UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CRUDE AND PARTIALLY PURIFIED MANNANASE ON THE FIBRE CONTENT OF PALM KERNAL CAKE/ EXPELLER

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By

SAFARUL BIN MUSTAPHA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Dr. Suraini Abd Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Palm Kernel Cake/Expeller (PKC/E) is an agricultural waste of palm oil mill which contributed about 2.2 million tones in 2007 in Malaysia. It is an important ingredient for the formulation of animal feed instead of corn and soybean husk. The PKC/E fibre composed mainly of galactomannan type of hemicelluloses, which can barely be consumed by non-ruminant (monogastric) animals. Mannanase is a major extracellular enzyme which is secreted to convert mannan into simple sugar of mannose. In this study, *Aspergilus niger* FTCC 5003, a mannanase producer and PKC/E as a sole carbon source were fermented in 500 mL shake flask using the optimised parameter conditions for 10 days and the culture broth were collected for hydrolysis purpose. Purification of the mannanase from submerged fermentation of PKC/E obtained from fermentation *A. niger* FTCC 5003 was also carried out. Crude and partially purified enzymes were employed in this study to degrade PKC/E using enzymatic hydrolysis technique to compare with fermented PKC/E. The characteristic of palm kernel cake under fermented and hydrolysed conditions were studied under Scanning Electron Microscope

(SEM) and the Near Infrared System (NIR) was used to perform proximate analysis of quantitative parameter.

SEM showed fermented PKC surface structure were significantly degraded compared with the hydrolyzed palm kernel. The results of Near infrared reflectance spectroscopy (NIRS) proved that the crude fiber (CF) of palm kernel cake was reduced by enzymatic hydrolysis. The ether extract (EE) or crude fat which contained fat soluble vitamin showed the highest in hydrolyzed palm kernel cake. Partial purification of mannanase enzyme, produced by *A. niger* FTCC 5003 was achieved by fractional precipitation with ammonium sulphate and Tangential Flow Filtration (TFF) ultrafiltration. The purification of mannanase collected from TFF ultrafiltration was achieved with 4.8 fold of purification and 32% recovery whereas the purification of mannanase precipitated by ammonium sulphate was achieved 9.89 fold of purification and 28% recovery. The molecular mass determinations of with partially purified mannanase were estimated as 32 kDa by SDS-PAGE. The optimal temperatures of the purified mannanase was 45°C and stable at 40-50°C, and the pH optimum was at pH 4-6. However, it was most stable at pH 3-7.

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

KESAN MANNANASE MENTAH DAN SEPARA TULEN KE ATAS KANDUNGAN SERAT DALAM HAMPAS ISIRUNG KELAPA SAWIT/ "EXPELLER"

Oleh

SAFARUL MUSTAPHA

February 2011

Pengerusi : Profesor Dr. Suraini Abd Aziz, PhD
Fakulti : Bioteknologi and Sains Biomolekul

Hampas Isirung Kelapa Sawit/**"**Expeller**"** (HIKS) merupakan sisa pertanian kilang minyak kelapa sawit yang memberi sumbangan sekitar 2.2 juta tan pada tahun 2007 di Malaysia. Ini merupakan unsur penting untuk formulasi makanan haiwan yang asalnya daripada jagung dan kulit soya. HIKS, terdiri terutamanya dari jenis galaktomannan dari hemiselulosa, yang hampir tidak boleh dicernakan oleh haiwan bukan-ruminan (monogastrik). Mannanase adalah enzim ekstrasel utama yang dikeluarkan untuk menukar mannan menjadi gula ringkas iaitu mannose. Dalam kajian ini, *Aspergilus niger* FTCC 5003 menghasilkan enzim mannanase dan HIKS sebagai sumber karbon tunggal yang difermentasikan di dalam kelalang kon 500 ml dengan menggunakan parameter yang optimum dalam masa 10 hari dan cecair kultur dikumpulkan untuk tujuan kajian hidrolisis. Penulenan enzim mannanase daripada fermentasi HIKS yang diperolehi daripada fermentasi *A. niger* FTCC 5003 juga dijalankan. Enzim mentah dan separa enzim tulen yang digunakan dalam kajian ini adalah untuk mendegradasikan HIKS

menggunakan teknik hidrolisis. Ciri-ciri HIKS dalam keadaan fermentasi dan hidrolisis di bawah mikroskop elektron (SEM) dikaji dan 'Near Infrared' (NIR) digunakan untuk melakukan analisis proksimat kuantitatif. SEM menunjukkan struktur permukaan HIKS fementasi yang telah dihuraikan secara signifikan jika dibandingkan dengan HIKS terhidrolisis. Keputusan daripada NIR telah membuktikan HIKS terhidrolis menunjukkan pengurangan serat kasarnya (CF). Protein kasar (CP) adalah tertinggi di dalam HIKS fermentasi dan ekstrak eter (EE) pula tertinggi didalam HIKS terhidrolisis. Penulenan separa enzim mannanase yang dihasilkan oleh *A. niger* FTCC 5003 dicapai daripada pemendapan dasar dengan amonium sulfat dan sistem ultrafiltrasi. Pengandaan penulenan enzim mannanase daripada ultrafiltrasi telah dicapai pada 4.8 ganda penulenan dan 32% takat protein diperolehi dan amonium sulfat mencapai 9.89 ganda penulenan dan 23% protein diperolehi. Penentuan jisim molekul enzim mannanase tulen daripada kuputusan gel elektroforesis (SDS-PAGE) diganggarkan pada 32 kDa. Suhu optimum enzim mannanase tulen adalah 45°C dan kestabilan pada 40-50°C dan optimum pH yang paling aktif pada pH 4-6 dan paling stabil pada pH 3-7.

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I certify that an Examination Committee met on **7 February 2011** to conduct the final examination of **Safarul Mustapha** on his **Masters** thesis entitled "**Effects of Crude and Partially Purified Mannanase on The Fibre Content In Palm Kernal Cake/Expeller**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as the fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institution.

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SAFARUL MUSTAPHA

Date: 7 February 2011

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