UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF MANNAN-DEGRADING ENZYMES BY *ASPERGILLUS NIGER* IN SOLID STATE AND SUBMERGED FERMENTATION FOR HYDROLYSIS OF PALM KERNEL CAKE

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By

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

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The production of mannan degrading enzymes under solid substrate fermentation and liquid fermentation by *Aspergillus niger* FTCC 5003 were carried using palm kernel cake (PKC) as the sole carbon source. The crude enzymes produced were then used in the enzymatic hydrolysis of PKC. In the preliminary study, it was observed that mannanase was the highest enzyme activity produced. Other enzymes produced were, amylase, xylanase, polygalacturonase and cellulases. Types of inoculum and extraction temperature play an important role in the production of mannanase under solid substrate fermentation (SSF). The optimum parameters for the mannanase production in submerged fermentation (SmF) under shake flask condition were 1×10^4 spores/mL, with 2% (w/v) of PKC incubated at 35ºC, and agitated at 200 rpm. While the optimum parameters for mannanase production in SSF were 1×10^6 spores/mL, with 50% (v/w) of initial moisture content, 2% of nitrogen in urea and incubated at 30ºC. Mannanase produced under optimum condition was 104 U/mL (SmF) and 1705 U/g PKC (SSF). Mannanase obtained from SSF was more heat stable as compared to

those obtained from SmF. Both types of mannanase had the same optimum pH, which was pH 7 and both had two different optimum temperatures, which were 30°C and 50°C for SmF; and 35°C and 55°C for SSF. For the enzymatic hydrolysis of PKC, the optimum condition for the hydrolysis was 10% of PKC, 100 U/mL enzyme concentration, at 45°C and pH 6.5 when mannanase obtained from SmF was used. On the other hand, when mannanase from SSF was used the optimum condition was obtained at 45°C, pH 7.5 with 10% PKC and 50 U/mL enzyme concentration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN ENZIM PENGHURAIAN MANAN MELALUI FERMENTASI KEADAAN PEPEJAL DAN CECAIR OLEH *ASPERGILLUS NIGER* UNTUK MENGHIDROLISIS HAMPAS ISIRONG KELAPA SAWIT

Oleh

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Penghasilan enzim penghuraian manan oleh *Aspergillus niger* melalui fermentasi substrate pepejal (SSF) and fermentasi cecair (SmF) dengan menggunakan hampas isisarong kelapa sawit sebagai sumber karbon utama. Enzim kasar yang dihasilkan digunakan untuk menghidrolisiskan PKC. Dalam permulaan penyelidikan ini, didapati mananase adalah enzim yang tertinggi dihasilkan. Enzim lain seperti amilase, xylanase, poligalaktoranase and celulase juga dihasiklan. Jenis inokulum dan suhu pnegekstrakan memainkan peranan penting dalam penghasilan mananase melalui SSF. Parameter optimum untuk penghasilan mananase melalui SmF dalam keadaan kelalang kon dengan menggunakan spora kepekatan 1×10^4 spore/mL dengan 2% (i/b) PKC, pengeraman pada suhu 35°C dan digoncang pada 200 rpm. Manakala parameter optimum untuk penghasilan mananase melalui SSF adalah dengan menggunakan spora kepekatan 1 x 10^6 spora/mL, 50% (i/b) kandungan kelembapan permulaan, 2% sumber nitrogen daripada urea dan pengeraman pada 30°C. Pada keadaan optimum, aktiviti mananase yang diperolehi adalah 104 U/mL (LF) dan 1705

U/g PKC (SSF). Mananase yang dihasilkan melalui SSF adalah lebih tahan kepada suhu bebanding dengan proses SmF. Selain itu, didapati bahawa kedua-dua mananase mempunyai pH optimum pada pH 7 dan kedua-dua enzim mempunyai dua suhu optimum, iaitu pada 30°C and 50°C untuk SmF; dan pada 35°C serta 55°C untuk SSF. PKC seterusnya digunakan untuk proses hidrolisis berenzim menggunakan enzim mannanase kasar yang dihasilkan melalui SmF dan SSF. Keadaan optimum untuk proses hidrolisis berenzim dengan menggunakan manannase yang dihasilkan melaluli proses SmF adalah 10% PKC, 100 U/mL enzim, pada suhu 45°C dan pH 6.5. Manakala keadaan optimum proses hidrolisis berenzim dengan menggunakan manannase yang diperolehi melaluli SSF adalah pada 45°C dan pH 7.5 dengan 10% PKC dan 50 U/mL enzim.

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I certify that an Examination Committee has met on 26 December 2006- to conduct the final examination of Lisa Ong Gaik Ai on her Doctor of Philosophy thesis entitle "Production of Mannan-degrading Enzymes by *Aspergillus niger* in Solid State and Submerged Fermentation for Hydrolysis of Palm Kernel Cake" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the Candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 14 JUNE 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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LISA ONG GAIK AI

Date: 23 APRIL 2007

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LIST OF ABBREVIATIONS

A_w	Water activity
BSA	Bovine serum albumin
CMCase	Carboxymethyl Cellulase
DNS	Dinitrosalicylic acid solution
DP	Degree of polymerisation
EU	European Union
FPase	Filter paper assay
FTCC	Food Technology Culture Collection
MARDI	Malaysia Agricultural Research and Development Institute
PKC	Palm kernel cake
PUF	Polyurethane foam
SmF	Submerged fermentation
SSF	Solid substrate fermentation

CHAPTER 1

INTRODUCTION

Malaysia is the world's largest producer of palm oil. According to MPOB (2005), oil palm alone covered an area of 3.80 million hectares in 2003. Most of the hectare belongs to the private sector which account for about 59% of total hectare while another 31% are government sponsored schemes. The smallholder constitutes only 10%. The by product from the palm oil industry especially in the extraction of palm kernel oil is the palm kernel cake (PKC). Most of the PKC are exported or being used locally as supplement in animal feed, which is normally used for ruminant. From the countries that import PKC, European Union (EU) countries absorb more than 85% of Malaysian PKC annually and the Netherlands is the biggest importer of Malaysian PKC among the EU countries. Asian countries which import Malaysian PKC are South Korea and Japan. In 2000, approximately 1.1 million tonnes or 90% of Malaysian PKC exports to the EU were taken by the Netherlands (Aspar, 2001).

The by-product of oil palm that is palm kernel cake is fibrous in nature. Degradation of fibre into simpler carbohydrate improves the nutritive value of the by-products for poultry. Natural degradation of fibre in the open and in the rumen involves many different microbes (Tenkanen *et al.*, 1997; Mohd-Jaafar *et al.*, 2001). Numerous sources of microbial cocktails are available commercially for speeding up the degradation process. Under proper fermentation condition, the degradation period may be reduced further with possible increase in efficiency.

PKC is obtained as a by-product after solvent or mechanical extraction of the oil from the palm kernel. In Malaysia, mechanical extraction by screw press is the most widely used. The solvent extraction process is generally not used currently due to its higher cost (Aspar, 2001). The 5-8 % fat content (Alimon, 2004) makes PKC as an energy feed. PKC also contains approximately 16% fibre. Fibre is considered an essential nutrient for dairy cattle, since cattle fed on insufficient fibre often develop metabolic or digestive problems (Miller and O'Dell, 1969). PKC is a valuable feed meal and a useful source of protein and energy for livestock and it is commonly used in animal feed for ruminants (Hutagulung, 1981; Awaludin, 2001), swine and poultry (Siew and Noraini, 1992).

Fishmeal and maize (corn), which constitute the major portion of poultry rations, are rather expensive and are the main reason for the high cost of diets. Attempts to cut cost have revolved around finding cheap and available substitutes, generally of agro-industrial origin. Brewers dried grains, wheat bran, oil palm slurry, and poultry manure are among the by-products that have been tried and have shown great promise (Osei and Amo, 1987). PKC has largely been neglected as feed ingredient for poultry. Nevertheless Nwokolo *et al*. (1976) found PKC to be a good source of protein for poultry. McDonald *et al*. (1982) suggested that PKC in the diets of poultry should be limited to 20%.

PKC has become a draw back for monogastric livestock especially poultry because the cell wall of PKC is composed of mainly linear and high crystalline mannan and a small quantity of galactomannan (Mohd-Jaafar and

Jarvis, 1992). Utilisation of filamentous fungi in solid substrate fermentation (SSF) for fibre digestibility improvement has been widely applied (Raimbault, 1998). *Aspergillus* and *Rhizopus* sp. are among the most important microorganisms used in SSF process due to their physiological, enzymological and biochemical properties and also due to the wide application in food and feed production (Noraini *et al.*, 2001). Besides that, *Aspergillus* has been reported by Ong *et al*. (2004), to produce abundant mannanase in the presence of PKC as sole carbon source in SSF. Enzymatic degradation of PKC now becomes an important and popular method in increasing PKC digestibility. The application of enzymes in hydrolysing mannan and galactomannan in poultry feed has been reported by Noraini *et al.* (2000).

A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilise enzymes at some stage or the other. Current developments in biotechnology are yielding new applications for enzymes. SSF holds tremendous potential for the enzymes production. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Pandey *et al.*, 1999). In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformation involving organic solvent media, mainly for bioactive compounds (Pandey, 1992a; Pandey *et al.*, 1999). This research was therefore concerned with the cultivation of selected fungus on PKC under submerged (SmF) and solid substrate (SSF) fermentation conditions. It was expected that the fungus will utilise the PKC by producing enzymes to degrade the polysaccharide content.

Hence, the objectives of this study were to:

i. screen for hydrolytic enzymes produced by *Aspergillus niger* under solid substrate fermentation (SSF) using palm kernel cake (PKC) as substrate,

ii. optimise the production of mannan-degrading enzyme through submerged and solid substrate fermentation by *A. niger* using PKC as substrate,

iii. partially characterise the crude enzymes produced,

iv. optimise the parameters for the hydrolysis of PKC using the crude enzymes preparation obtained from both techniques of fermentation.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Kernel Cake (PKC)

Palm kernel cake (PKC), is a by-product from kernel oil extraction, which constitutes about 50% of the original palm kernel. It can be obtained by either solvent or mechanical extraction. The cake produced by solvent extraction is dry, gritty and unpalatable to animals. However, it is less subjected to rancidity due to lower oil content and it can be stored for longer time than that processed by screw press (Yeong *et al*., 1981). The left over residue is usually dark brown in colour and has a pleasant odour (Babjee *et al*., 1985).

PKC or sometimes referred to as palm kernel expeller (PKE) is obtained as a by product from the extraction of palm kernel oil via the mechanical process. The production of PKC involves the grinding of palm kernels followed by screw pressing with or without an intermediary flaking and cooking stages (Tang, 2001). Figure 2.1 showed the simplified flow chart on the mechanical extraction of palm kernel oil.

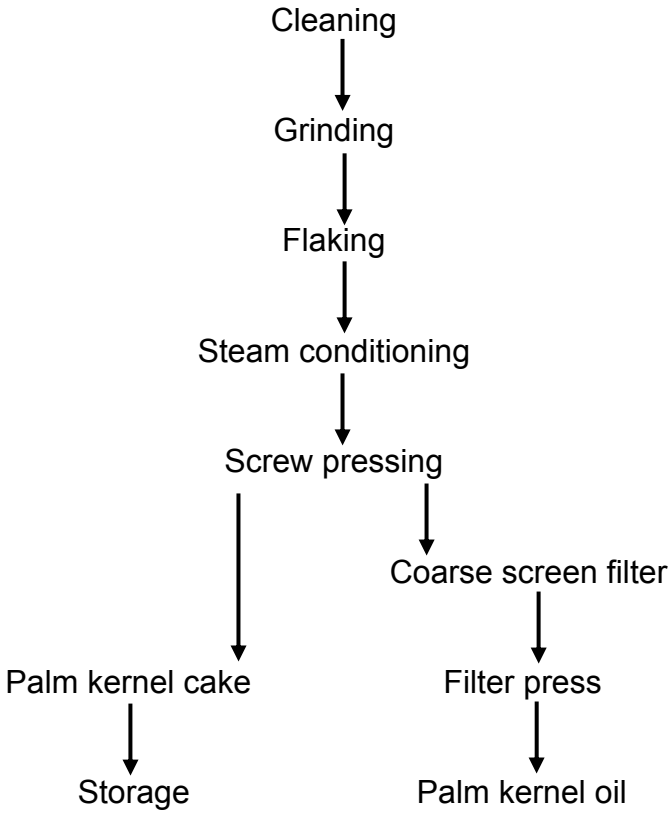

Figure 2.1 Mechanical extraction of palm kernel oil

2.1.1 General Characteristic and Nutritional Values of Palm Kernel Cake

PKC is valuable in supplying protein and energy as the proximate analyses of PKC are shown in Table 2.1. It is being exported to Europe for feeding dairy cattle. PKC contains about 16 - 18% crude protein, which is higher than that in rice bran. The nutritive values are found to be close to rice bran, wheat bran and coconut oil meal (Yeong *et al*., 1981; Mat, 1983). Nevertheless, the protein content of PKC is considered sufficient to meet the requirements of most ruminants. To some extent, the protein level in PKC may also meet the requirement of certain classes of poultry, such as breeder and layer hens, provided that lysine and methionine are supplemented (Alimon, 2004).