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Application of response surface methodology for protein enrichment of cassava peel as animal feed by the white-rot fungus *Panus tigrinus* M609RQY

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ABSTRACT

Response surface methodology (RSM) was employed to optimize the process conditions for production of protein-enriched animal feed from cassava peel by a locally isolated white rot fungus *Panus tigrinus*.

Face-Centered Central Composite Design (FCCCD) with three variables (pH, inoculum size and moisture content) was used to determine the effect of these operational parameters on the protein increase of cassava peel as animal feed under solid-state fermentation. A significant quadratic model was obtained for protein increase using this design. Results of the statistical analysis showed that a significant ($P < 0.05$) linear effect was obtained for moisture content, while only the interaction effect between moisture content and inoculum size was significant ($P < 0.01$). The optimum process combination was found to be 75% (v/w) of moisture content, a pH of 5.3 and 7% (v/w) inoculum size. A maximum increase of protein (55.16%) was obtained during 15-day of solid-state fermentation.

1. Introduction

Cassava peel, a thin brown outer covering with a thicker leathery parenchymatous inner covering is the first waste generated during the processing of cassava to either food or other industrial products. Cassava is the third most important source of calorie after wheat and rice in the developing countries (FAO, 2008). This peel could make up to 10–20% of the wet weight of the roots (Obadina, Oyewole, Abiola, & Sanni, 2006), indicating its enormous potential for biotechnological and industrial processes considering its abundant availability and low cost. However, these peels are regarded as waste and are openly dumped causing serious environmental problem associated with their decomposition. Very small quantity is used for animal feeding, due to its low protein content, high level of hydrocyanide and high crude fiber content. Successful efforts have been made in enhancing its protein content by microbial techniques (Aderemi & Nworgu, 2007; Okpako, Ntui, Osuagwu, & Obasi, 2008).

On the other hand, most of the fungi used in the previous studies are spores forming in nature, which can cause respiratory problems due to their small sizes or secrete mycotoxins, thus making their use difficult in animal feeding (Peiji, Yinbo, Xin, Mingtian, & Yongcheng, 1997). In light of this, bioconversion using edible non-sporulating white rot fungi could be more efficient in meeting these problems. One promising white rot fungi is the locally isolated *Panus tigrinus*, which secretes the three lignin modifying enzymes, degrades cell wall components and synthesizes microbial protein without spores formation.

Maximum production of enzymes is one of the main goals of bioprocesses which involve strains development, medium optimization and process optimization. These are vital tools for development of fermentation processes at industries. The use of One Factor at A Time (OFAT) optimization method has been the main method used in optimization of bioprocesses for animal feed production, with a major disadvantage that it does not consider interaction (Ahmed, Ahmad, & Saeed, 2010; Shojaosadati, Faraidouni, Madadi-Nouei, & Mohamadpour, 1999). This has led to the adoption of statistical experiment design (RSM) that can efficiently analyzed the effects of several independent variables and also consider interactions among the variables in relation to the response (dependent variable) (Myers & Montgomery, 2002).

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Operational parameters such as moisture content, inoculum size and pH were identified as most crucial in the production of protein enriched cassava peel as animal feed due to their effects on microbial growth, substrate utilization and enzyme production in solid state fermentation (SSF). Report has shown that sufficient amount of inoculum is required for optimal microbial growth, substrate utilization, and degradation while excessive inoculum may create competition for nutrients thus affecting microbial growth and performance (Sabu, Pandey, Jaafar, Daud, & Szakacs, 2005). In a medium of high moisture content, oxygen transfer is hindered due to reduced porosity of substrate, thereby limiting the growth within substrate (Patel, Gupte, & Gupte, 2009). However, in a condition of insufficient water level, optimal fungal activity is suppressed. The pH of a medium is highly dependent on the microorganism and the nature of the substrate used. Considering the buffering effects of most lignocellulosic material, determining the optimum pH is highly crucial for efficient cell growth and enzyme production.

There are scarse reports on the use of RSM for optimization of process parameters during solid state fermentation of agro-industrial residues to animal feed. Sharma and Arora (2010) used RSM to optimize moisture level, concentration of NH_4Cl and malt extract during solid state fermentation of wheat straw as animal feed by *Phlebia floridensis* using lignin degradation profile and production of different lignocellulolytic enzymes as response. They reported that with the increase in moisture content, the lignocellulolytic enzymes production increased in the presence of almost equal amount of NH_4Cl and malt extract. Under the optimized conditions, the in vitro digestibility has also been reported to increase by almost 50% with a loss of 27.6% in lignin Zhu, Zhang, Zhang, and Huang, (2011) also applied RSM to optimize additional glucose, $CuSO_4$ and initial moisture content during SSF of corn stover by *Trametes versicolor* to enhance ligninolysis and increase crude protein content. Under the optimized conditions, the crude protein content of corn stover was doubled with maximum lignin and hemicelluloses degradation of up to 34.8 and 21.9%, respectively and maximum cellulose loss of less than 10.5%.

Hence, the aim of this study is to determine the effects of operational parameters (moisture content, inoculum size and pH) to identify their optimum levels using response surface methodology on the production of protein-enriched cassava peel as animal feed by a locally isolated white rot fungus *P. tigrinus*.

2. Materials and methods

2.1. Sample collection and preparation

Cassava peels were collected from a small scale Kerepek (local snack) industry in Kuala Langat, Selangor, Malaysia. They were immediately washed to remove sand, tuber head and dried at 60 °C in an air forced oven for 48 h to avoid deterioration and growth of unwanted microbes. The dried cassava peel was milled by using a grinding machine with sieve (1 mm) (Model D-79219 staufen, IKA-WERKE GMBH & Co. KG Germany) and stored in air tight container.

2.2. Microorganism and inoculum preparation

The white rot fungus *P. tigrinus* M609RQY was locally isolated (Tijani, Jamal, Alam, & Mirghani, 2011) maintained on malt extract agar (MEA) (Merck) plate at 4 °C and subcultured forth nightly (every two weeks) on a new plate to prolong its life. Inoculum suspension was prepared by washing 4 MEA plates cultured for 7 days at 30 °C with 60 ml of sterilized distilled water to yield a concentration of 0.865 g/l of biomass.

2.3. Solid state fermentation (SSF)

The fermentation media (20 g) were prepared in 250 ml Erlenmeyer flasks, comprising of 5.14 g of substrate (cassava peel), 0.86 g of co-substrate (wheat flour) and 14 ml of moisture content (liquid concentration). The moisture content consisted of 11.8 ml of distilled water (which varies according to the experiment design), 1.2 ml of inoculum (which varies according to the experiment design) and 1 ml of mineral solution. The composition of the mineral solution was based on a previous study (Tijani, Jamal, Alam, & Mirghani, 2012) as follows (g/l): $(NH_4)_2SO_4$, 1.5; $MgSO_4.H_2O$, 0.45; KH_2PO_4, 0.8 and $MnSO_4$, 0.05. Initial pH of the media was adjusted according to the experimental design using 1 M HCl or 1 M NaOH before autoclaving at 121 °C for 20 min. The flasks consisting of the media were allowed to cool down to room temperature before aseptically inoculating them and were incubated at 30 °C for 15 days. Samples were carried out in triplicates.

2.4. Response surface methodology (face centered central composite design)

Response surface methodology is a powerful mathematical technique used in evaluating the relationship that exists between variables and their response. It involves three major steps: (i) carrying out statistical design of experiment, (ii) calculating the coefficient of the variables in a mathematical model, (iii) predicting the response and validating the adequacy of the model (Myers & Montgomery, 2002). Face centered central composite design (FCCCD) under the response surface methodology was used to evaluate the effect of process parameters on the production of protein enriched cassava peel as animal feed. The boundary levels for each parameter were as follows: pH (3 and 7), inoculum (4% and 8%) and moisture content (60% and 80%) with protein content (mg/g) as enrichment indicator, was taken as response to the design. Seventeen experimental runs with three center points were generated including the response surface plot by using the statistical software package Design-Expert 6.0.8 (Stat Ease Inc., Minneapolis, USA). The three parameters were chosen as the most crucial variables and were denoted as *A* (pH), *B* (inoculum) and *C* (moisture content) as shown in Table 1. In order to determine the relationship that exist between the dependent and independent variables, a second order polynomial equation was fitted to the data by multiple regression procedure.

Table 1
Actual values of FCCCD experiment with three parameters showing the experimental and predicted response for protein enrichment of cassava peel.

Run	*A*: pH	*B*: Inoculum size (%)	*C*: Moisture content (%)	Protein (Exp.) (mg/g)	Protein (Predict.) (mg/g)
1	5.3	6	70	83.32	76.81
2	5.3	6	70	85.62	76.81
3	3.3	8	60	46.58	50.00
4	7.3	8	80	81.49	80.50
5	7.3	6	70	71.24	76.49
6	5.3	6	60	56.35	64.35
7	3.3	8	80	73.60	77.47
8	5.3	8	70	74.68	72.37
9	5.5	6	80	94.12	76.08
10	3.3	4	60	64.95	56.93
11	3.3	6	70	69.62	73.45
12	7.3	8	60	51.71	50.38
13	7.3	4	60	59.36	59.96
14	3.3	4	80	50.70	50.28
15	7.3	4	80	56.83	53.41
16	5.3	4	70	52.16	63.56
17	5.3	6	70	79.66	76.81

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