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ORIGINAL ARTICLE

Optimization of production conditions for β-mannanase using apple pomace as raw material in solid-state fermentation

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Abstract Apple pomace is a wasted resource produced in China in large quantities, disposal of which has caused serious environmental problems. In order to make the best of this residue, apple pomace together with cottonseed powder was used as a raw material to produce β-mannanase in solid-state fermentation (SSF) by *Aspergillus niger* SN-09. Optimization of fermentation conditions for maximizing β-mannanase production was carried out using Plackett-Burman and Central Composite designs. A mixture of apple pomace and cottonseed powder (3:2, w/w) with 59.2 % (w/w) initial moisture, together with certain ionic compounds and salts, proved to be the optimal medium. The test fungi were inoculated in the optimized medium and incubated at 30°C for 48 h. The activity of β-mannanase reached 561.3 U/g, an increase of 45.7 % compared with that in basal medium, and reached the same level of production as that achieved using wheat bran and soybean meal as raw materials as in most factories in China. This is the first report of the use of apple pomace as a raw material to produce β-mannanase in SSF. This will not only reduce the production cost of β-mannanase, but also represents a new and effective way to make the best use of apple pomace, which can consequently help to reduce the environmental pollution caused by this waste.

Keywords β-mannanase · Apple pomace · Cottonseed powder · Response surface methodology · Solid-state fermentation

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Introduction

Mannan, together with xylan and galactans, is a main component of hemicelluloses—a complex group of branched and linear polysaccharide present widely in plant cell walls (Chun and Chen 2004). The main chain of mannan is constituted of β-1,4-linked D-mannopyranose and D-glucopyranose units, partially substituted by β-1,6-linked D-galactosyl side groups (Stoll et al. 1999). In common feed ingredients, such as soybean meal and corn, there is a considerable amount of mannan, which cannot be digested and absorbed due to the absence of mannanase in the digestive enzymes of domestic animals. In addition, mannans also have anti-nutritional effects, and have adverse impacts on livestock. They can increase the viscosity of gastrointestinal contents, which affects digestion and absorption of nutrients directly and also can cause diarrhea (Jackson 2001).

However, the anti-nutritional effects of the mannan substances present in feedstuff can be eliminated by addition of β-mannanase as a feed additive. β-mannanase (EC 3.2.1.78) can hydrolyze mannan substances into manno-oligosaccharides, thus decreasing the viscosity of the gastrointestinal contents and improving the digestion and absorption of nutrition in feedstuff (Burke and Cairney 1997). Furthermore, oligosaccharides can play a major role in regulation of the animal intestine (Wu et al. 2005). For all these reasons, the use of β-mannanase as a feed additive is of significant importance. In addition, β-mannanase has various other applications in many other fields, such as the pulp and paper industries, oil drilling, and in the detergent and food industries (Dhawan and Kaur 2007).

At present, producing β-mannanase from fungi such as *Aspergillus niger*, *Trichoderma reesei* and *Sclerotium rolfsii* has attracted most attention, although mannans can also be

decomposed by many other microorganisms (Abdeshahian et al. 2010; Gubitz et al. 1996; Hagglund et al. 2003). Many general substrates, such as wheat bran, bean gum, guar gum and konjac flour, have been adopted as raw materials to produce β-mannanase in both solid-state fermentation (SSF) and liquid fermentation (Abdeshahian et al. 2010; Dhawan and Kaur 2007; Kurakake and Komaki 2001). However, to date there are almost no reports of apple pomace being adopted as a raw material to produce β-mannanase.

Apple pomace is the solid residue produced in the process of juice extraction from apples, and more than 100 million tons of this waste resource is produced in China every year (Cheng et al. 2008). Apple pomace is rich in carbohydrates, vitamins, minerals, fiber and other nutrients, but 25–30 % of the available apple pomace is used as feed directly or for deep processing, while the rest is abandoned as waste, which gives off an acid smell and causes serious pollution (Joshi and Devender 2006; Sun et al. 2009). In order to make the best use of apple pomace and to solve the environmental problem, apple pomace has been adopted as a raw material in the production of pectin, ethanol, lactic acid, citric acid, hydrogen and some enzymes such as cellulase, pectinase and xylanase (Dhillon et al. 2011a, b; Gassara et al. 2010; Hui et al. 2010; Joshi et al. 2006; Kumar and Chauhan 2010). However, no study has been carried out so far on the use of apple pomace to produce β-mannanase.

In this paper, in order to reduce the industrial cost of β-mannanase production, apple pomace was adopted for the first time as a main raw material in SSF by *Aspergillus niger* instead of wheat bran, bean gum, konjac flour and the other general substrates mentioned above, and Plackett-Burman design and response surface methodology (RSM) were employed to optimize medium composition and fermentation conditions.

Materials and methods

Microorganism and inoculum preparation

Aspergillus niger strain SN-09 was provided by the laboratory of the Life Science Institute of the Shandong Agriculture University in China. It was cultivated on potato dextrose agar (PDA) slants at 4°C. The stock culture was inoculated into wheat bran medium in a 250 ml flask and incubated at 30°C for 72 h. It was then mixed with 100 mL sterile water, stirred for 15 min, then separated with two-double sterile gauze. The filtered solution diluted to 1×10^7 spores/mL in sterile water was used as the inoculum in the study. Direct microscopic counts were taken using a blood counting chamber.

Materials and solid-state fermentation

Apple pomace (obtained from a local company in Yantai District of Shandong Province, China) was triturated in a hammer mill and then sifted. The fraction that passed through a 30-mesh sieve but was retained by a 50-mesh sieve was used as raw material in this study. The moisture content, total protein content and crude fiber content was 9 %, 6 % and 15 %, respectively.

A mixture of apple pomace and cottonseed powder (1:1, w/w) was adopted as the basic medium. The initial moisture content was adjusted to 50 % (w/w), and the pH value was maintained at its natural level, i.e., pH 5.5. SSF was carried out in 250 mL conical flasks containing 20 g medium, and sealed with a six-layer sterile gauze. After autoclaving at 121°C for 30 min and then cooling to 40°C, the culture medium was inoculated with 1 mL of inoculum prepared as above. The inoculated medium was incubated at 30°C for 48 h, and shaken gently at intervals during the incubation. The fermented medium was dried in a ventilated oven at 45°C for 24 h and subsequently processed into flour for β-mannanase activity analysis. Three replications were adopted for each test.

Single factor designs

Since the ratio of carbon to nitrogen (C/N) in apple pomace is higher than the optimum required for growth of the microorganism, supplementation with an extra nitrogen source was necessary. The purpose of the single factor experiments was to determine which kind of nitrogen source was best and how much should be supplemented. To screen an optimal organic nitrogen source, the fermentation experiments were performed with 50 % (w/w) apple pomace and 50 % (w/w) of one of a variety of nitrogen sources including soybean meal, cottonseed powder, wheat bran, corn syrup and brewer's spent grains. To determine how much of the selected organic nitrogen source should be supplemented, it was mixed well with apple pomace at different ratios, and then used as culture medium. To select optimal quick-acting nitrogen sources, non-protein nitrogen sources, including $(NH_4)_2SO_4$, urea, NH_4NO_3, $NaNO_3$ and NH_4Cl, were added to the medium at the level of 1 % (w/w), respectively. All the SSF experiments were carried out as described above. All treatments were run in triplicate, and the data from each experiment were subjected to the SAS statistical package (Version 8.2, SAS, Cary, NC) for ANOVA, and an LSD post hoc test was used to determine significant differences ($P<0.05$) amongst treatments.

Plackett-Burman design

The Plackett-Burman design was used to screen the most significant fermentation parameters affecting the production of β-mannanase, as it is known to be of great use in

identifying the important nutrients among various nutrients in relatively few experiments as compared with the one-factor-at-a-time technique. The ranges and levels of the variables investigated in this study (in both coded and natural values) are given in Table 1. The test variables were coded according to the following equation.

$$X_i=(x_i-x_o)/\Delta x_i \quad (1)$$

where X_i is the coded value of an independent variable, x_i is the real value of an independent variable, x_0 is the real value of an independent variable at the center point, and Δx_i is the step change value. The activity of β-mannanase was considered as the dependent variable or response (y_i). Design Expert version 7.1 software (Stat-Ease, Minneapolis, MN) was employed for regression analysis of data. All data presented are mean values of triplicate experiments, n=3.

Central composite design

The factors, including $(NH_4)_2SO_4$, KH_2PO_4 and initial moisture content, that were significant in the Plackett-Burman experiment, were used to select the optimized conditions for β-mannanase production using a central composite design (CCD) and RSM. They were tested at five coded levels (−1.682, −1, 0, 1, and 1.682; ranges and levels given in Table 2). The test was essentially a full 2^4 factorial design with six axial points (or so-called star points) and nine replicates at the center, resulting in a total number of 23 experiments. The quadratic model for predicting the optimal point was expressed according to the following equation:

$$y = b_o + \sum b_i Z_i + \sum b_{ii} Z_i^2 + \sum b_{ij} Z_i Z_j \quad (2)$$

where y is the response variable (the activity of β-mannanase); b_0, b_i, b_{ii}, b_{ij} are constant coefficients; Z_i and Z_j are the coded levels of the independent variables. Design Expert version 7.1 software (Stat-Ease) was used for regression analysis of data to obtain optimal working parameters and to generate response surface graphs. All data presented are mean values of triplicate experiments, n=3.

Table 1 Coded and uncoded values of factors in the Plackett-Burman design

Variable	Factor	Level		
		−1	0	1
X_1	$(NH_4)_2SO_4$ (%, w/w)	0.5	1.0	1.5
X_2	Initial moisture content (%, w/w)	45	50	55
X_3	$CaCl_2$ (%, w/w)	0.1	0.2	0.3
X_4	$MgCl_2$ (%, w/w)	0.050	0.075	0.100
X_5	KH_2PO_4 (%, w/w)	0.050	0.0625	0.075
X_6	$ZnSO_4$ (%, w/w)	0.05	0.075	0.10
X_7	$MnSO_4$ (%, w/w)	0.05	0.075	0.10
X_8	$FeSO_4$ (%, w/w)	0.05	0.075	0.10
X_9 – X_{11}	Dummy variables			

Effect of incubation time on enzyme production

The fermentation was performed with all the parameters kept at their optimized levels. A total of 39 conical flasks were used in the experiment, and the fermentation processes of 3 flasks were terminated every 12 h (1–24 h) or 6 h (24–84 h). The fermented substrate was mixed well, dried in an oven at 45°C for 24 h, and then used as samples for related analyses.

Assay of β-mannanase activity

Fermented substrate (1 g) was ground with 10 mL 0.1 M sodium citrate buffer (pH 6.0) to extract β-mannanase. A 0.2-mL aliquot of the extract was incubated with 1.8 mL of a solution containing locust bean gum (0.05 %, w/v) in 0.1 M sodium citrate buffer (pH 6.0) at 50°C for 5 min. The reaction was stopped by adding 3 mL DNS (3,5-dinitrosalicylic acid) solution, and then diluted to 25 mL. The absorbance at 540 nm was read in an Ultrospec 4300 pro UV-Visible Spectrophotometer (Pharmacia, Uppsala, Sweden), with reaction solution containing boiled enzyme-extract as a control (Ozturk et al. 2010). One unit of β-mannanase activity was defined as the amount of enzyme required to release 1 μmol D-mannose in 1 min. Each sample had three parallel determinations.

Results and discussion

Effect of organic nitrogen source on production of β-mannanase

The source of organic nitrogen had a great effect on the ability of *A. niger* SN-09 to produce β-mannanase (Fig. 1). According to ANOVA and Tukey's test results, when using soybean meal or cottonseed powder as the nitrogen source, the activity of β-mannanase was significantly (P<0.01) higher than when using other nitrogen sources including wheat bran, corn syrup and brewer's spent grains. The reason might be that the former had higher protein content than the latter, which was conducive to the growth and enzyme-producing ability of the test fungus. However, there was no significant difference in the activity of β-mannanase between the treatments using cottonseed powder or soybean meal as nitrogen source (P>0.05). Since the price of cottonseed powder is much lower than that of soybean meal in China, it was reasonable to selected cottonseed powder as the best and most economical organic nitrogen source.

Table 2 Experimental ranges and levels of the independent variables in central composite design (CCD)

Variable	Factor	Level				
		−1.682	−1	0	1	1.682
Z_1	$(NH_4)_2SO_4$ (%, w/w)	0.318	1.000	2.000	3.000	3.682
Z_2	KH_2PO_4 (%, w/w)	0.016	0.050	0.100	0.150	0.184
Z_3	Initial moisture content (%, w/w)	51.6	55	60	65	68.4

Effect of ratio of apple pomace to cottonseed powder on production of β-mannanase

The ratio of apple pomace to cottonseed powder in the medium had a crucial effect on the growth of fungal mycelia and the production of β-mannanase. The data presented in Fig. 2 reveal that β-mannanase activity increased as the ratio of apple pomace to cottonseed powder increased, achieving its highest value when the ratio was 1:1 (w/w), and then decreasing as the ratio increased further. However, the activity of β-mannanase did not differ significantly ($P>0.05$) when the ratio of apple pomace to cottonseed powder changed from 2:3 (w/w) to 3:2. The ratio 3:2 (w/w) was chosen as the optimum value instead of 1:1 (w/w) in subsequent tests because we hoped to adopt apple pomace as a raw materials as much as possible in order to reduce production costs and make the best use of this agricultural waste.

Effect of quick-acting nitrogen sources on production of β-mannanase

Unlike a protein nitrogen source, non-protein nitrogen sources can be utilized more rapidly by microorganisms. It was necessary to add a quick-acting nitrogen source to the medium in order to shorten the period of fermentation and increase the biomass by converting it into protein. As shown in Fig. 3, the addition of $(NH_4)_2SO_4$ and urea could both significantly increase β-mannanase activity ($P<0.05$), and the maximum value was obtained when 1 % (w/w) $(NH_4)_2SO_4$ was added to the substrate. However, the difference between the effects of $(NH_4)_2SO_4$ and urea was not significant ($P>0.05$).

Plackett-Burman design

The results of the Plackett-Burman experiment are shown in Table 3. Experimental data were subjected to regression analysis by Design-Expert (version 7.1 software, Stat-Ease). The obtained regression equation was as follows:

$$y = 365.86 + 45.39X_1 + 81.70X_2 + 21.42X_3 + 7.63X_4 + 36.67X_5 + 15.61X_6 - 9.08X_7 - 17.79X_8 \quad (3)$$

where y is β-mannanase activity (U/g), and X_1–X_8 are the coded values of the independent variables.

Variance analysis revealed that the P-value of the equation was 0.0330 and that the model was significant at the 5 % level, indicating that the fit of the model was better. Determination of the coefficient of the

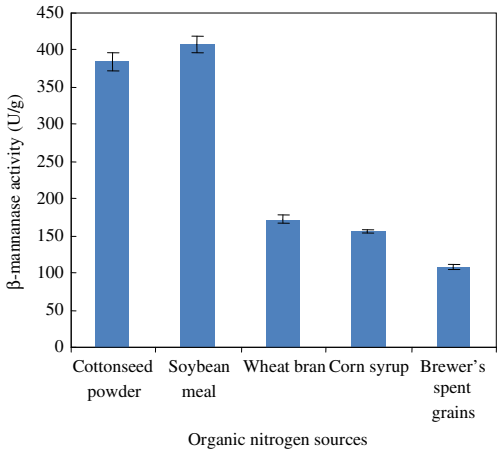

Fig. 1 Effect of organic nitrogen source on production of β-mannose by *Aspergillus niger* SN-09 in solid-state fermentation (SSF). The radio of apple pomace to organic nitrogen sources was 1:1 (w/w) and the initial moisture content 50 % (w/w). *Error bars* SD

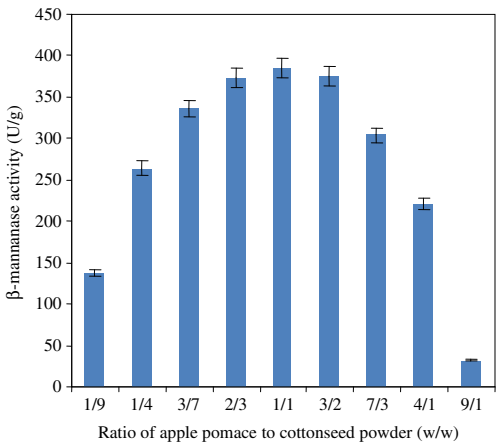

Fig. 2 Effect of the ratio of apple pomace to cottonseed powder on the production of β-mannanase by *A. niger* SN-09 in SSF. The initial moisture content of the medium was 50 % (w/w). *Error bars* SD

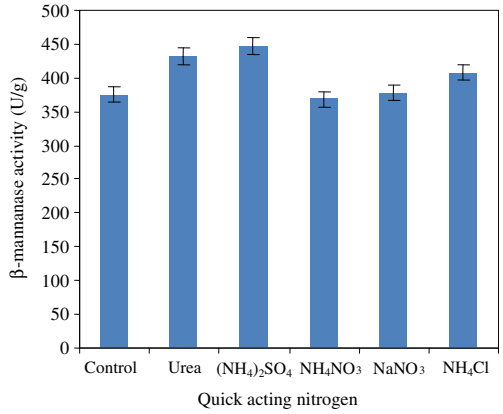

Fig. 3 Effect of quick-acting nitrogen sources on production of β-mannanase by *A. niger* SN-09 in SSF. The initial moisture content of the medium was 50 % (w/w). *Error bars* SD

model (R^2) was 0.9696, suggesting that 96.96 % of the variation in the experiment could be interpreted by the model, while only 3.04 % of variation could not be interpreted by it.

Coefficients of the equation were analyzed by variance analysis (Table 4). The effect of initial moisture content in the medium on the production of β-mannanase was positive, and achieved a highly significant level ($P<0.01$). Effects of $(NH_4)_2SO_4$ and KH_2PO_4 were both positive and achieved significant level ($P<0.05$), while the other factors were not significant at the 5 % level ($P>0.05$). Significant factors , including initial moisture content, $(NH_4)_2SO_4$ and KH_2PO_4, were further optimized using RSM to determine optimum levels, while the optimal levels of non-significant factors were determined directly according to their positive or negative regression coefficients.

Table 4 Regression analysis for the production of β-mannanase in the Plackett-Burman design

Source	Coefficient	*F*-value	Probability > *F*
Model	–	11.95	0.0330
Intercept	365.8631	–	–
$(NH_4)_2SO_4$	45.3869	17.52	0.0248
Initial moisture content	81.69643	56.78	0.0048
$CaCl_2$	21.42262	3.90	0.1426
$MgCl_2$	7.625	0.49	0.5326
KH_2PO_4	36.67262	11.44	0.0430
$ZnSO_4$	15.6131	2.07	0.2455
$MnSO_4$	-9.07738	0.70	0.4639
$FeSO_4$	-17.7917	2.69	0.1993

Central composite design

The results of the CCD are shown in Table 5. The quadratic regression model was found to be as follows:

$$y = 565.47 - 6.15Z_1 + 2.99Z_2 - 10.51Z_3 + 1.63Z_1Z_2 - 7.07Z_1Z_3 + 1.83Z_2Z_3 - 28.72Z_1^2 - 7.00Z_2^2 - 26.40Z_3^2 \quad (4)$$

where y is the activity of β-mannanase (U/g), and Z_1, Z_2 and Z_3 are coded independent variables for $(NH_4)_2SO_4$, KH_2PO_4, and initial moisture content, respectively.

Model fitting was estimated by variance analysis. The *P*-value of model was < 0.0001, indicating that the model was significant at the 1 % level; and the *P*-value of lack of fit was 0.9135, indicating that lack of fit was not significant at the 5 % level. The coefficient (R^2) of the model was determined as

Table 3 Results of Plackett-Burman design for the production of β-mannanase

Run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	β-Mannanase activity(U/g)
1	1	−1	1	−1	−1	−1	1	1	−1	1	−1	243.50
2	1	1	−1	1	−1	−1	−1	1	−1	−1	1	422.14
3	−1	1	1	−1	1	−1	−1	−1	−1	−1	−1	435.21
4	1	−1	1	1	−1	1	−1	−1	1	−1	−1	352.43
5	1	1	−1	1	1	−1	1	−1	1	1	−1	504.93
6	1	1	1	−1	1	1	−1	1	1	1	1	579.00
7	−1	1	1	1	−1	1	1	−1	−1	1	1	430.86
8	−1	−1	1	1	1	−1	1	1	1	−1	1	282.71
9	−1	−1	−1	1	1	1	−1	1	1	1	−1	247.86
10	1	−1	−1	−1	1	1	1	−1	−1	1	1	365.50
11	−1	1	−1	−1	−1	1	1	1	1	−1	1	313.21
12	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	213.00

Table 5 Experimental design and results of the full factorial CCD for the production of β-mannanase

Run	Z_1	Z_2	Z_3	β-Mannanase activity (U/g)
1	−1	−1	−1	511.9
2	1	−1	−1	503.1
3	−1	1	−1	510.5
4	1	1	−1	514.9
5	−1	−1	1	504.6
6	1	−1	1	474.2
7	−1	1	1	517.2
8	1	1	1	486.6
9	−1.682	0	0	490.5
10	1.682	0	0	479.3
11	0	−1.682	0	544.7
12	0	1.682	0	547.9
13	0	0	−1.682	517.0
14	0	0	1.682	465.9
15	0	0	0	540.1
16	0	0	0	589.6
17	0	0	0	567.8
18	0	0	0	566.5
19	0	0	0	549.9
20	0	0	0	562.1
21	0	0	0	556.3
22	0	0	0	587.5
23	0	0	0	569.2

0.9184, suggesting that 91.84 % of variation in the experiment could be interpreted by the model, while only 8.16 % of variation could not be interpreted by it. All of these analyses confirmed that the model was a good fit.

Regression coefficients of the equation denoted the impact of various factors on the value of effect, so with larger *F*-value of regression coefficients and smaller *P*-values, the coefficient was more significant. From the variance analysis (Table 6), the quadratic terms of $(NH_4)_2SO_4$ and initial moisture content were extremely significant ($P<0.01$), i.e., the effects of $(NH_4)_2SO_4$ and initial moisture content on the activity of β-mannanase were significant, while KH_2PO_4 was not significant in the selected range. The optimal values of the test variables in coded unit area calculated by solving the quadratic regression equation above were shown as follows: Z_1=0-0.08, Z_2=0.18, Z_3=−0.18. After decoding, the true value was obtained: 1.92 % (w/w) $(NH_4)_2SO_4$, 0.11 % (w/w) KH_2PO_4, and 59.1 % (w/w) initial moisture content. Under the optimum conditions above, the maximum activity of β-mannanase predicted by the model was 566.9 U/g. The statistical conclusions above could be further explained by a three-dimensional surface diagram drawn according to the above model. The maximum value in the response surface plot shown in Fig. 4 was the optimum value predicted by the model.

Ten sets of validation experiments were designed to verify the accuracy of the model, and each experiment was repeated at least three times. One of them was performed with the optimized conditions mentioned above, and the other nine were carried out around the optimized conditions stochastically. All experimental values were in very good agreement with predicted values, indicating that the model was accurate and effective in explaining the actual fermentation process. The experimental value was 561.3±23.72 U/g under optimized conditions, increased by 45.7 % compared to that in basal medium, which indicated that RSM was an effective means of optimizing the culture medium.

Table 6 Regression results from data of CCD experiments

Source	Sum of squares	df	Coefficient	*F*-Value	*P*-Value Prob > F
Model[a]	27,308.59	9		16.25	< 0.0001**
Z_1	517.35	1	−6.15	2.77	0.1199
Z_2	122.28	1	2.99	0.65	0.4329
Z_3	1,509.75	1	−10.51	8.09	0.0138*
Z_1Z_2	21.16	1	1.63	0.11	0.7418
Z_1Z_3	400.30	1	−7.07	2.14	0.1669
Z_2Z_3	26.68	1	1.83	0.14	0.7115
Z_1^2	13,104.72	1	−28.72	70.19	< 0.0001**
Z_2^2	778.95	1	−7.00	4.17	0.0619
Z_3^2	11,074.70	1	−26.40	59.32	< 0.0001**
Residual	2,427.18	13			
Lack of fit	357.70	5		0.28	0.9135
Pure Error	2,069.48	8			
Total	29,735.77	22			

* Significant at 5 % level,
** Significant at 1 % level
[a] Z_1: $(NH_4)_2SO_4$, Z_2: KH_2PO_4, Z_3: Initial moisture content

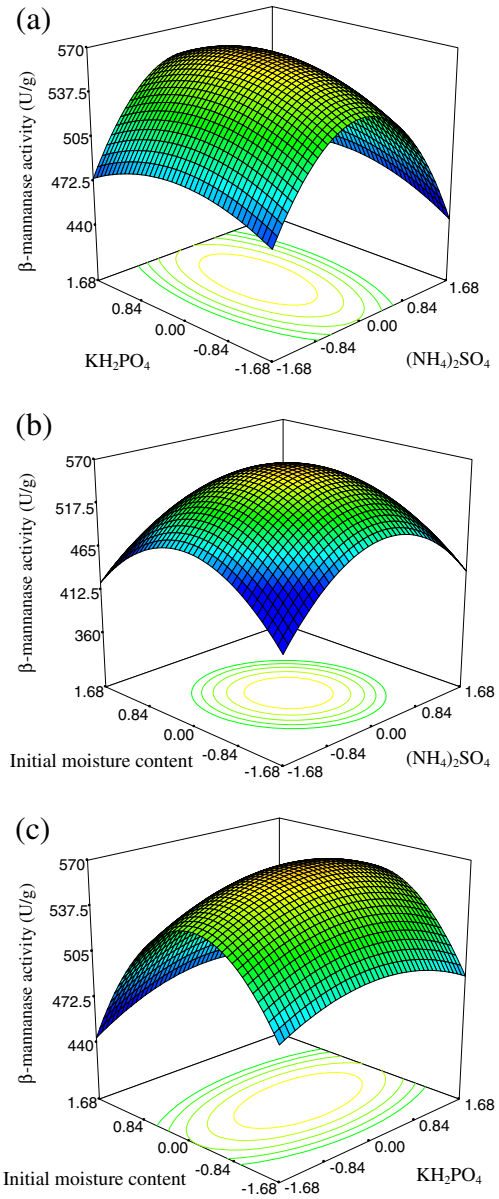

Fig. 4 Response surface plot for the activity of β-mannanase in terms of the effects of **a** $(NH_4)_2SO_4$ and KH_2PO_4, **b** $(NH_4)_2SO_4$ and initial moisture, and **c** KH_2PO_4 and initial moisture. Factors not included in the axes were fixed at their respective optimum levels

Effect of incubation time on enzyme production and dry matter weight loss

Direct determination of biomass in solid medium is very difficult because it is impossible to separate the organism from the substrate. Terebiznik and Pilosof (1999) pointed out that biomass is highly correlated with the loss of dry matter weight. Therefore, dry matter weight loss was used as a measure of cell growth in this study. The time course profiles of the production of β-mannanase are shown in Fig. 5. At the initial period (0–24 h), the loss of dry matter weight was at a micro scale and the change in enzyme activity was very small. During the fermentation period of 24–48 h, the fungus grew fast, resulting in a rapid increase in both dry matter weight loss and β-mannanase activity. During the next fermentation period (48–72 h), growth of the fungus slowed down, as did the change in dry matter weight loss and β-mannanase activity. The activity of β-mannanase reached a peak at 72 h, and then began to decrease slowly. However, spores began to appear at 48 h, and a few hours later a large number of spores were produced, which turned the substrate black and had a negative effect on product characteristics. In addition, the longer the incubation time, the lower the yield obtained, so the fermentation should be terminated at 48 h to avoid excessive loss of dry weight matter, which was caused mainly by the respiratory metabolism of the fungus.

The relationship between the production of β-mannanase and the growth of test fungi could also be obtained from time-course profiles; β-mannanase activity was correlated highly with dry matter weight loss during the fermentation time of 24–48 h. The linear correlation equation was as follows:

$$y = 18.2x - 17.65 \quad (5)$$

where y is the activity of β-mannanase (U/g), and x the dry matter weight loss (%). The R^2 of the equation was calculated to be 0.987, indicating that 98.7 % of the variability in the activity of β-mannanase could be explained by the equation.

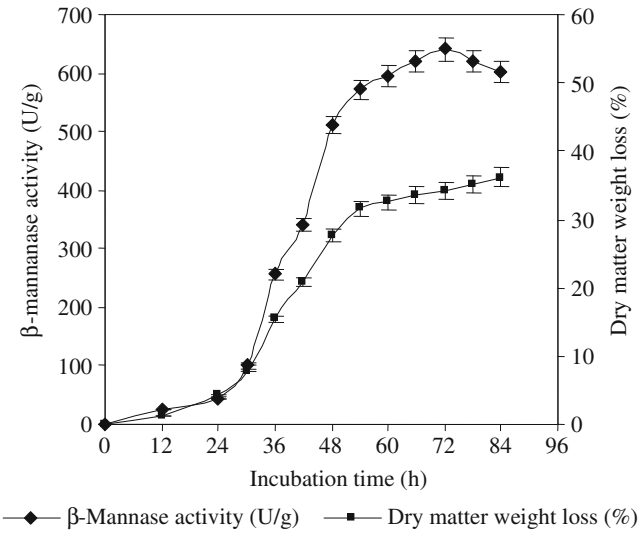

Fig. 5 Time-course profile of β-mannanase production and dry weight loss rate by *A. niger* SN-09 in SSF. *Error bars* SD

Conclusion

In this study, apple pomace was used as raw material to produce β-mannanase in order to make the best of the agricultural waste and reduce the production cost of β-mannanase. SSF technology using *A. niger* SN-09 was adopted due to the low cost of equipment, high volumetric productivity and decreased operational expenditure (Araya et al. 2007). RSM was found to be effective in optimizing medium composition for β-mannanase production by *A. niger* SN-09 in SSF. The results showed that the optimum medium composition was apple pomace and cottonseed powder 3:2 (w/w), urea 1.95 % (w/w), KH_2PO_4 0.1 % (w/w), initial water content 59.2 % (w/w), $CaCl_2$ 0.2 % (w/w), $MgCl_2$ 0.1 % (w/w). Under optimized conditions, validation experiments produced an enzymatic activity of β-mannanase of 561.3±23.72 U/g dry content—an increase of 45.7 % compared with basal medium, and reaching the average enzyme production level achieved on wheat bran and soybean meal-based materials reported in the literature (Kurakake and Komaki 2001; van Zyl et al. 2009).

The study is of significance in allowing the full utilization of apple pomace, and serves as a model for developing innovative techniques to make the best of similar agricultural and industrial wastes.

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