PRODUCTION OF MONO- AND DIACYLGLYCEROLS FROM THE ESTERIFICATION OF PALM OIL FATTY ACID DISTILLATE CATALYZED BY IMMOBILIZED RICE BRAN LIPASE IN A PACKED BED REACTOR

By

CHONG FUI CHIN

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

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Dedicated to my beloved Chong family and my dear, Woei Lieh
for their love and encouragement

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

PRODUCTION OF MONO- AND DIACYLGLYCEROLS FROM THE ESTERIFICATION OF PALM OIL FATTY ACID DISTILLATE CATALYSED BY IMMOBILIZED RICE BRAN LIPASE IN A PACKED BED REACTOR

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Rice bran lipase (RBL) was preserved and immobilized onto the rice bran by treating with phosphate buffer. The activities of RBL were determined by the esterification assay using oleic acid and glycerol (molar ratio of 2:1) as substrate and hexane as reaction medium. The effects of immobilization on the stability of RBL at higher temperature and in organic solvent were examined. The untreated and immobilized RBL exhibited the highest esterification activity of 1.25 and 3.55 U/g at 45 and 65ºC respectively. At 75°C, the untreated RBL completely lost its esterification activity whereas the immobilized lipase still posses 48% esterification activity. The immobilized RBL retained a relative esterification activity above 80% after 16 hours of incubation in hexane compared to only 50% for that of the untreated RBL. As a result, the immobilized RBL was selected for the subsequent experiment of esterification of free fatty acid from palm oil fatty acid distillate (PFAD) with glycerol in hexane to produce acylglycerols (MAG and DAG). The esterification mixture: PFAD (20 g), glycerol (3.35 g) (as glycerol/fatty acid ratio 1:2 molar), hexane (40 g), silica gel (10 g) and immobilized RBL (100 g) was well mixed by

shaking at 100 rpm. The maximum degree of esterification, 69.8%, was achieved at 65ºC after 2 hours reaction.

A packed bed reactor (PBR) was designed and constructed based on the esterification result obtained from the shaked flask experiment. The developed PBR in this study had an efficient heating system, and a water removal system to remove the reaction water from the esterification reaction. The material of construction of the jacketed thermo-stated packed bed vessel is stainless steel (SUS 304), which is resistant to corrosion and non-toxic to the process enzyme. The reactor is mobile and easy to operate, where the loading and unloading process of the immobilized enzyme is simple to carry out. The result of performance test showed that the heating system installed is efficient to control the temperature in the packed enzyme bed.

The intensified esterification reaction was operated by circulating of 10 l reaction mixtures (PFAD and glycerol) in hexane through a packed bed column filled with 10 kg of immobilized RBL. The process parameters that can influence the performance of PBR such as reaction time, reaction temperature and type of water removal agent were investigated. The esterification reaction in PBR was optimum at reaction temperature of 65ºC and the use of silica gels (1 kg) as water removal agents. The product of esterification (MAG and DAG) was qualitatively identified by rapid thin-layer chromatography (TLC) method. The performance of PBR was generally better than that in shaked flask in terms of maximum degree of esterification and reaction time required. The intensified esterification of PFAD and glycerol catalysed by immobilized RBL in the self fabricated PBR had resulted a comparable 61% in maximum degree of esterification and 0.5 h shorter in reaction time than that in

shaked flask, at reaction temperature of 65ºC and the use of silica gels (1 kg) as water removal agent.

Since both rice bran and PFAD are agroindustrial residues and abundantly available in Malaysia, the successful of application of RBL as biocatalyst for the esterification of FFA from PFAD with glycerol in hexane to produce MAG and DAG has a very important impact both on the economy and environmental aspect of rice and palm oil process industry.

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

PENGHASILAN MONO- DAN DIASILGLISEROL MELALUI PENGESTERAN ASID LEMAK SULINGAN KELAPA SAWIT TERMANGKIN OLEH LIPASE BRAN BERAS TERPEGUN DI DALAM REAKTOR LAPISAN TERPADAT

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Lipase bran beras (RBL) telah dipegun ke atas bran beras dengan rawatan penimbal fosfat. Aktiviti lipase bran beras ditentukan dengan assai pengesteran menggunakan asid oleik dan gliserol (2:1 nisbah molar) sebagai substrat dan hexane sebagai medium tindak balas. Kesan proses kepegunan terhadap kestabilan lipase bran beras pada suhu yang tinggi dan dalam pelarut organik telah ditelitikan. Lipase bran beras tanpa rawatan dan yang terpegun menunjukkan aktiviti pengesteran paling tinggi, 1.25 dan 3.55 U/g pada suhu 45 dan 65ºC masing-masing. Pada suhu 75ºC, lipase bran beras tanpa rawatan kehilangan segala aktiviti pengesteran manakala bagi yang terpegun masih mempunyai aktiviti pengesteran setinggi 48%. Kepegunan lipase bran beras masih mengekalkan relatif aktiviti pengesteran lebih daripada 80% setelah direndam dalam hexane selama 16 jam berbanding dengan hanya 50% bagi lipase tanpa rawatan. Oleh itu, lipase bran beras terpegun dipilih untuk ujikaji proses pengesteran asid lemak bebas dari minyak sulingan kelapa sawit (PFAD) dan gliserol di dalam pelarut hexane untuk menghasilkan asilgliserol (MAG dan DAG). Bahan yang digunakan dalam proses pengesteran ialah PFAD (20 g), gliserol (3.35 g)

(dalam nisbah molar gliserol/asid lemak: 1:2), hexane (40 g), gel silika (10 g) dan lipase bran beras terpegun (100 g). Bahan campuran ini dicampur secara rata dengan pengoncangan pada 100 rpm. Darjah pengesteran maksimum setinggi 69.8% telah dicapai setelah 2 jam tindak balas pada 65ºC.

Sebuah reaktor lapisan terpadat (PBR) telah direkabentuk dan dibina berdasarkan keputusan yang didapati dari ujikaji dalam kelalang goncang. PBR yang direkabentuk dalam penyelidikan ini dilengkapi dengan sistem pemanasan yang berkesan dan sistem penghindaran air untuk menghindarkan air daripada aktiviti pengesteran. Bahan binaan PBR ini ialah keluli nirkarat (SUS 304), di mana ia tahan daripada kakisan dan tidak bertoksik kepada enzim. Reaktor ini mudah dialih dan senang dikendali, di mana process pemuatan ke dalam dan pemunggahan daripada reaktor untuk enzim terpegun adalah mudah. Keputusan ujikaji prestasi menunjukkan sistem pemanasan yang dipasang itu dapat mengawal suhu dalam reaktor dengan berkesan.

Proses pengesteran dioperasi dengan menyembur 10 l bahan tindak balas (asid lemak dan gliserol) dalam hexane ke dalam tangki yang dipadatkan dengan 10 kg lipase bran beras terpegun. Parameter proses yang mempengaruhi pretasi PBR seperti suhu, masa dan jenis agen penyerap air telah disiasat. Tindak balas pengesteran dalam PBR itu adalah optimal pada suhu 65ºC, dan penggunaan gel silika (1kg) sebagai agen penyerap air. Produk pengesteran (MAG dan DAG) dikesan secara kualitatif melalui analisasi kromatografi lapisan nipis (TLC). Prestasi PBR yang direkacipta itu adalah lebih baik daripada kelalang goncang dari segi darjah pengesteran dan masa tindak balas yang diperlukan. Proses pengesteran asid lemak bebas dari minyak sulingan

kelapa sawit (PFAD) dan gliserol yang intensif di dalam PBR membawa darjah pengesteran maksimum sebanyak 61% dan penyingkatan masa ujikaji sebanyak 0.5 h berbanding dengan eksperimen di dalam kelalang goncang pada suhu tindak balas 65ºC dan penggunaan gel silika (1 kg) sebagai agen penyerap air.

Oleh kerana kedua-dua bran beras dan PFAD adalah hasil sampingan industri pertanian yang mudah didapati, kejayaan penggunaan lipase bran beras sebagai biomangkin untuk pengesteran asid lemak bebas dari PFAD dengan gliserol dalam hexane untuk menghasilkan MAG dan DAG memberi kesan positif terhadap ekonomi dan aspek alam sekitar dalam industri pemprosesan padi dan minyak kelapa sawit.

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I certify that an Examination Committee has met on 28th March 2006 to conduct the final examination of Chong Fui Chin on her Master of Science thesis entitled "Production of Mono- and Diacylglycerols from the Esterification of Palm Oil Fatty Acid Distillate Catalyzed by Immobilized Rice Bran Lipase in a Packed Bed Reactor" 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 relevant degree. Members of the Examination Committee are as follows:

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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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CHONG FUI CHIN

Date:

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