UNIVERSITI PUTRA MALAYSIA

# ENZYME-CATALYZED SYNTHESIS AND CHARACTERIZATION OF DIHYDROXYSTEARIC ACID ESTER FROM PALM-BASED DIHYDROXYSTEARIC ACID AND MONOHYDRIC ALCOHOL

ROILA AWANG

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DOCTOR OF PHILOSOPHY
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UPM

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By

ROILA AWANG

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**ENZYME-CATALYZED SYNTHESIS AND CHARACTERIZATION OF DIHYDROXYSTEARIC ACID ESTER FROM PALM-BASED DIHYDROXYSTEARIC ACID AND MONOHYDRIC ALCOHOL**

**By**

**ROILA AWANG**

**Chairman : Professor Dr. Mahiran Basri**

**Faculty : Science and Environmental Studies**

Enzymatic synthesis of dihydroxystearic acid ester (DHSA ester) from dihydroxystearic acid (DHSA) and monohydric alcohol in organic solvent were investigated. Five commercial immobilized enzyme (Lipozyme IM, Novozym 435, Termamyl, Savinase and Lipolase) were tested for their suitability for the reaction. Among the enzymes tested, Lipozyme IM and Novozym 435 were chosen for optimization studies because of their higher specific activity. The effect of various reaction parameters such as time, temperature, organic solvent, amount of enzyme, mole ratio of substrates, thermodynamic water activity ($a_w$) and structure of substrates were studied to determine optimal condition for the production of DHSA ester.

The optimal conditions for DHSA ester synthesis using Lipozyme IM and Novozym 435 were obtained at reaction time of 3h, temperature of 50°C,

mole ratio of substrates, 2.0 and amount of enzyme, 10-20% (w/w). The maximum conversion for Lipozyme IM and Novozym 435 at optimal condition was 92.4% and 94.9% respectively without removal of water in the reaction mixture. The results clearly demonstrated that both enzymes are well suited for the preparation of DHSA ester in organic media. This esterification reaction follows Michaelis-Menten kinetics as observed from the relationship of initial rate of the reaction, both as a function of enzyme and of substrate concentration. The kinetics of the enzymatic reaction is suggested to agree with a Ping-Pong Bi Bi mechanism. In a 5L batch reactor, up to 90% conversion was obtained at the optimal condition. Lipozyme IM remained active after repeated used of 12 times.

Characteristics and properties of DHSA ester were also evaluated, which include heat and colour stability, solubility, irritancy, dispersibility as well as emulsification properties. Purified DHSA ester showed higher heat stability compared to crude DHSA ester. Solubility of DHSA ester in methanol and ethanol is comparatively lower as compared to the solubility at higher chain length of alcohol. This compound is non-irritant to the skin. DHSA ester seems to have some emulsifying properties in water-in-oil system with high or low content of oil phase. The composition of the oil phase is important, as castor oil seems the most compatible oil. DHSA ester could be used as stabilizer or co-emulsifier in oil-in water emulsion system.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat bagi mendapatkan ijazah Doktor Falsafah

**SINTESIS DAN PENCIRIAN ESTER ASID DIHIDROKSISTEARIK DARI ASID DIHIDROKSISTEARIK BERASASKAN SAWIT DAN ALKOHOL MONOHIDRIK DENGAN MENGGUNAKAN ENZIM SEBAGAI MANGKIN**

**Oleh**

**ROILA AWANG**

**Pengerusi : Profesor Dr. Mahiran Basri**

**Fakulti : Sains dan Pengajian Alam Sekitar**

Ester asid dihidroksistearik (DHSA ester) telah disediakan melalui proses pengesteran diantara asid dihidroksistearik (DHSA) dan alkohol monohidrik dalam pelarut organik menggunakan enzim sebagai mangkin. Lima jenis enzim (Lipozyme IM, Novozym 435, Termamyl, Savinase dan Lipolase) telah diuji kesesuaiannya dalam tindakbalas ini. Diantara enzim yang diuji, Lipozyme IM dan Novozym 435 telah dipilih untuk kajian pengoptimuman berdasarkan aktivitinya yang tinggi. Kesan pelbagai parameter seperti masa tindakbalas, suhu tindakbalas, pelarut organik, amaun enzim, nisbah bahan tindakbalas dan aktiviti termodinamik air telah dikaji.

Keadaan optimum bagi penyediaan ester DHSA menggunakan Lipozyme IM dan Novozym 435 adalah seperti berikut: masa tindakbalas, 3 jam;

suhu tindakbalas 50°C; nisbah mol bahan tindakbalas, 2.0 dan amaun enzim, 10%. Peratus penghasilan ester DHSA untuk tindakbalas yang menggunakan Lipozyme IM dan Novozym 435 pada keadaan optimum adalah 92.4% dan 94.9%. Keputusan ini menunjukkan kedua-dua enzim ini sesuai digunakan untuk penyediaan ester DHSA dalam pelarut organik. Berdasarkan hubungan kadar tindakbalas terhadap kepekatan enzim dan bahan tindakbalas, tindakbalas pengesteran ini mengikut kinetik Michaelis-Menten, yang mana kinetik tindakbalas ini dicadangkan bersesuaian dengan mekanisma 'Ping Pong Bi Bi'.

Ester DHSA dikaji untuk mengetahui sifat-sifat termasuk kestabilan haba dan warna, kelarutan, iritasi, kesebaran dan juga sifat-sifat emulsifikasi. Ester DHSA tulen menunjukkan kadar kestabilan terhadap haba yang lebih tinggi berbanding ester DHSA mentah. Kelarutan ester DHSA di dalam metanol dan etanol adalah lebih rendah jika dibandingkan dengan kelarutan dalam alkohol yang mempunyai rantai yang lebih panjang. Bahan ini tidak menyebabkan iritasi kepada kulit. Ester DHSA menunjukkan sifat-sifat pengemulsi dalam sistem emulsi air dalam minyak dengan kandungan fasa minyak yang tinggi atau rendah. Komposisi fasa minyak adalah penting yang mana didapati minyak jarak adalah yang paling sesuai. Ester ini juga boleh digunakan sebagai bahan penstabil atau agen sampingan emulsi (co-emulsifier) dalam sistem emulsi minyak dalam air.

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I certify that an Examination Committee met on 16th September 2002 to conduct the final examination of Roila Awang on her Doctor of Philosophy thesis entitled "Enzyme-Catalyzed Synthesis and Characterization of Dihydroxystearic Acid Ester from Palm-Based Dihydroxystearic Acid and Monohydric Alcohol" 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 follows:

**Abdul Rahman Manas, Ph.D.**
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

**Mahiran Basri, Ph.D.**
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

**Abu Bakar Salleh, Ph.D.**
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

**Salmiah Ahmad, Ph.D.**
Senior Principal Research Officer
Advanced Oleochemical Technology Centre
Malaysian Palm Oil Board
(Member)

**Ibrahim Che Omar, Ph.D.**
Professor
School of Chemistry
Universiti Sains Malaysia
(Independent Examiner)

**SHAMSHER MOHAMAD RAMADILI, Ph.D**
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 OCT

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of Supervisory Committee are as follows:

**Mahiran Basri, Ph.D.**
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairperson)

**Abu Bakar Salleh, Ph.D.**
Professor
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

**Salmiah Ahmad, Ph.D.**
Senior Principal Research Officer
Advanced Oleochemical Technology Centre
Malaysian Palm Oil Board
(Member)

**AINI IDERIS, Ph.D.**
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 9 JAN 2003

## 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.

**ROILA AWANG**

Date: 29/10/02

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