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BIOCONVERSION OF PALM KERNEL CAKE AND ITS EVALUATION AS AN AQUAFEED INGREDIENT

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DEDICATED TO

MY HUSBAND

AND

MY SONS

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Palm kernel cake (PKC) is one of the by-products of the oil palm industry. Malaysia being the world's largest producer of oil palm produces over a million tones of PKC annually. Traditionally, PKC is used as an ingredient in ruminant feed and its use for non-ruminants is usually in low amounts due to problems of digestibility. In this study, an attempt was made to microbially enrich the PKC protein content using: *Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Aspergillus niger and Sclerotium rolfsii* fungi. In the solid-state fermentation (SSF), effects of inoculum concentrations (1, 2 and 3%), moisture levels (41, 44 and 47%) and pH levels (3.5, 4.0, 4.5, and 5.0) were evaluated. Protein content of PKC increased significantly ($P \leq 0.05$) coupled with a significant reduction in cellulose and hemicellulose contents by all the fungi used. The highest protein increase of 33% was obtained using *T. longibrachiatum* fermented PKC compared with 18% in unfermented PKC. The effect of moisture content was more critical compared to pH. Fermentation increased the analysed total amino acids (14 to 25%) and mostly the unsaturated ones (oleic and linoleic acids). The extracellular enzymes activity

secreted, such as β-D-mannanase, β-mannosidase, α-galactosidase, endoglucanase, and filter paper cellulase (Fpase) by hydrolysing of PKC polysaccharides was evaluated by monitoring the amount of reducing sugar released the crude enzyme extracts. A wide range of enzyme activities was obtained from the various fungi, while the amount of reducing sugar released ranged from 0.05 g/ml for *S. rolfsii* to 1.05 g/ml for *A. niger*. The *S. rolfsii* and *A. niger* were good producers of mannan-degrading enzymes when cultured with enriched nutrient medium containing PKC as the carbon source. The scaled up protein enrichment of PKC using SSF was carried out using *T. longibrachiatum* in the plastic bag and in a rotating drum bioreactor made from transparent PVC at 47% moisture level and pH 4.5. The bioreactor fermented PKC gave a protein (31.78%) value higher than that of plastic bag fermentation due the difficulty in controlling temperature and insufficient of aeration of the latter. The formulation of test diets for fish using red tilapia was done by incorporation of 10 to 40% of fermented PKC in the diets. Feed digestibility as well as overall growth of fish was evaluated for eight-weeks. Digestibility of diets (53 to 74%) was lower in comparison with reference diet (81%). Fish on test diets also showed lower body weight gain (53 to 105%) compared to reference diet (114%) and inferior feed conversion ratio (3.0 to 5.3), compared to reference diet (2.9). However, carcass of fish on test diets had higher phosphorus and calcium content compared to those on reference diet. In conclusion, PKC is a good carbon source for the production of cellulose and hemicellulose degrading enzymes. Its protein content can be increased through fungal fermentation and fish feeds can contain this protein enriched PKC up to 10% of diet as a partial fish meal and binder replacement.

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BIOPENUKARAN HAMPAS ISIRONG SAWIT DAN PENILAIANNYA SEBAGAI BAHAN MAKANAN AKUAKULTUR

Oleh

ILUYEMI FLORENCE BUCHI

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Hampas isirong sawit adalah salah satu hasil sampingan industri kelapa sawit. Malaysia merupakan pengeluar kelapa sawit utama dunia dengan menghasilkan lebih satu juta ton isirong sawit setiap tahun. Secara tradisinya, hampas isirong sawit ini digunakan sebagai bahan dalam kandungan makanan ruminan dan dimakan dalam jumlah yang rendah untuk makanan bukan-ruminan kerana masalah penghadaman. Dalam kajian ini, usaha telah dilakukan bagi memperkayakan kandungan protein hampas isirong sawit secara mikrob dengan menggunakan: kulat *Trichoderma harzianum, Trichoderma koningii, Trichoderma longibrachiatum, Aspergillus niger and Sclerotium rolfsii*. Dalam keadaan fermentasi pepejal (SSF), kesan kepekatan inoculum (1, 2 dan 3%) tahap kelembapan (41, 44 dan 47%) dan tahap pH (3.5, 4.0, 4.5, dan 5.0) telah dinilai. Kandungan protein hampas isirong sawit bertambah dengan ketaranya ($P \leq 0.05$) diikuti dengan pengurangan yang serupa dalam kandungan selulosa dan hemiselulosa dengan menggunakan kesemua kulat tersebut. Pertambahan protein yang tertinggi sebanyak 33% telah diperolehi dengan

menggunakan hampas isirong sawit yang difermentasikan dengan *T. longibrachiatum* jika dibandingkan dengan 18% dalam isirong sawit yang tidak difermentasikan. Kesan kandungan kelembapan didapati lebih kritikal dibandingkan dengan pH. Fermentasi telah meninggikan jumlah asid amino (14 ke 25%) dan kebanyakannya yang tidak tepu (asid olik dan linolik). Aktiviti enzim ektraselular yang dikeluakan, seperti β-D mannanase, β-mannosidase, α-galactosidase, endoglucanase and Fpase melalui hidrolisis hampas isirong sawit telah dinilai dengan melihat jumlah gula terturun yang dikeluarkan dari ekstrak enzim mentah. Berbagai aktiviti enzim telah didapati daripada pelbagai kulat dengan jumlah gula terturun yang dikeluarkan dalam lingkungan 0.05 g/ml untuk *S. rolfsii* ke 1.05 g/ml untuk *A. niger*. *S. rolfsii* dan *A. niger* merupakan pengeluar terbaik bagi enzim mannan apabila dikultur dengan media yang diperkayakan dengan hampas isirong sawit sebagai sumber karbon. Peningkatan kandungan protein hampas isirong sawit dengan menggunakan SSF telah dijalankan dengan menggunakan *T. longibrachiatum* di dalam karung plastik dan dalam drum bioreaktor yang berputar yang diperbuat daripada PVC lutsinar pada tahap kelembapan 47% dan pH 4.5. Kandungan protein hampas isirong sawit yang difermentasi di dalam bioreaktor adalah lebih tinggi jika dibandingkan dengan fermentasi dalam karung plastik oleh kerana kesukaran untuk mengawal suhu dan kekurangan pengudaraan. Formulasi ujian pemakanan dengan menggunakan anak ikan tilapia merah dilakukan dengan memasukan 10 ke 40% isirong sawit difermentasi dalam pemakanan tersebut. Penghadaman makanan dan pertumbuhan ikan secara keseluruhannya telah dinilai selama lapan minggu. Penghadaman pemakanan (53 ke 74%) adalah rendah jika dibandingkan dengan bahan makanan rujukan (81%). Bahan makanan yang diuji juga menunjukkan kadar

pertambahan berat bedan yang rendah (53 ke 105%) berbanding dengan bahan makanan rujukan (114%) dan nisbah kadar perubahan pemakanan yang rendah (3.0 ke 5.3) berbanding dengan makanan rujukan (2.9). Walau bagaimanapun, karkas ikan yang diuji mengandungi unsur fosforus dan kalsium yang tinggi dibandingkan dengan yang berada dalam ikan yang dirawat dengan bahan makanan rujukan. Sebagai kesimpulannya, hampas isirong sawit adalah suatu sumber karbon yang baik untuk pengeluaran enzim penguraian selulosa dan hemiselulosa. Kandungan proteinnya boleh ditambah melalui fermentasi kulat dan pemakanan ikan boleh mengandunyi isirong yang diperkaya sehingga tahap 10% sebagai penggantian sebahagian daripada makanan ikan dan pengikat.

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	: Acid detergent Fibre
ADL	: Acid detergent Lignin
ANOVA	: Analysis of variance
BW	: Body weight
CF	: Crude Fibre
CP	: Crude Protein
D	: Diet
DE	: Digestible Energy
DM	: Dry matter
EE	: Ether Extract
FAO	: Food and Agricultural Organization of the United Nations
FCR	: Feed conversion ratio
GC	: Gas Chromatography
HPLC	: High Pressure Liquid Chromatography
LSD	: Least Significant Difference
LSF	: Liquid solid fermentation
NDF	: Neutral detergent Fibre
NSP	: Non-starch polysaccharides
PER	: Protein efficiency ratio
PKC	: Palm kernel cake
PITC	: Phenyl-isothiocyanate
PORLA	: Palm Oil Registration and Licensing Authority

PTC : Phenyl-thiocarbamine

PUFA : Polyunsaturated fatty acid

SGR : Specific growth rate

SSF : Solid state fermentation

TL-PKC : *Trichoderma longibrachiatum* fermented palm kernel cake

UPM : Universiti Putra Malaysia

CHAPTER I

INTRODUCTION

The concept of bioconversion of organic solid substrates into useful products is not new. It has been used for many centuries, long before the underlying microbiological, biochemical and bioengineering principles were understood. Thus, the early developments were based on local traditional practices, rather than on accurate scientific basis. Recently, a lot of scientific research and developments are being carried out either to upgrade or optimise traditional technologies or to develop new ones for maximum utilization of organic solid substrates for the production of feed, food, fuels and fertilizers. At present, many types of processes are being carried out on large scale. Examples are indigenous fermented foods (Steinkraus, 1983), mushroom production (Hatch and Finger, 1979), composting (Diaz et al., 1982), production of fuels, such as methane and ethanol (NRC, 1981), and the production of certain enzymes (Kim et al., 1985). Production of microbial biomass for animal feed has been carried out mainly on pilot scale (Durand and Chereau, 1988).

Lignocellulosic materials are the main organic substrates that are being used for the production of microbial biomass. This is because most of the organic carbon that is produced as plant biomass is accumulated in lignocelluloses, which is the main structural component of plant cell wall. Chemically, lignocelluloses materials are

composed of three major components, i.e. extraneous substances, polysaccharides and lignin (Janes, 1969). Extraneous substances are made up of ash, terpenes, resins and phenols (Rydholm, 1965; Janes, 1969). The polysaccharide component is comprised of mainly cellulose, some hemicellulose together with small quantities of starch, pectin and water-soluble polysaccharides, such as arabinogalactans. Lignin is essentially a 3-dimensional phenylpropane polymer with phenylpropane units held together by ether and carbon-carbon bonds. In lignocelluloses materials, the percentage of lignin can be as high as 29% in conifers and 26% in broad-leaved species on a dry matter basis (Leonowicz et al., 1988).

Palm kernel cake (PKC) which is a residue obtained from the extraction of palm kernel oil is an abundant and low-cost organic raw material in Malaysia as well as other countries where oil palm is cultivated and processed in large scale. Presently, Malaysia is the largest producer of oil palm. The cultivated area is estimated at about 2.3 million hectares with production of oil registering 8 million tonnes in 2000 (PORLA, 2001). The PKC production has been increasing correspondingly. For example, in 1999, 1.2 million tonnes of PKC was produced. This value however has increased to 1.6 million in the year 2000. The PKC therefore represents an under-utilized residue in Malaysia. The polysaccharides of most lignocelluloses, which are composed of predominantly cellulose, produce glucose as the major monomer upon complete hydrolysis. In contrast, polysaccharides of PKC produced mainly mannose and some quantities of glucose and galactose upon complete hydrolysis. This is because PKC polysaccharides are composed mainly of hemicelluloses, which are

essentially linear mannan, closely bound to small quantities of galactomannan (Daud and Jarvis 1992).

The complexity of lignocelluloses reduces their nutritional quality for animal consumption (Kim, 1981). Microbiological technologies can help upgrade such lignocelluloses substrates to increase their biological value in animal feeding. In nature, microorganisms, mainly bacteria and fungi live on lignocelluloses. Therefore, upgrading usually involves the conversion of these lignocelluloses to microbial biomass by cultivating fungi, yeasts or bacteria on them with or without pre-treatment of the lignocelluloses substrate (Zadrazil, 1980; Taniguchi et al., 1982). Solid-state cultivation of microorganisms is the preferred method since it mimics the conditions that are prevalent under natural conditions. During solid-state fermentation, microbial growth is carried out on water insoluble substrates in the absence of free water. This process offers some advantages, such as simpler technology, use of a more concentrated substrate, low waste water output and improved product recovery since the solid mass coming out of the bioreactor would be the final product.

Bacteria and yeasts grow on the surface film of these solid substrates much like in free liquid. Filamentous fungi, however, can grow in the absence of free water, utilizing the bound water of the substrate (Tengerdy, 1985). Therefore, fungi are the most efficient decomposers of lignocelluloses. Various species belonging to the genera *Aspergillus, Penicillum, Trichoderma* and *Fusarium* are commonly used. The fungi most investigated for animal feed are *Trichoderma spp*. (Tengerdy, 1985),