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IMPROVEMENT OF
THE NUTRITIVE VALUE OF PALM KERNEL CAKE
BY FUNGAL AND EXTRUSION TREATMENTS

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APPROVAL SHEET

This project entitles "**IMPROVEMENT OF THE NUTRITIVE VALUE OF PALM KERNEL CAKE BY FUNGAL AND EXTRUSION TREATMENTS**" prepared and submitted by FAM JYE PING in partial fulfilment of the requirement for the degree of Bachelor of Science (Honours) in Biochemistry is hereby accepted.

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ABSTRACT

Palm kernel cake (PKC) is an agro-industrial by-product used widely as animal feed, but has limitated use in poultry feeds due to its high fibre content. The objective of the project was to enhance the nutritive value of PKC through solid state fermentation (SSF) using the fungus *Rhizopus oligosporus* and extrusion. In this study, central composite design (CCD) of response surface methodology (RSM) was used to design the fermentation conditions. Four independent variables investigated were moisture content, incubation time, temperature, and inoculum density. Thirty runs with different fermentation conditions were evaluated to treat PKC with *R. oligosporus,* and to measure the interested responses (reducing sugar, soluble protein, crude fibre and neutral detergent fibre). Through the CCD approach, the results were used to produce second order polynomial equations for each response to proceed to the optimization of fermentation condition. The optimized fermentation conditions were moisture level of 68.7 ml/50 g substrate, incubation duration of 5.9 days, incubation temperature of 34°C and inoculum density of $1x10^7$ spore/ml. Under optimized condition, the nutritive values of fermented PKC were 8.25±0.04 mg/g of reducing sugar, 10.48±0.66 mg/g of soluble protein, 15.9±0.78 % of crude fibre, 57.6±0.495 % of neutral detergent fibre as compared to 7.77±0.25 mg/g of reducing sugar, 2.88±0.16 mg/g of soluble protein, 20.42±0.98 % of crude fibre and 73.7±0.72 % of neutral detergent fibre for unfermented PKC. Extruded PKC was also used as substrate for SSF under optimized fermentation condition. The reducing sugar and soluble protein content of fermented extruded-PKC has increased 80.9 % and 138.5 % , while crude fibre and neutral detergent fibre has decreased 23.4% and 23.6%, respectively, as compared to unfermented extruded-PKC. The results showed that the nutritive value of PKC was enhanced by solid state fermentation with *R. oligosporus*, while extrusion process did not improve fermentation further to enhance the nutritive value of PKC.

ABSTRAK

Bungkil isirung sawit adalah hasil sampingan agro-industri yang digunakan secara meluas sebagai makanan haiwan, namun mempunyai penggunaan terhad terhadap makanan haiwan ternakan kerana mempunyai kandungan serat yang tinggi. Objektif projek ini adalah untuk meningkatkan nilai nutrisi pemakanan PKC melalui fermentasi keadaan pepejal dengan menggunakan kulat *Rhizopus oligosporus*. Dalam kajian ini, rekabentuk komposit pusat (CCD) dalam kaedah permukaan sambutan (RSM) telah digunakan untuk merekabentuk keadaan penapaian. Empat pembolehubah yang dikaji ialah kelembapan, tempoh pengeraman, suhu dan ketumpatan inokulum. Tiga puluh eksperimen dengan keadaan fermentasi berbeza telah diuji terhadap *R. oligosporus* pada PKC, dan untuk mengukur empat tindak balas (gula penurun, protein larut, serat kasar dan serat detergen neutral). Dengan menggunakan CCD, keputusan telah digunakan untuk menghasilkan persamaan kuadratik kedua bagi setiap tindak balas untuk pengoptimumankan keadaan penapaian. Keadaan penapaian yang optimum adalah tahap kelembapan 68.7 ml/50g substrat, tempoh inkubasi 5.9 hari, suhu pengeraman 34°C dan kepadatan inokulum daripada $1x10^7$ spora/ml. Pada keadaan optimum, nilai pemakanan PKC ditapai adalah 8.25±0.04 mg/g gula penurun, 10.48 ±0.66 mg/g protein larut, 15.9±0.78 % serat kasar, 57.6±0.50 % serat detergen neutral berbanding dengan 7.77±0.25 mg/g gula penurun, 2.88±0.16 mg/g protein larut, 20.4±0.98 % serat kasar, 73.7±0.72 % serat detergen neutral dalam PKC yang tidak difermentasi. PKC yang tersemperit telah digunakan sebagai substrat untuk SSF di bawah keadaan penapaian yang optimum. PKC yang tersemperit dan difermentasi mengandungi gula penurun dan protein larut 80.6% dan 138.5% yang lebih tinggi, manakala serat kasar dan serat detergen neutral adalah 23.4% dan 23.6% lebih rendah berbanding dengan PKC yang tersemperit dan tidak difermentasi. Hasil kajian menunjukkan bahawa nilai pemakanan PKC telah dipertingkatkan melalui fermentasi keadaan pepejal *R. oligosporus*, manakala proses penyemperitan tidak membantu meningkatkan nilai pemakanan PKC.

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LIST OF ABBREVIATIONS

Abbreviation	Full Interpretation
%	Percent
$(NH_4)_2SO_4$	Ammonium sulphate
°C	Degree celsius
µl	Microlitre
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CCD	Central composite design
DNSA	3,5-dinitrosalylic acid
et al	And others
g	Gram
KH_2PO_4	Potassium phosphate monobasic
mg	Milligram
$MgSO_4 \cdot 7H_2O$	Magnesium sulphate heptahydrate
ml	Millilitre
NDF	Neutral detergent fibre
PKC	Palm kernel cake
RSM	Response surface methodology
SSF	Solid state fermentation
UV	Ultraviolet
v/v	Volume/ volume
Vis	Visible
w/v	Weight/ volume

CHAPTER 1

INTRODUCTION

1.1 Background

The livestock production in Malaysia especially poultry was expanding in the last few years. At present, poultry production depends heavily on imported feeds such as soybean and corn (Raghavan, 2000). The ever increasing costs of feeds affect production cost which in turn burden the consumers.

In Malaysia, palm oil industry is an abundant and significant agricultural sector that contributes toward economy. Malaysia produced 12 million metric tons of palm oil and about 2.5 million metric tons of palm kernel cake (PKC) in 2004 (Young, 2006). Most of the PKC produced in Malaysia are exported to European Union countries, Japan, South Korea and even New Zealand at low prices for the purpose of dairy cattle feed (Hishamuddin, 2001). PKC is widely used as feed ingredient to ruminants (Chin, 2008), but to a limited extent for poultry due to its high fibre content. Due to the rising cost in imported feeds, local poultry producers may need to rely significantly on available feed ingredients like PKC. However, suitable treatments have to be developed to improve its nutritive value before it can be used at a higher inclusion rate in poultry diet.

Many studies have been carried out in order to enhance the nutritive value of PKC. Physical, chemical and biological methods have been employed to enhance the nutrient

composition of PKC. In recent years, solid state fermentation (SSF) by using various bacteria and fungi has been shown to be a practical technique to enrich the nutritive value of PKC. SSF is a process where microorganisms are cultivated on solid substrate in the presence of little amount of water (Van de Lagemaat and Pyle, 2001). The examples of fungi that have been used in SSF are *Aspergillus niger*, *Trichoderma harzianum* and *Rhizopus oryzae* (Ramin *et al*., 2010) and the example of bacteria used is *Bacillus* 7DY7 (Wong *et al*., 2010). In general, the solid-state fermented PKC contains higher protein content and lower cellulose and hemicellulose concentration, but the use of different types of microorganism shows different levels of improvement for each nutrient composition of PKC.

1.2 Problem Statement

The development of poultry production is supported by a large amount of animal feeds. In poultry, 70% of production cost come from feed cost. Malaysia is heavily dependent on imported feedstuff such as corn, soybean meal and fishmeal which costing around RM 2.5 billion every year. PKC has been used mainly as feed for ruminants, but it may become an alternative feed ingredient for poultry, provided it can be nutritionally improved. Suitable methods need to be investigated to overcome the high fibre and low protein content of PKC.

1.3 Hypothesis

The hypothesis of this study was that solid state fermentation using *Rhizopus oligosporus* according to response surface methodology (RSM) and extrusion enhanced the nutritive value of PKC.

1.4 Objectives

The general objective of this particular project was to improve the nutritive value of PKC by solid state fermentation with *Rhizopus oligosporus* based on RSM composite design and extrusion.

The specific objectives were:

1. To optimize fermentation condition using response surface methodology (RSM) with moisture content, incubation duration, temperature and inoculum density as the independent variables.
2. To determine the effect of both extrusion and solid state fermentation on nutrient composition of PKC.

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