 72h and inoculum quantity 20%.

On the basis of crude ash result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: A> C > D > B. The optimum process parameter was $A_3B_2C_1D_1$, which means substrate ratio of FSD:WB = 2:1, temperature 28℃, time 48h and inoculum quantity 10%.

Fermentation of Flos Sophorae Dregs by Aspergillus Niger to Production Animal Feed

Just as in the above fermentative use of Rhizopus oryzae, we produce animal feed from Flos Sophorae dregs using solid state fermentation process with Aspergillus niger. According to the crude fiber, crude protein and crude ash, the results were obtained as shown in **Table 7-9**, we quantitatively analyzed the relationship between the parameters and levels of the fermentation process by Aspergillus niger.

Table 9. The crude ash result of orthogonal design for optimization of Aspergillus niger fermentation process

Level	Factors				Crude ash %
	A	B	C	D	
1	1:1 (A1)	26 (B1)	48 (C1)	10 (D1)	9.305
2	1:1 (A1)	28 (B2)	72 (C2)	15 (D2)	5.769
3	1:1 (A1)	30 (B3)	96 (C3)	20 (D3)	6.084
4	3:2 (A2)	26 (B1)	72 (C2)	20 (D3)	5.443
5	3:2 (A2)	28 (B2)	96 (C3)	10 (D1)	4.756
6	3:2 (A2)	30 (B3)	48 (C1)	15 (D2)	5.807
7	2:1 (A3)	26 (B1)	96 (C3)	15 (D2)	5.301
8	2:1 (A3)	28 (B2)	48 (C1)	20 (D3)	8.288
9	2:1 (A3)	30 (B3)	72 (C2)	10 (D1)	5.109
K1	7.053	6.683	7.800	6.390	
K2	5.335	6.271	5.440	5.626	
K3	6.233	5.667	5.380	6.605	
Range	1.718	1.016	2.420	0.979	

Table 10. Fermentation of Flos Sophorae Dregs by The Optimal Process

	Substrate ratio FSD:WB	Temperature℃	Timeh	Inoculation quantity%	Crude fiber%	Crude protein%	Crude ash%
Rhizopus oryzae	2:1	28	72	15	11.31	42.7	5.62
Aspergillus niger	2:1	30	96	15	9.77	34.6	5.21
Flos Sophorae Dregs	-	-	-	-	22.76	20.7	7.40

On the basis of crude fiber result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: D > A > B > C. The optimum process parameter was $A_3B_3C_2D_2$, which means substrate ratio of FSD:WB = 2:1, temperature 30℃, time 72h and inoculum quantity 15%.

On the basis of crude protein result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: A> C > B >D. The optimum process parameter was $A_3B_1C_3D_2$, which means substrate ratio of FSD:WB = 2:1, temperature 26℃, time 96h and inoculum quantity 15%.

On the basis of crude ash result analysis, the sequence of effects on composite grade to evaluate the fermentation process was: C > A > B > D. The optimum process parameter was $A_2B_3C_3D_2$, which means substrate ratio of FSD:WB = 3:2, temperature 30℃, time 96h and inoculum quantity 15%.

Fermentation of Flos Sophorae Dregs by The Optimal Process

In accordance with the optimal process parameters, Flos Sophorae Dregs were fermented three times by Rhizopus oryzae or Aspergillus niger, the results were shown in **Table 10**.

The grade standard of the fermentation product of Flos Sophorae Dregs is guided by GB 6432-6439 "Quality index and classification standard of rice bran for feed" (**Table 3**). Compared with the quality of Flos Sophorae Dregs, we can find that the crude protein content of Flos Sophorae dregs is 20.7%, reach the first-class standards, so the dregs has very high utilization value. The content of crude fiber is 22.76%, does not reach the third class, high fiber affects taste and is not conducive to digestion and absorption. We use solid state biological fermentation to treat dregs, the main purpose is to reduce the crude fiber content and further improve the crude protein content. We choose Rhizopus oryzae and Aspergillus niger which are easy to grow in the dregs and the culture conditions are simple (Ai et al. 2012, Wang et al. 2008).

In the research of using Rhizopus oryzae fermented dregs, the crude fiber content of nine experimental groups show that crude fiber content is reduced after fermentation (**Table 4**); while, the crude protein content of nine experimental groups show that crude protein content have a small amount of increase after fermentation (**Table 5**). Comprehensive analysis of **Table 4-6**, the most influential factor is substrate ratio of FSD:WB, the impact of inoculation quantity is the smallest; Based on the results obtained, the optimum composition was $A_3B_2C_2D_2$, the optimal process parameters were summarized as shown in **Table 10**.

In the research of using Aspergillus niger fermented dregs, the crude fiber content of nine experimental groups show that crude fiber content is reduced after fermentation (**Table 7**), compared with the data from Rhizopus oryzae fermentation (**Table 4**), the crude fiber content is reduced even more after fermentation with Aspergillus niger. The crude protein content of nine experimental groups show that crude protein content have a small amount of increase after fermentation (**Table 8**), compared with the data from

Rhizopus oryzae fermentation (**Table 5**), the crude protein content is basically the same. Comprehensive analysis of **Table 7-9**, the quantitative analysis of different experimental results, the biggest influencing factors are different, consider the purpose of the study is mainly to reduce the crude fiber content of dregs, so the most influential factors on the crude fiber content is the inoculum quantity (**Table 7**). In addition, statistical analysis of the three results obtained the best process conditions, inoculation quantity is the same which is D2, 15%. Based on the results obtained, the optimum composition was $A_3B_3C_3D_2$, the optimal process parameters were summarized as shown in **Table 10**.

Comparing the data in **Table 10**, it can be seen that in Rhizopus oryzae fermentation experiment group: crude fiber content decreased 50.3%; crude protein content increased 106.3%; crude ash content decreased 24.1%; in Aspergillus niger fermentation experiment group: crude fiber content decreased 57.1%; crude protein content increased 67.1%; crude ash content decreased 29.6%. Utilizing Rhizopus oryzae fermented Flos Sophorae dregs, crude protein and crude ash has reached the first-class standard, and crude fiber has reached the third-class standard; Utilizing Aspergillus niger fermented Flos Sophorae dregs, crude protein and crude ash has reached the first-class standard, and crude fiber has reached the secondary standard.

CONCLUSION

In this ecological research, we produce animal feed from Flos Sophorae dregs using solid state fermentation process with Rhizopus oryzae and Aspergillus niger, in Rhizopus oryzae fermentation experiment group: crude fiber content decreased 50.3%; crude protein content increased 106.3%; crude ash content decreased 24.1%; in Aspergillus niger fermentation experiment group: crude fiber content decreased 57.1%; crude protein content increased 67.1%; crude ash content decreased 29.6%.

Utilizing Rhizopus oryzae fermented Flos Sophorae dregs, crude protein and crude ash has reached the first-class standard, and crude fiber has reached the third-class standard; Utilizing Aspergillus niger fermented Flos Sophorae dregs, crude protein and crude ash has reached the first-class standard, and crude fiber has reached the secondary standard. This study can not only bring considerable economic benefits to medicine factory by utilizing dregs, but also ecologically improve air, water quality and living environment, and open up new fields for the sustainable development of pharmaceutical enterprises.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (Grants No. 11475020 and 8151114029), Chaoyang District collaborative innovation project (Grant No. XC1608), Beijing Union University Graduate Program.

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