



UNIVERSITI PUTRA MALAYSIA

**FERMENTATION OF PALM KERNEL EXPELLER USING LOCAL FUNGAL
ENZYMES FOR PRODUCTION OF POTENTIAL PREBIOTICS**

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By

CHEN WEI LI

**Thesis Submitted to the School of Graduate Study, Universiti Putra Malaysia in
Fulfilment of the Requirement for the Degree of Master of Science**

January 2014

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Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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January 2014

Chairperson: Liang Juan Boo, PhD
Institute: Institute of Tropical Agriculture

The rapid expansion of livestock industry to meet the increasing demand for animal protein has stimulated the search for appropriate agro by-products as alternative feed resources. Among the available by-products, palm kernel expeller (PKE) has been identified to be of great potential, because large quantity of this by-product from the palm oil industry is being produced annually in Malaysia. Many attempts have been made to reduce the high non-starch polysaccharides (NSP) of PKE to a tolerable level for monogastric animal, particularly poultry diets. This includes the use of exogenous enzymes, which has been proven rather successful in reducing the NSP contents, but when the enzyme treated PKE was fed to the animal, results in term of animal performance was inconsistent. In addition, scanty research data suggested that PKE can be a potential source for prebiotic instead of only as a source of dietary energy for monogastric animals. Thus, to overcome the current over-dependent on the use of commercial enzymes, which are normally imported at high cost to hydrolyse the NSP, the primary objective of this thesis was to increase the production of prebiotic oligosaccharides from PKE by using enzyme produced by indigenous fungi isolated from PKE itself.

Ten fungi were isolated from local PKE. From a preliminary screening, three of the ten isolates which had the highest cellulase, mannanase, and xylanase activity were selected and identified using molecular analysis of fungal internal transcribed spacer (ITS) region. One isolate showed similarities to *Paecilomyces variotii*, while the other two showed similarity to *Aspergillus terreus*. Enzymes extracted from each of the above strain were tested for their ability to degrade PKE, as measured by the total reducing sugar production at the end of the solid state fermentation (SSF). Enzymes produced from isolate *A. terreus K1* were most efficient in degrading the NSP of PKE into soluble sugar was selected for subsequent optimization process.

Response surface methodology (RSM) was employed to optimize critical factors (temperature, moisture, inoculum, and pH) that affect SSF. A four-factor and five-level central composite design (CCD) was employed. Using PKE as sole substrate, a maximum cellulase (18.05 U/g DM) was produced at 30 °C, 61% moisture, pH 5.3, and 7.5×10^5 spores/g PKE while maximum mannanase activity (42.03 U/g DM) was obtained at 31 °C, 61% moisture, pH 6.4, and 6% spores, whereas maximum xylanase (339.68 U/g DM) was obtained at 29 °C, 70% moisture, pH 4.6, and 7.7×10^5 spores/g PKE. In order to maximize the co-production of all the three enzymes

simultaneously, CCD with three responses was used. It was predicted that maximum endoglucanase, mannanase, and xylanase (17.37, 41.24, and 265.57 U/g DM, respectively) can be produced by fermenting PKE at 30.5 °C, 62.7% moisture, pH 5.8, and 6.0×10^5 spores/g PKE. Verification of the predicted condition was conducted in triplicate, and the enzyme activities obtained (19.97, 44.12, and 262.01 U/g DM, respectively) were close to the predicted values.

The effectiveness of enzyme treatment to improve the prebiotics potential of PKE was evaluated using rats as animal model. Data of HPLC analysis showed that monosaccharides content increased 3 folds in the PKE_{ENZ} (enzyme-treated PKE) and 4 folds in SPKE_{ENZ} (steam + enzyme treated PKE) treatments as compared to the untreated PKE. These increases were reflected by the higher mannanoligosaccharides in the PKE_{ENZ} and SPKE_{ENZ} samples (3.95 and 8.07 mg/g PKE, respectively) compared to untreated PKE-extract (1.79 mg/g PKE). *In vitro* study showed that the different PKE-extracts used in this study were able to support growth of three different strains of *Lactobacillus sp.*. However, their growth varied significantly among species and PKE-extracts used ($P < 0.05$), with *L. brevis* I 218 had the highest growth compared to the other two strains (*L. salivarius* I 24 and *L. gallinarum* I16), and its growth was the highest when incubated in the SPKE_{ENZ} extract. In order to confirm the results of the *in vitro* study, an *in vivo* study were conducted using Sprague-Dawley rats fed standard rodent diet supplemented with the different PKE extracts. Results of the *in vivo* study showed that all the PKE-extracts tested can support growth of beneficial bacteria (*Lactobacillus* and *Bifidobacterium*), however, only rats in the SPKE_{ENZ} treatment group had significantly higher population of *Lactobacillus* and *Bifidobacterium* and lower population of *E. coli* compared to the control group.

In conclusion, this thesis revealed that enzyme produced from fungi isolated from local PKE can be used to hydrolyse the NSP of PKE into mono- and oligo-saccharides and provided *in vitro* and *in vivo* data to show that they possess prebiotic properties. Comparison between different treatment-methods of PKE showed that pre-treatment by steaming prior to enzymatic treatment could further enhance the beneficial effects of PKE-extract as prebiotics.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAPAIAN ISIRONG KELAPA SAWIT DENGAN MENGGUNAKAN ENZIM KULAT TEMPATAN BAGI PENGHASILAN PREBIOTIK

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Perkembangan yang pesat dalam industri penternakan untuk memenuhi permintaan protein haiwan telah mendorong kecenderungan untuk mencari produk sampingan agro yang sesuai sebagai sumber makanan alternatif. Antaranya ialah ekstrak isirong kelapa sawit (PKE) yang telah dikenalpasti mempunyai potensi yang tinggi kerana ia telah dihasilkan dalam kuantiti yang banyak oleh industri kelapa sawit setiap tahun di Malaysia. Banyak percubaan penyelidikan telah dijalankan untuk mengurangkan kandungan polisakarida bukan kanji (NSP) ke tahap yang boleh diterima oleh haiwan monogastrik, terutamanya sebagai makanan ternakan. Ini merangkumi penggunaan enzim luaran yang telah terbukti agak berjaya dalam mengurangkan kandungan NSP, tetapi memberi keputusan yang tidak konsisten dari segi prestasi pertumbuhan haiwan. Selain itu, beberapa data penyelidikan mencadangkan bahawa PKE berpotensi untuk digunakan sebagai sumber prebiotik selain daripada penggunaannya sebagai sumber tenaga. Oleh itu, untuk mengatasi pergantungan yang berlebihan kepada enzim-enzim komersial yang biasanya diimport pada kos yang tinggi untuk hidrolisis NSP, objektif utama kajian ini adalah untuk meningkatkan penghasilan prebiotik oligosakarida dengan menggunakan enzim yang dihasilkan oleh kulat yang diasingkan daripada PKE.

Sepuluh kulat telah dipencil daripada PKE. Dari pemeriksaan awal, tiga kulat daripada sepuluh kulat yang mempunyai aktiviti selulase, mananase, dan xilanase tertinggi dipilih dan dikenalpasti berasaskan teknik analisis molekul "Internal Transcribed Spacer" (ITS) kulat tersebut. Berdasarkan keputusan analisis, isolat F3 menunjukkan persamaan dengan spesies *Paecilomyces variotii*, manakala isolat F4 dan K1 menunjukkan persamaan dengan spesies *Aspergillus terreus*. Keupayaan degradasi PKE bagi setiap enzim yang diekstrak daripada sepuluh isolat tersebut telah diuji berdasarkan penghasilan jumlah kandungan gula penurun selepas fermentasi keadaan pepejal (SSF). Daripada hasil ujian atas, enzim ekstrak dari isolat K1 (*A. terreus* K1) telah menunjukkan keupayaan degradasi PKE kepada gula pelarut yang paling cekap dan ia dipilih untuk proses optimasi faktor-faktor fermentasi yang berikutnya.

Kaedah "Response Surface Methodology" (RSM) telah digunakan untuk mengoptimasi faktor-faktor kritikal (suhu, kelembapan, inokulum, dan pH) yang mempengaruhi kecekapan SSF. "Central composite design" (CCD) telah dirangka bagi keempat-empat faktor pada lima tahap. Dengan menggunakan PKE sebagai

substrat tunggal, aktiviti selulase maksimum (18.05 U/g DM) telah dihasilkan melalui fermentasi pada suhu 30 °C, kelembapan 61%, pH 5.3 dengan menggunakan 7.5×10^5 spora/g PKE. Aktiviti mananase maksimum (42.03 U/g DM) telah diperolehi melalui fermentasi PKE pada suhu 31 °C, kelembapan 61%, pH 6.4 dengan menggunakan 6.0×10^5 spora/g PKE, manakala aktiviti xilanase maksimum (339.68 U/g DM) telah dicapai pada suhu 29 °C, kelembapan 70%, pH 4.6 dengan menggunakan 7.7×10^5 spores/g PKE. Bagi memaksimumkan pengeluaran ketiga-tiga enzim secara serentak, analisis dilakukan dengan menggunakan ketiga-tiga enzim aktiviti sebagai respon. Adalah diramalkan aktiviti selulase, mananase dan xilanase maksimum (17.37, 41.24, dan 265.57 U/g DM, masing-masing) boleh dicapai dengan menfermentasi PKE pada suhu 30.5 °C, kelembapan 62.7%, pH 5.8, dan 6% *A. terreus* K1 spora. Pengesahan ramalan faktor-faktor fermentasi telah dijalankan sebanyak tiga kali dan hasil kajian telah menunjukkan aktiviti ketiga-tiga enzim yang diperolehi (19.97, 44.12, dan 262.01 U/g DM, masing-masing) adalah berdekatan dengan nilai-nilai yang diramalkan.

Keberkesanan rawatan enzim untuk meningkatkan potensi PKE sebagai prebiotik telah dinilai dengan menggunakan tikus sebagai model haiwan. Analisis HPLC menunjukkan bahawa kandungan monosakarida meningkat 3 kali ganda selepas fermentasi PKE dengan enzim (ekstrak PKE_{ENZ}) dan 4 kali ganda selepas fermentasi PKE (autoklaf) dengan rawatan enzim (ekstrak SPKE_{ENZ}) berbanding ekstrak PKE tanpa rawatan enzim. Peningkatan kandungan monosakarida ini juga serasi dengan keputusan kandungan mannanoligosakarida dalam ekstrak PKE_{ENZ} dan ekstrak SPKE_{ENZ} (3.95 dan 8.07 mg/g PKE, masing-masing) berbanding ekstrak PKE (1.79 mg/g PKE). Dalam kajian *in vitro*, ketiga-tiga ekstrak PKE dapat menyokong pertumbuhan *Lactobacillus sp.* Walaubagaimanapun, kadar pertumbuhan berbeza antara spesies dan ekstrak PKE ($P < 0.05$), di mana *L. brevis* I 218 menunjukkan pertumbuhan yang terbaik berbanding dengan spesies yang lain (*L. salivarius* I 24 dan *L. gallinarum* I 16), dan pertumbuhan tertinggi dicatatkan apabila *L. brevis* I 218 dieram dalam ekstrak SPKE_{ENZ}. Untuk mengesahkan keputusan *in vitro*, kajian *in vivo* telah dijalankan dengan menggunakan tikus Sprague-Dawley yang diberi makanan tikus piawai dengan ekstrak PKE yang berlainan. Keputusan kajian *in vivo* menunjukkan bahawa ketiga-tiga ekstrak PKE boleh menyokong pertumbuhan mikroflora (*Lactobacillus* dan *Bifidobacterium*). Walaubagaimanapun, hanya tikus yang diberi makanan ekstrak SPKE_{ENZ} menunjukkan peningkatan populasi *Lactobacillus* dan *Bifidobacterium* yang tertinggi, dan populasi *E. coli* yang terendah berbanding dengan kumpulan kawalan.

Kesimpulannya, tesis ini menunjukkan enzim yang dihasilkan dari kulat yang dipencilkan daripada PKE boleh digunakan untuk mendegradasi polisakarida bukan kanji (NSP) kepada mono- dan oligo- sakarida. Kajian *in vitro* dan *in vivo* telah menunjukkan ekstrak PKE boleh digunakan sebagai sumber prebiotik. Manakala, perbandingan antara cara pra-rawatan PKE menunjukkan bahawa pra-rawatan secara autoklaf sebelum rawatan enzim diperlukan untuk menyerlahkan faedah-faedah ekstrak PKE sebagai prebiotik dengan sepenuhnya.

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I certify that a Thesis Examination Committee has met on 10 January 2014 to conduct the final examination of Chen Wei Li on her thesis entitled "Fermentation of palm kernel expeller using local fungi enzymes for production of potential prebiotics" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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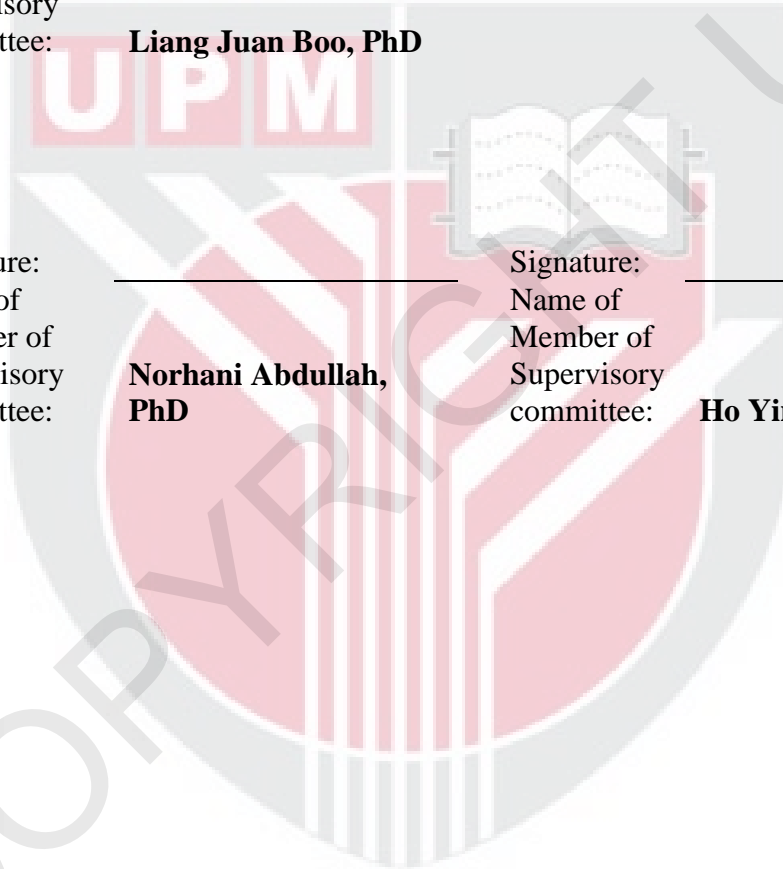


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