



## Optimization of medium composition for soluble dietary fibre production by *Ganoderma lucidum* using lotus root pomace

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### ABSTRACT

Statistical experimental designs were applied for the optimization of medium constituents for soluble dietary fiber (SDF) production by *Ganoderma lucidum* in solid state fermentation (SSF) using lotus root pomace (LRP) as the substrate. Factors including wheat bran, MgSO<sub>4</sub>, FeSO<sub>4</sub> and VB<sub>1</sub> were identified by Plackett-Burman design (PBD) as significant for SDF production. The path of steepest ascent design (SAD) was undertaken to determine the optimal region of four significant factors. To determine the optimal values of the significant variables, Box-Behnken design (BBD) and response surface analysis were employed. The optimum conditions were found to be the 5g LRP, 1.77 g wheat bran, 0.029 g/L MgSO<sub>4</sub>, 0.011 g/L FeSO<sub>4</sub> and 0.052 g/L VB<sub>1</sub>. SDF production at these optimum conditions was 0.113 g/g DW substrate. Compositional analysis showed that SDF was composed of rhamnose, xylose, mannose, galactose and glucose in a molar ratio of 1.2:1.0:1.6:7.2:56.9.

**Key words:** Box-Behnken design, *Ganoderma lucidum*, Lotus root pomace, Plackett-Burman design, Soluble dietary fiber

Dietary fiber (DF), a non-starch polysaccharide indigestible in the small intestine but fermentable in the colon, has received much attention due to its health benefits (Coleman *et al.* 2013, Trinidad *et al.* 2006, Walker 2013). DF is divided into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) according to its water solubility (Theuwissen and Mensink 2008). Although IDF has some physiological effects such as increasing the fecal bulk and reducing the gastrointestinal transit time (Goni and Martin Carron 1998), SDF has more important functions such as reducing the risk of cardiovascular disease, lowering blood serum cholesterol and promoting gut microflora (Isken *et al.* 2010, Jenkins *et al.* 2000). Therefore, the proportion of SDF in DF determines the quality of DF (Chawla and Patil 2010) and it is very meaningful that converting IDF into SDF. At present, the method of chemical treatment is used for the conversion of IDF into SDF, which is not only unsafe but also helpless for the increase of sensorial properties of the final product (Esposito *et al.* 2005). So, enzymes produced by microbe are used for solving these problems. For instance, enzymes produced by selected yeast strains and *Trichoderma* species are used to obtain a

significant increase of SDF (Moore *et al.* 2007, Napolitano *et al.* 2006).

Lotus root (*Nelumbo nucifera* Gaertn.) is a well-known aquatic vegetable in China and its total annual output more than 2 megaton. Nevertheless, about 1.2 megaton lotus root pomace (LRP) is generated during the processing per year (Guan *et al.* 2010). These LRP represent a major disposal problem for the industry, intended as an animal feed or disposed to the soil as a waste. Therefore, an eco-friendly and economically sustainable technology is needed for the management of the LRP. Our preliminary study showed that LRP contained large amounts of DF, besides starch, reducing sugars and vitamin, but disappointingly, IDF account for substantial majority in DF. *Ganoderma lucidum* (Fr.) Krast (Polyporaceae), a fungus named “Lingzhi” in China, is a famous Traditional Chinese Medicine for curing a series of diseases (Hsieh and Yang 2004), has been shown to be an efficient converter compared with *Hydnoid hymenium* and *Oyster mushroom* in our preliminary study (data not shown). Hence an attempt is made in this paper to conversion of IDF into SDF by *Ganoderma lucidum* using LRP as a major substrate.

Plackett-Burman design (PBD), Steepest ascent design (SAD) and Response Surface Methodology (RSM) have been successfully combined applied in medium composition (Feng *et al.* 2011, Liu *et al.* 2011, Meng *et al.* 2012).

The objectives of this study were application of PBD, SAD and BBD to optimize the medium composition in production of SDF from *Ganoderma lucidum* via SSF using

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LRP as the major substrates.

## MATERIALS AND METHODS

*G. lucidum* was obtained from the Institute of Edible Fungus of Jiangsu Academy of Agricultural Sciences (Nanjing, China). The stock culture was maintained on potato dextrose agar (PDA) slants and was sub-cultured every month, and the slants were incubated at 28°C for 6 d and stored at 4°C. *G. lucidum* was initially grown on PDA medium in a petri dish, and was then transferred to the seed culture medium by punching out 4 pieces of the agar plate (4 mm×4 mm) culture with a sterilized self-design cutter. The seed culture was grown in a 250 ml Erlenmeyer flask containing 50 ml of liquid potato dextrose medium at 28°C for 5 d.

The experiments of SSF were performed in 250 ml Erlenmeyer flasks with 5 g LRP moistened with 12.5 ml distilled water as the basal medium. The fermentation substrate was composed of 5 g LRP and nutritional additions (Table 1 and 2). The metal ions were added as culture solution of different concentrations at the solid-liquid ratio of 2.5 (data obtained from a previous experiment, not shown here). The substrate was sterilized at 121°C for 20 min. After cooling, 10 ml seed culture of *G. lucidum* was added to the substrate and was incubated at 28°C for 7 d.

Eight variables in SSF substrates were screened by PBD to select the significant ones affecting SDF production. Four significant variables selected by PBD were further optimized by SAD to search their optimum range of concentrations. Based on the results of SAD experiment, the concentrations of significant variables were further optimized by BBD (Table 4).

The SSF sample was well ground and extracted in distilled water at 90°C for 30 min. The suspension was then centrifuged at 10 000 rpm for 15 min and the supernatant was mixed with four volumes of anhydrous ethanol, stirred vigorously and kept overnight at 4°C. The precipitate from the anhydrous ethanol dispersion was collected by filtration, washed sequentially with ethanol (80%, v/v) and acetone, and dried at 105°C to a constant weight to afford SDF product (Selvendran and Susan Pont 1980).

To determine the monosaccharide composition of SDF,

5 mg SDF was hydrolyzed with 2 ml of 2 M trifluoroacetic acid (TFA) at 120°C for 2 h. The hydrolyzate was repeatedly co-concentrated with methanol to dryness and converted to its trimethylsilyl (TMS) derivative. The TMS derivative of hydrolyzate was prepared by adding 1 ml pyridine, 0.4 ml hexamethyldisilazane and 0.2 ml trimethylchlorosilane and by heating at 80°C for 30 min. The TMS derivatives of standard sugars were prepared in the same way (Larre Larrouy *et al.* 2003). After cooling, samples were analyzed on HP 6890 GC equipped with flame ionization detector (FID) and a HP-5 fused silica capillary column (30 m×0.32 mm×0.25 mm).

All experiments were carried out in triplicate and the average of SDF production was taken as the responsive value. Analysis of variance (ANOVA), regression analysis was done and contour plots were drawn by the statistical software package, Design-Expert 7.0 (Stat-Ease, Inc., Minneapolis, MN, USA).

## RESULTS AND DISCUSSION

### Pre-screening of substrates for SDF production in SSF

Our preliminary study (data not shown) showed when LRP was used as the sole substrate, *G. lucidum* could not grow well and the final SDF production was 0.035g/g DW. The low SDF production indicated that other ingredients should be added to the substrate. Table 2 presents Wheat bran, MgSO<sub>4</sub>, FeSO<sub>4</sub> and VB<sub>1</sub> were the significant variables affecting SDF production. The polynomial model describing SDF production established by regression could be presented as:

$$SDF=0.054 + 0.013 X_1 - 0.011X_7 + 0.008X_8 \quad (1)$$

where  $X_1$ ,  $X_5$ ,  $X_7$  and  $X_8$  were actual values of wheat bran, MgSO<sub>4</sub>, FeSO<sub>4</sub> and VB<sub>1</sub>, respectively.

The *F*-value and *P*-value of the model was 19.38 and 0.0166 respectively, and the determination coefficient *R*<sup>2</sup> was 0.9810. These results indicated a good prediction of the model.

Among the significant variables, wheat bran served as a supplementary substrate to LRP for high SDF production. The small porosity of LRP leaves insufficient space for air circulation, and the big porosity of wheat bran helps solve this problem. The enhancing effect was also utilized previous studies (Hsieh and Yang 2004, Yang *et al.* 2003). Based on the results obtained from PBD, Mg<sup>2+</sup> and Fe<sup>2+</sup> were found to have significant negative effects on SDF production. This finding was consistent with the result by Weinberg *et al.* (Weinberg 1978). VB<sub>1</sub> was also a significant positive variable affecting SDF production. This was probably because vitamins could produce a growth response at very low concentration and had a typical catalytic function in the cell as coenzymes or constituents of coenzymes (Chen *et al.* 2008).

### Optimization of SDF production in SSF

Table 3 evident that the highest SDF production was

Table 1 Experimental range of eight variables at two levels in the PBD

Variables	Units	Symbol code	Experimental value	
			Low	High
Wheat bran	g	$X_1$	0	1.50
Soybean mealy	g	$X_2$	0	0.75
Rice bran	g	$X_3$	0	1.50
KH <sub>2</sub> PO <sub>4</sub>	g/L	$X_4$	0	1.00
MgSO <sub>4</sub>	g/L	$X_5$	0	0.10
CaCl <sub>2</sub>	g/L	$X_6$	0	0.05
FeSO <sub>4</sub>	g/L	$X_7$	0	0.04
VB <sub>1</sub>	g/L	$X_8$	0	0.05

Table 2 PBD matrix for eight variables with actual value of SDF production

Trials	Variables screened by PBD								SDF (g/g DW)
	X <sub>1</sub> ⊙~	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub> ⊙~	X <sub>6</sub>	X <sub>7</sub> ⊙~	X <sub>8</sub> ⊙~	
1	1	1	1	-1	-1	-1	1	-1	0.048
2	1	1	-1	-1	-1	1	-1	1	0.020
3	1	-1	1	1	-1	1	1	1	0.087
4	-1	-1	-1	-1	-1	-1	-1	-1	0.044
5	1	-1	-1	-1	1	-1	1	1	0.015
6	-1	-1	-1	1	-1	1	1	-1	0.023
7	-1	1	1	1	-1	-1	-1	1	0.038
8	1	-1	1	1	1	-1	-1	-1	0.112
9	-1	1	1	-1	1	1	1	-1	0.046
10	-1	-1	1	-1	1	1	-1	1	0.077
11	-1	1	-1	1	1	-1	1	1	0.069
12	1	1	-1	1	1	1	-1	-1	0.068

The variables with \* was significant ( $p<0.05$ ). Note: X<sub>3</sub> (rice bran) makes little contribution (0.32%) on the model, so we remove it to improve the goodness of fit of the model equation when modeling.

obtained in the seventh step, indicating that it was approaching the neighborhood of the optimum SDF production. Therefore, the combination of the values at the seventh step was selected as the central point of BBD.

*Optimization of significant variables by BBD*

Based on the results of PBD and SAD experiment, the concentrations of the four significant variables were further optimized by BBD.

Table 4 showed the experimental design and the corresponding results of BBD. Multiple regression analysis of the experimental data suggested that the predicted response (SDF production) could be expressed in terms of the following second-order polynomial equation:

$$SDF = -1.195 - 1.000 AC - 3.000 AD - 0.126 A_2 - 119.815 B^2 - 1026.620 C^2 \tag{2}$$

Table 3 The experiment design of SAD and corresponding results

Code	Wheat bran (g)	MgSO <sub>4</sub> (g/L)	FeSO <sub>4</sub> (g/L)	VB <sub>1</sub> (g/L)	SDF (g/g DW)
1	0.75	0.060	0.024	0.025	0.061±0.003 <sup>cde</sup>
2	0.90	0.055	0.022	0.030	0.066±0.003 <sup>cd</sup>
3	1.05	0.050	0.020	0.035	0.065±0.008 <sup>cd</sup>
4	1.20	0.045	0.018	0.040	0.054±0.008 <sup>e</sup>
5	1.35	0.040	0.016	0.045	0.059±0.014 <sup>de</sup>
6	1.50	0.035	0.014	0.050	0.079±0.004 <sup>b</sup>
7	1.65	0.030	0.012	0.055	0.088±0.000 <sup>a</sup>
8	1.80	0.025	0.010	0.060	0.057±0.009 <sup>de</sup>
9	1.95	0.020	0.008	0.065	0.066±0.004 <sup>cd</sup>
10	2.10	0.015	0.006	0.070	0.069±0.004 <sup>c</sup>
11	2.25	0.010	0.004	0.075	0.056±0.010 <sup>e</sup>

Different letters in the line showed different significance ( $p<0.05$ )

Table 4 BBD matrix to research the interaction between the four significant variables and the value of SDF determined

Run	Wheat bran (g)	MgSO <sub>4</sub> (g/L)	FeSO <sub>4</sub> (g/L)	VB <sub>1</sub> (g/L)	VB <sub>1</sub> (g/g DW)
1	1.20	0.030	0.012	0.040	0.078
2	2.10	0.015	0.012	0.055	0.094
3	1.20	0.030	0.018	0.055	0.067
4	2.10	0.030	0.012	0.040	0.074
5	1.65	0.030	0.006	0.070	0.074
6	2.10	0.045	0.012	0.055	0.091
7	1.65	0.030	0.012	0.055	0.113
8	1.65	0.015	0.018	0.055	0.069
9	1.20	0.030	0.012	0.070	0.069
10	1.65	0.015	0.012	0.040	0.071
11	1.65	0.015	0.006	0.055	0.067
12	2.10	0.030	0.006	0.055	0.089
13	1.20	0.045	0.012	0.055	0.069
14	1.65	0.045	0.012	0.040	0.114
15	1.65	0.030	0.012	0.113	0.053
16	2.10	0.030	0.012	0.070	0.058
17	1.20	0.030	0.006	0.055	0.051
18	2.10	0.030	0.018	0.055	0.069
19	1.65	0.030	0.018	0.040	0.013
20	1.65	0.015	0.012	0.070	0.032
21	1.65	0.030	0.012	0.055	0.113
22	1.65	0.030	0.006	0.040	0.045
23	1.65	0.030	0.012	0.055	0.032
24	1.65	0.030	0.018	0.070	0.033
25	1.65	0.045	0.012	0.070	0.067
26	1.65	0.045	0.006	0.055	0.047
27	1.65	0.030	0.012	0.055	0.038
28	1.20	0.015	0.012	0.055	0.113
29	1.65	0.045	0.018	0.055	0.032

where A, B, C and D stood for wheat bran, MgSO<sub>4</sub>, FeSO<sub>4</sub> and VB<sub>1</sub>, respectively.

Table 5, indicated that the model had a high capacity of prediction. AC, AD, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup> were significant model terms, indicating that the interactions between wheat bran and FeSO<sub>4</sub>, wheat bran and VB<sub>1</sub> were significant.

The significant effects of wheat bran and FeSO<sub>4</sub>, at a constant MgSO<sub>4</sub> and VB<sub>1</sub> concentration of 0.030 g/L and 0.055 g/L, on SDF yield were illustrated in Fig 1 b. At the fixed FeSO<sub>4</sub> concentration, the SDF yield increased rapidly with wheat bran addition at the beginning and reached its peak value when the amount of bran added was 1.77g. At a fixed wheat bran addition, FeSO<sub>4</sub> increased SDF production which reached its peak value when the concentration of FeSO<sub>4</sub> was 0.011 g/L. Fig 1 c shows the significant effect of the interaction between wheat bran and VB<sub>1</sub> on SDF production at a constant MgSO<sub>4</sub> (0.030 g/L) and FeSO<sub>4</sub> (0.012 g/L). At a fixed VB<sub>1</sub> concentration, wheat bran increase SDF production which reached its peak when wheat bran was 1.83 g/L. When wheat bran addition was set, VB<sub>1</sub> promoted SDF production which reached its peak value when VB<sub>1</sub> concentration was 0.052 g/L.

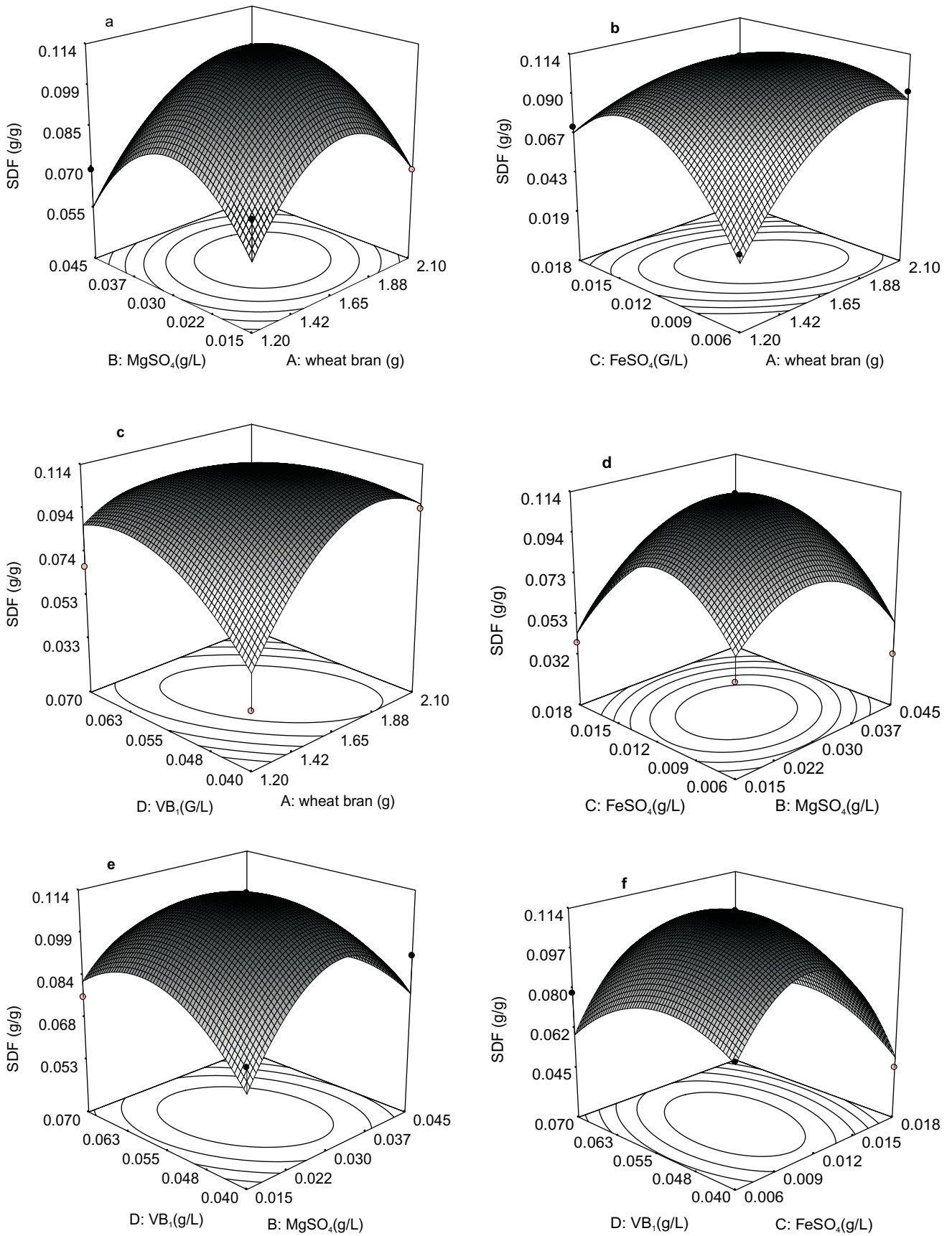


Fig 1 Response surface plots showing interactions between: (a) wheat bran and  $MgSO_4$ , (b) wheat bran and  $FeSO_4$ , (c) wheat bran and  $VB_1$ , (d)  $MgSO_4$  and  $FeSO_4$ , (e)  $MgSO_4$  and  $VB_1$ , (f)  $MgSO_4$  and  $VB_1$  on SDF production using SSF of *G. lucidum*.

Table 5 Source model, sum of squares, df, mean squares, F and P values of the model in BBD

Source model	Sum of squares	Degree of freedom	Mean squares	F-value	P-value
Model	0.010	14	7.407E-004	2.96	0.0452
A	1.141E-004	1	1.141E-004	0.46	0.5149
B	1.841E-004	1	1.841E-004	0.74	0.4112
C	5.467E-004	1	5.467E-004	2.18	0.1702
D	3.675E-005	1	3.675E-005	0.15	0.7096
AB	9.000E-006	1	9.000E-006	0.036	0.8534
AC	2.916E-003	1	2.916E-003	11.65	0.0066
AD	1.640E-003	1	1.640E-003	6.55	0.0248
BC	4.225E-005	1	4.225E-005	0.17	0.6898
BD	4.000E-004	1	4.000E-004	1.60	0.2348
CD	1.000E-004	1	1.000E-004	0.40	0.5415
A <sup>2</sup>	1.830E-003	1	1.830E-003	7.31	0.0222
B <sup>2</sup>	2.052E-003	1	2.052E-003	8.20	0.0169
C <sup>2</sup>	3.857E-003	1	3.857E-003	15.41	0.0028
D <sup>2</sup>	7.648E-004	1	7.648E-004	3.06	0.1110
Residual	2.503E-003	10	2.503E-003		
Cor Total	0.013	24			

R<sup>2</sup> = 0.8056, adequate precision = 7.596

The interactions between wheat bran and MgSO<sub>4</sub>, MgSO<sub>4</sub> and FeSO<sub>4</sub>, MgSO<sub>4</sub> and VB<sub>1</sub>, FeSO<sub>4</sub> and VB<sub>1</sub> were not significant according to BBD analysis (Fig 1a, Fig 1d, Fig 1e and Fig 1f). The corresponding response plots identified the interactions between the four variables with respect to each response.

The model also predicted the maximum SDF production (0.114 g/g DW) to be at a combined addition of wheat bran 1.77 g, MgSO<sub>4</sub> 0.029 g/L, FeSO<sub>4</sub> 0.011 g/L and VB<sub>1</sub> 0.052 g/L.

#### Verification of the optimal condition

In order to verify the adequacy of the model equation, experiments were carried out using the predicted medium components (LRP 5 g, wheat bran 1.77 g, MgSO<sub>4</sub> 0.029 g/L, FeSO<sub>4</sub> 0.011 g/L, and VB<sub>1</sub> 0.052 g/L). Under the optimized medium, the actual SDF production reached 0.113 g/g DW, which was in good agreement with the predicted value (0.114 g/g DW). This indicated that RSM was effective in optimization of SDF production from *G. lucidum* by SSF.

#### Composition analysis of SDF

To determine the monosaccharide composition, SDF was hydrolyzed, converted to TMS derivatives and analyzed on GC. The contents of carbohydrate and protein in the final SDF production were 75.61% and 0.47%, respectively. Compositional analysis showed that SDF was composed of rhamnose, xylose, mannose, galactose and glucose in a molar ratio of 1.2:1.0:1.6:7.2:56.9. This result was similar with that of other investigators (Bao *et al.* 2002, Lai and Yang 2007).

The present study involved the use of statistical

experimental designs to optimize the medium composition in production of SDF from *Ganoderma lucidum* via SSF using LRP as the major substrates. Four variables: wheat bran, MgSO<sub>4</sub>, FeSO<sub>4</sub> and VB<sub>1</sub> were identified as significant for SDF production. The optimum medium were found and produced 3.4-fold higher levels of SDF than unoptimized medium. Compositional analysis showed that SDF was composed of rhamnose, xylose, mannose, galactose and glucose. This research reveals the great potential of *Ganoderma lucidum* for the conversion of IDF into SDF from a low grade agro waste like LRP.

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