UNIVERSITI PUTRA MALAYSIA

LIPASE-CATALYZED PREPARATION OF GALACTOSE OLEATE ESTER IN IONIC LIQUID

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

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DEDICATION

"To my beloved parents and siblings, for the unconditional love, blessing, strength and support "

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

LIPASE-CATALYZED PREPARATION OF GALACTOSE OLEATE ESTER IN IONIC LIQUID

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October 2012

Chair: Emilia bt Abd Malek, PhD

Faculty: Science

Lipase-catalyzed preparation of galactose oleate ester was performed in 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]) ionic liquid with the addition of dimethylsulfoxide (DMSO) as a solubilizing agent and co-solvent, and Lipozyme RM IM (lipase from *Rhizomucor miehei* immobilized on macroporous anion exchange resin) as the biocatalyst. Galactose oleate ester was purified and characterized by thin layer chromatography (TLC), fourier transform-infrared spectroscopy (FT-IR), high performance liquid chromatography (HPLC), direct injection-mass spectrometry (DI-MS), liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR). Optimal preparation conditions with a high conversion rate (87%) was obtained in DMSO:[Bmim][BF_4] (1:20, v:v) with 2% (w/w) Lipozyme RM IM loaded for 2 h, at 60°C, with a stirring speed of 300 rpm and a molar ratio of galactose to

oleic acid of 1:3.

The formation of galactose oleate ester was evaluated through kinetic study using Michaelis-Menten kinetic model. The Ping-Pong Bi-Bi mechanism with one substrate inhibition was adopted as it best explained the experimental findings. The kinetic results showed the K_m values (galactose = 0.02905 mmol/mL.mg and oleic acid = 0.00025 mmol/mL.mg). The low Michaelis constant values showed that the Lipozyme RM IM has higher affinity towards both substrates. The overall results on the physicochemical studies such as hydrophile lipophile balance (HLB) value, physical state, refractive index and surface tension illustrated that the ester possess suitability for industrial application as emulsifiers and surfactant.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat bagi mendapatkan Ijazah Sarjana Sains

PENYEDIAAN ESTER GALAKTOSA OLEAT BERMANGKIN LIPASE DI DALAM CECAIR IONIK

Oleh

HANIM SALAMI BINTI MOHD SAUPI

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Penyediaan ester galaktosa oleat bermangkin lipase telah dilakukan di dalam cecair ionik 1-butil-3-metilimidazolium tetrafluoroborat ([Bmim][BF_4]) dengan tambahan dimetilsulfoksida (DMSO) sebagai agen pelarut dan ko-pelarut dan Lipozyme RM IM (lipase dari *Rhizomucor miehei* dipegunkan di atas resin tukaran anion makroporos) sebagai biomangkin. Ester galaktosa oleat telah ditulenkan dan dicirikan dengan kromatografi lapisan nipis (TLC), spektroskopi infra merah transformasi Fourier (FT-IR), kromatografi cecair prestasi tinggi (HPLC), suntikan terus-spektroskopi jisim (DI-MS), kromatografi cecair-spektrometri jisim (LC-MS) dan resonan magnetik nuklear (NMR) untuk mengesahkan identiti produk. Keadaan optimum penyediaan dengan kadar penukaran yang tinggi (87%) telah diperolehi dalam DMSO:[Bmim][BF_4] (1:20, v:v) dengan 2% (w/w) Lipozyme RM IM dimuatkan selama 2 jam, pada suhu 60°C, dengan

kelajuan putaran pengaduk 300 rpm dan nisbah molar galaktosa kepada asid oleik 1:3.

Pembentukan ester galaktosa oleat telah dinilai melalui kajian kinetik menggunakan model kinetik Michaelis-Menten. Mekanisma Ping-Pong Bi-Bi dengan satu perencatan substrat telah diambil sebagai yang terbaik menjelaskan penemuan eksperimen. Keputusan kinetik menunjukkan nilai K_m (galaktosa = 0.02905 mmol/mL.mg dan asid oleik = 0.00025 mmol/mL.mg). Nilai pemalar Michaelis yang rendah menunjukkan bahawa Lipozyme IM IM mempunyai afiniti yang lebih tinggi terhadap kedua-dua substrat. Keputusan keseluruhan kajian fizikokimia seperti nilai keseimbangan hidrofilik lipofilik (HLB), keadaan fizikal, indeks biasan dan tegangan permukaan, menggambarkan bahawa ester mempunyai kesesuaian untuk kegunaan industri sebagai pengemulsi dan surfaktan.

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I certify that a Thesis Examination Committee has met on (insert the date of viva voce) to conduct the final examination of Hanim Salami binti Mohd Saupi on her thesis entitled "Lipase-Catalyzed Synthesis of Galactose Oleate Ester in Ionic Liquid" 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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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 at any other institution.

HANIM SALAMI BINTI MOHD SAUPI

Date: 23rd October 2012

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

[Bmim][BF_4]	1-Butyl-3-methylimidazolium tetrafluoroborate
[Bmim][PF_6]	1-Butyl-3-methylimidazolium hexafluorophosphate
[Bmim][Tf_2N]	1-Butyl-3-methylimidazolium trifluoromethylsulfonyl imide
[Bmim][TfO]	1-Butyl-3-methylimidazolium trifluoromethanesulfonate
br.	broad
Br	Bromide
Cl	Chloride
CRL	*Candida rugosa* lipase
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
HPLC	High Performance Liquid Chromatography
HLB	Hydrophile Lipophile Balance
ILs	Ionic liquids
KOH	Potassium hydroxide
LCMS	Liquid Chromatography Mass Spectrometry
Novozym 435	Immobilized lipase from *Candida antartica* B
Lipozyme RM IM	Immobilized lipase from *Mucor miehei*
Lipozyme TL IM	Immobilized lipase from *Thermomyces lanuginosus*
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance

O/W	Oil-in-water
NaOH	Sodium hydroxide
SEM	Scanning Electron Micoscopy
TLC	Thin Layer Chromatography
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

Sugar fatty acid ester has characteristics of non-ionic and biodegradable surfactants. These surfactants have very good emulsifying, stabilizing or conditioning effects. Sugar ester consists of a carbohydrate as hydrophilic group and fatty acid as lipophilic component (Maruyama *et al.*, 2002). Sugar ester surfactant is widely applied in pharmaceutical, detergent and pharmaceutical industry because of their amphiphilic properties (Ganske and Bornscheuer, 2005). An advantage of using biosurfactant is that in addition to their efficacy, sugar fatty acid ester is still being considered as natural (Mutua and Akoh, 1993).

There are two ways to synthesize sugar esters that are commonly used; chemical catalyzed method or enzymatically-catalyzed method. Enzymatic synthesis of sugar ester using lipase as biocatalyst causes great impact and a turning point to chemical synthesis since chemical-catalyzed method lead to major problem such as colored product, harsh reaction conditions and low conversion with low selectivity. Lipase usage has enabled high conversion of pure product because of its specificity, catalyzing reaction on specific sites of a molecule. Hence, this method has high potential to substitute chemical synthesis in the surfactant production (Song and Wei, 2002; Yu *et al.*, 2008).

Organic solvents (eg. *tert*-butanol, acetone and acetonitrile) are often used since enzymes remain active in these solvents. However, the usage of organic solvents in sugar ester synthesis has some limitations due to low solubility of sugar in these solvents. Moreover, in order to practice green chemistry and technology, volatile organic solvents must be replaced or reduced since it is harmful to human and environment (Walsh *et al.*, 2009; Ganske and Bornscheuer, 2005).

Ionic liquids (ILs) were recently showed as possible substitute of organic solvents for application in biocatalytic reactions. ILs are non-volatile, non-inflammable and thermally stable. They possess special characteristics in their properties that can be tunable depending on the composition of cations and anions. Commercial anhydrous ILs with tetrafluoroborate ($[BF_4]$), hexafluorophosphate ($[PF_6]$), bis(trifluoromethylsulfonyl)imide ($[NTf_2]$) and trifluoromethanesulfonate ([TfO]) anions have been recently used in carbohydrate ester synthesis as reaction media (Ganske and Bornscheuer, 2005; Kim *et al.*, 2003; Park and Kazlauskas, 2001).

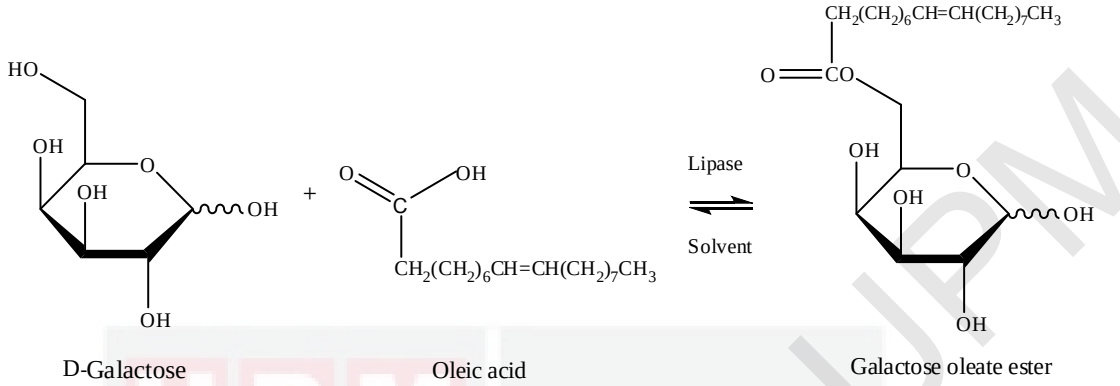
Enzyme stability and selectivity have been found to be high in these anion based ILs. However, carbohydrate solubility remains a major problem in ILs (Park and Kazlauskas, 2003; [a]Lee *et al.*, 2008). The addition of co-solvents from polar organic solvents may improve the solubility of carbohydrates in ILs. Polar organic solvents that are suitable as co-slovent should be able to dissolve sugar and maintain enzyme activity (Ganske and Bornscheuer, 2005).

Galactose is an essential monosaccharide. It is a basic substrate for the biosynthesis of macromolecules in the body. Fatty acid used in this synthesis is palm oil based with wide range of chain length. Palm oil fatty acid have a variety applications in industry and easily available. It may represent a good source of acyl donors during enzymatic synthesis of ester (Liu *et al.*, 2000).

Oleic acids are monounsaturated long chains fatty acids and are widely used as a substitute for saturated fatty acids to reduce potential dietary problems. They also exhibit antioxidant properties by preventing lipoprotein oxidative modification that leads to atherosclerosis (Lopez-Huertas *et al.*, 2010; Sola *et al.*, 1997).

Galactose oleate ester was specifically chosen to be synthesized since there were only a few reports on the synthesis of sugar ester from galactose and oleic acid, especially in ILs as reaction media. Some researchers have reported up to 60% conversion using galactose and divinyl adipate as substrates with DMF as reaction medium with reaction time of 7 days at 35°C (Reyes-Duarte *et al.*, 2005). In addition, long chain sugar esters are widely applied as surfactants and only monoester and diester are relevant for cosmetic applications (Kitagawa *et al.*, 1999).

The chemical reaction for preparation of galactose oleate ester was described as follows:



D-Galactose Oleic acid Galactose oleate ester

The main aim of this study is to preparee sugar fatty acid ester by lipase-catalyzed process at optimized reaction conditions. In addition, ester has high commercial value and can be utilized in various industries. An ester of galactose and oleic acid, *i.e.* galactose oleate is expected to exhibit positive characteristics from both substrates for use in the food industry and others.

To date, there are no published reports on synthesis of galactose oleate ester in ionic liquids. The specific objectives are as follows:

1) To prepare and characterize sugar fatty acid ester by lipase-catalyzed process
2) To optimize the esterification reaction conditions
3) To analyze the kinetics of the lipase-catalyzed reaction of oleic acid with galactose
4) To study the physicochemical characteristics of synthesized sugar fatty acid ester

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