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MICROBIAL LIPASE PRODUCTION OPTIMIZATION, CHARACTERIZATION AND APPLICATION IN COCONUT OIL MODIFICATION

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

MICROBIAL LIPASE PRODUCTION OPTIMIZATION, CHARACTERIZATION AND APPLICATION IN COCONUT OIL MODIFICATION

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Chair: Professor Lai Oi Ming, PhD

Faculty: Biotechnology and Biomolecular Sciences

Lipases are the enzymes that catalyze the hydrolysis of fats and oils and can be found widely in nature. These lipases, especially when from microbial sources are preferred and extensively used especially in biotechnological applications and commercial industries.

Nowadays, the use of antibiotics have been thought to contribute to the emergence of antibiotic-resistant microorganisms and a new market for natural food additives with antimicrobial properties has been opened. One of the most popular elements with strong anti microbial activities are medium-chain fatty acid glycerides and medium-chain free fatty acids (MCFA) particularly lauric acid.

Coconut oil is considered as a good source of MCFA as it consisted of about 50% lauric acid in triglyceride form. Medium chain triglycerides (MCTGs) could be hydrolyzed to medium chain glyceride derivatives and MCFAs including lauric acid

using lipases. Therefore, in this study bacterial and fungal lipases were produced, optimized and characterized. Certain possible applications in coconut oil modification were also investigated. Different fermentation systems were also developed to get the highest lipolytic activity and functionality in coconut oil modification.

Among different lipase producing bacterial isolates, three Gram-positive cocci (*Staphylococcus xylosus, S. sciuri, S. aureus*) and one Gram-negative short rod (*Acinetobacter* sp.) were selected based on their highest lipase production activity and better lipase characteristics. Bacterial isolates were found to be new strains according to the BIOLOG and DSMZ identification. All strains were able to produce lipase in submerged fermentation (SmF) but only *Acinetobacter* sp. showed the same capability on coconut solid state fermentation (SSF).

The use of fungi for the production of commercially important products has increased rapidly over the past half century. Two lipolytic filamentous fungal strains, *Geotrichum candidum* ATCC 34614 and *G. candidum* local isolate were investigated in the case of lipase production in both SmF and SSF. Lipase production for *G. candidum* ATCC 34614 was 15 times higher than the bacterial isolates while, for the local strain it was in the same range with bacterial isolates. Both fungal strains revealed great potential on coconut SSF.

Modeling studies on culture parameters optimization for lipase production of these microorganisms were performed using response surface methodology (RSM) and artificial neural networks (ANNs). Based on the obtained optimum conditions,

lipases were produced in SmF for characterization studies. The results demonstrated good characteristics for all lipases as they were found to be thermostable, acid-base tolerant and solvent-detergent stable. Lipases were then used to perform hydrolysis reaction on coconut oil.

A coconut solid-state fermentation system was developed in order to apply the produced lipase directly on substrate without any downstream processing. Both fungal strains SSF lipases showed high functionality on coconut oil MCTG conversion into MCDG, MCMG and MCFA. The oil conversion percentages reached to 78% and 76% after optimization for *G. candidum* ATCC 34614 and *G. candidum* local strain, respectively. The local strain showed higher lipase functionality under extreme conditions of moisture and oil content compared to the former strain that revealed no activity in those conditions. Microscopic studies demonstrated that the local strain can grow faster and better on coconut solid culture with penetration capability compared to *G. candidum* ATCC 34614. Coconut oil was successfully modified by *G. candidum* lipases under solid state fermentation of coconut. The bacterial strain (*Acinetobacter* sp.) did not reveal any activity in coconut oil modification due to its short shelf life on coconut solid culture.

Modified coconut oils obtained from direct fermentation of fungal lipase during SSF process were characterized for its antimicrobial activity and thermal characteristics. The optimized modified coconut oils extracted from *G. candidum* ATCC 34614 and *G. candidum* local strain revealed 95 and 90 % antimicrobial activity against *S. aureus* and 90 and 85% against *E. coli*, respectively. The contributions of high level lauric acid together with medium chain mono- and di- glycerides in modified

coconut oils were the key factors for antimicrobial activity. The solid and submerged fermented coconuts also showed bactericidal effects. Differential scanning calorimetry of modified coconut oils showed lower melting points compared to the normal coconut oil.

G. candidum ATCC 34614 cultures analysis using HS-SPME/GC-MS also showed that 46 and 37 aromatic compounds were produced during the SmF and SSF, respectively. The produced aromatic compounds were mainly esters with fruity and flora notes in the modified coconut samples indicating successful hydrolysis of the lipases.

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

LIPASE MIKROB
PENGHASILAN PENGOPTIMUMAN, PENCIRIAN DAN DALAM PENGUBAHSUAN MINYAK KELAPA APLIKASI

Oleh

ANAHITA KHORAMNIA

Julai 2012

Pengerusi: Profesor Lai Oi Ming, PhD

Fakulti: Bioteknologi Dan Sains Biomolekul

Lipase adalah enzim yang memangkinkan tindak balas terutamanya hidrolisis lemak dan minyak. Lipase boleh didapati secara meluas di alam sekitar. Walau bagaimanapun, lipase dari samber mikrob lebih digemari dan digunakan secara meluas terutamanya dalam industri biotecknologi dan industri komersil.

Pada masa kini, pengunaan antibiotik telah menyebabkan kemunculan mikroorganisma yang rintans-antibiotic dan ini membuka pasaran baru kepada makanan aditif yang mempunyai ciri-ciri anti-mikrob. Salah satu elemen yang paling popular yang mempunyai cirri-ciri anti-mikrob yang kuat ialah gliserida acid lemak rantai sederhana dan acid lemak rantai sederhana (MCFA) terutamanya asik lauric.

Minyak kelapa dianggap sebagai sumber MCFA yang terdiri daripada kira-kira 50% acid laurik dalam bentuk trigliserida. A trigliserida rantai sederhan boleh dihidrolisis kepada gliserida terbitan rantai sederhana dan MCFA dengan menggunakan enzim

lipase. Dalam kajian ini, lipase dari bacteria dan kulat telah dihasil, dioptimum dan dicirikan untuk diaplikasi dalam pengubahsuaian minyak kelapa. Sistem fermentasi yang berbeza telah dibangunkan untuk mendapat amaun lipase yang paling tinggi dan sejurusnya digunakan untuk pengubasuaian minyak kelapa.

Antara bakteria yang didapati boleh menghasilkan lipase ialah: 3 Gram positif cocci (*Staphylococcus xylosus, S. sciuri, S. aureus*) dan 1 Gram negative rod pendek (*Acenitobacter sp.*). Bakteria-bakteria ini dipilih berdasarkan kemampuan mereka menghasilkan kandungan lipase yang tinggi dan mempunyai ciri-ciri yang lebih baik. Isolat bakteria ini merupakan strain baru berdasarkan kepada sistem BIOLOG dan DSMZ. Semua strain mampu menghasilkan lipase dalam fermentasi tenggelam manakala hanya *Aceinitobacter sp*. nenunjukkan keupayaan yang sama dalam sistem penapaian pepejal kelapa.

Pengunaan fungi dalam pengeluaran komersil produk adalah penting dan telah meningkat dengan pesat sejak setengah abad yang lalu. Dua strain lipolitic kulat *Geotrichum candidum* ATCC 34614 dan *G. candidum* tempatan disiasat dalam kebolehan untuk menghasilkan lipase di kedua-dua sistem SmF dan SSF. *G. candidum* ATCC 36414 dapat menghasilkan lebih 15 kali ganda kandungan lipase berbanding dengan isolat bakteria manakala strain tempatan adalah dalam lingkungan yang setara dengan isolat bakteria. Kedua-dua strain fungi ini mempunyai potensi untuk digunakan dalam sistem fermentasi pepejal.

Kajian pemodelan untuk menyiasat parameter yang optimum bagi pengeluaran lipase daripada microorganisma telah dijalankan dengan menggunakan kaedah gerak balas

permukaan (RSM) dan rangkaian neural tiruan (ANN). Lipase telah dihasilkan dalam sistem fermentasi pepejal untuk tujuan pencirian, berdasarkan keadaan optimum yang diperolehi. Keputusan menujukkan lipase ini mempunyai semua ciri-ciri yang baik seperti tahan panas, toleran kepada asid,bes dan pelarut-detergen. Dengan ini, lipase ini telah digunakan untuk menjalankan tindak balas hidrolisis ke atas minyak kelapa.

Sistem fermentasi pepejal telah dibangunkan untuk menghasilkan lipase secara langsung di atas substrat tanpa sebarang pemprosesan hiliran. Kedua-dua strain kulat yang menghasilkan lipase dalam penapaian pepejal menunjukkan fungsi yang tinggi dalam penukaran minyak kepada MCTG, MCDG, MCMG dan MCFA. Penukaran minyak sampai 78% dan 76% didapati selepas pengoptimuman bagi ATCC 36414 dan *G. candidum* strain tempatan. Strain tempatan menunjukkan fungsi lipase yang lebih tinggi dalam keadaan kelembapan dan minyak kandungan yang tinggi berbanding dengan strain *G. candidum* tempatan yang tidak menunjukkan sebarang aktiviti lipase. Kajian mikroskopik menunjukkan bahawa strain tempatan tumbuh lebih cepat and baik berbanding dengan strain ATCC 36414. Minyak kelapa berjaya ditukar dengan menggunakan lipase *G. candidum* dalam sistem fermentasi pepejal. Bakteria strain (*Acinetobacter sp*.) lipase tidak menunjukkan sebarang aktiviti dalam pengubahsuaian minyak kelapa disebabkan oleh hayat yang singkat apabila diaplikasikan dalam sistem fermentasi pepejal.

Minyak kelapa yang diubahsuai daripada kulat dalam kaedah fermentasi pepejal telah dicirikan dan menunjukkan ciri anti-mikrob dan toleransi terhadap suhu. Miinyak kelapa yang diubahsuai dengan menggunakan *G. candidum* ATCC 34614

dan *G. candidum* tempatan mennujukkan 95 dan 90% aktiviti anti-mikrob masing – masing manakala *S.aureus* dan *E.coli* mempunyai aktiviti anti-mikrob sebanyak 90% and 85% masing-masing. kandunsan asid laurik yang tinggi bersama-sama dengan rantai serderhana mono- dan di-gliserida merupakan faktor yang menyebabkan aktiviti anti-mikrob ini. Fermentasi pepejal dan fermentasi tenggelam juga menunjukkan aktiviti anti-mikrob. Analisa DSC menunjukkan bahawa minyak kelapa yang diubahsuai mempunyai tahap lebur yang sedikit berbanding dengan minyak kelapa biasa dan berpotensi untuk dijadikan bahan pengemulsi.

G. candidum ATCC 36414 di analsia dengan mengunakan HS-SPME dan GC-MS dan menunjukkan bahawa 46 dan 37 bahan aroma wujud bagi kedua-dua sistem fermentasi pepejal and penapaian tenggelam. Bahan aroma ini tediri daripada ester dengan nota buah-buahan dan bunga-bungaan dalam sampel minyak kelapa yang diabahsuci, menunjukan lipase berjaya menghidrolisis sampel tersebut.

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I certify that an Examination Committee has met on 02.07.2012 to conduct the final examination of **Anahita Khoramnia** on his Doctor of Philosophy thesis entitled "**Microbial lipases production: optimization characterization and application in coconut oil modification**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Doctor of Philosophy**. 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 acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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ANAHITA KHORAMNIA

Date: 02 July 2012

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