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OPTIMIZATION OF LIPASE CATALYSED SYNTHESIS OF XYLOSE CAPROATE ESTER IN ORGANIC SOLVENT

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OPTIMIZATION OF LIPASE CATALYSED SYNTHESIS OF XYLOSE CAPROATE ESTER IN ORGANIC SOLVENT

By

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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 Science

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November 2016

Chairman : Emilia Abd Malek, PhD
Faculty : Science

Lipase catalysed synthesis of xylose caproate ester was performed by condensation of xylose, an aldopentose, and caproic acid in organic solvents. A dual-solvent system containing dimethylsulfoxide (DMSO) and acetone (1:10, v/v) was used to determine the optimal conditions for the reaction. Different reaction parameters (solvent system, reaction time, substrate molar ratio and the amount of enzyme loaded) were studied. The highest conversion rate (64%) was obtained within 24 hours with the optimal conditions of 16% (w/v) Novozym 435 and a molar ratio of xylose to caproic acid of 1:4 (mmol). Xylose caproate ester was purified and characterized by thin layer chromatography (TLC), high performance liquid chromatography (HPLC), fourier transform infrared spectroscopy (FTIR), gas chromatography flame ionization (GCFID), gas chromatography mass spectrometry (GCMS) and nuclear magnetic resonance (NMR). The pure product existed as a waxy solid form with bright yellow color. The melting point, hydrophilic lipophipic value (HLB), and solubility of the product was evaluated.

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

KEADAAN OPTIMA SINTESIS ESTER XILOSA KAPROAT BERMANGKINKAN LIPASE DALAM PELARUT ORGANIK

Oleh

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Sintesis bermangkinkan lipase untuk menghasilkan ester xilosa kaproat telah dilakukan secara kondensasi xilosa, salah satu daripada aldopentosa dan asid kaproik dalam pelarut organik. Sistem dwi-pelarut yang mengandungi dimetilsulfoksida (DMSO) dan aseton (1:10, v/v) telah digunakan untuk menentukan keadaan optimum tindak balas. Parameter tindak balas yang berbeza (sistem pelarut, masa tindak balas, nisbah molar substrat dan jumlah enzim dimuatkan) telah dikaji. Kadar penukaran tertinggi (64%) telah diperolehi dalam tempoh 24 jam dengan keadaan optimum 16% (w/v) Novozym 435, dan nisbah molar xilosa terhadap asid kaproik sebanyak 1: 4 (mmol). Ester xilosa kaproat telah ditulenkan akan dicirikan secara Kromatografi Lapisan Nipis (TLC), Kromatografi Cecair Prestasi Tinggi (HPLC), Spektroskopi Inframerah (FTIR), Kromatografi Gas Pengesan Nyalaan Ion (GCFID), Kromatografi Gas Spektrometri Jisim (GCMS) dan Spektroskopi Resonan Magnetik Nukleus (NMR). Hasil yang tulen wujud dalam bentuk pepejal berlilin dengan warna kuning terang. Takat lebur, Nilai Hidrofilik Lipofilik (HLB), dan keterlarutan produk telah dinilai.

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

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LIST OF ABBREVIATIONS

DMSO	Dimethyl sulfoxide
HLB value	Hydrophilic lipophilic balance value
AE	Polyoxyethylene alkyl ethers
APE	Polyoxylethylene alkylphenyl ethers
2M2B	2-methyl-2-butanol
TBA	Tert-butyl alcohol
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
GCFID	Gas chromatography with flame ion detector
GCMS	Gas chromatography mass spectrometry
NMR	Nuclear magnetic resonance
CAL-B	*Candida antarctica* lipase B
rpm	Revolution perminutes
NaOH	Sodium hydroxide
aq	Aqueous
R_f	Retention factor
ELSD	Evaporative light scattering detector
UATR	Universal attenuated-total reflectance
IL	Ionic liquid
EMK	Ethyl methyl ketone
RSM	Response surface methodology
MS	Mass spectrometer
M^+	Molecular ion
m/z	Mass to charge ratio
FTIR	Fourier transform infrared spectroscopy

Na^+	Sodium ion
Ser	Serine
His	Histidine
FDA	Food and Drug Administration
ace	acetone
hxn	hexane

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Sugar esters are non-ionic surface active agents consist of sugar as hydrophilic group and fatty acid as hydrophobic group. Non-ionic refers to the uncharged group in polar head. Sugar esters are not new material in research field. They have been well known since, 1960s and getting advance until now. In recent years, sugar ester has gained popular attention (Ganske & Bornscheuer, 2005; Hu & Li, 2011; Kobayashi, 2011; Yang & Huang, 2012; Yoo *et al.,* 2007) due to their versatility as non-ionic surfactants (Yoo *et al.,* 2007) and present with wide range of hydrophilic-lipophilic balance value.

They are available with a range of properties: oil soluble (0-6), water dispersible (7-9) and water soluble (10-18) (Szuts *et al.,* 2007). Besides, sugar ester was reported as harmless (Abdulmalek *et al.,* 2012), non-irritant and non-toxic to skin and eyes (Šabeder *et al.,* 2006), biodegradable to environment (Gumel *et al.,* 2011), odorless (Ruela *et al.,* 2013) and tasteless (Lee *et al.,* 2008). These specific properties have made them suitable to be used as foodstuffs, cosmetics, pharmaceutical technology, biotechnological industries and medical products (Habulin *et al.,* 2008; Szuts *et al.,* 2007). While the properties as antimicrobial, antitumoral (Adnani *et al.,* 2011), antibacterial, miticidal, and insecticidal (Gumel *et al.,* 2011) have been reported and might open new market (Šabeder *et al.,* 2006).

Sugar esters are synthesised by esterification of sugar with fatty acid. Synthesis of ester can be carried out either enzymatically or chemically. Problems in chemical pathway can be overcome by using enzyme such as lipase for the synthesis of sugar esters. The main advantages of enzymatic pathway are the specificity and regioselectivity. Theoretically, lipase catalysed reaction can introduce fatty acid into specific position of sugar. Besides performed under mild reaction condition, they need fewer number of separating and refining steps.

Recently, enzymatic reaction has been performed in organic solvent. In this case, the low solubility of sugar in organic media can be solve by using polar solvent. However, high polar solvent tend to denature the enzyme. Thus, another approach was carried out by previous researcher by using dual solvent system in the reaction synthesis (Abdulmalek *et al.,* 2012).

The addition of fatty acid to sugar is to improve the compatibility of sugar with fats or oils. Equation 1, showed the formation of sugar ester via esterification

reaction between a sugar ($C_n(H_2O)_n$) and a fatty acid (RCO_2H) (Gumel *et al.,* 2011)

$$C_n(H_2O)_n + RCO_2H \rightleftharpoons C_n(H_2O)_{n-1}(OCOR) + H_2O \text{.....} \quad \text{Equation 1}$$

Water should be remove from esterification reaction to shift the equilibrium of the reaction away from hydrolysis. Hydrolysis can affect the percentage yield of product due to excess amount of water.

Xylose known as a simple sugar contains five carbon atoms. Xylose have been used as food additives and food produce for decades. Recently xylose known as versatile sugar because it has similar application as their hydrolysis product (xylitol) : source of diabetic patience, non- nutritive sweetener,additives in color photograph and in fermentation process (Murthy *et al.,*2005). Publish report showed that, native hemicellulose contain 85-90% of xylose (Samayam & Schall, 2010) and are abundantly available as cheap and renewable feedstock. This sugar also became popular in Japan and Europe in the 1960s. It was successfully introduced in the United States upon approval by the Food and Drug Administration (FDA) in 1963.

An earlier report showed synthesis reaction between 1,2-*O*-isopropylidene-D-xylofuranose and arachidonic acid, produce xylose-5-arachidonate (Ward *et al.,* 1997). Then, another research team reported that xylose was almost unreacted with long chain fatty acid (Vitisant, 2012). However, the detailed result of the xylose ester conversion was not stated in the paper since they didn't focus on xylose ester synthesis. Later, a published report conveyed on synthesis and optimization of xylose laurate and xylose stearate ester using statistical experimental design (Bouzaouit & Bidjou-haiour, 2015). Murray co-workers and Bidjou-Haiour co-workers has different ester position, however both surface tension value was convenient with each other (Bidjou-Haiour & Klai, 2013; Murray *et al.,* 2000).

From investigation, there were lack of published reports on xylose ester. In this project, xylose ester was synthesized via enzymatic route using short-chain fatty acid (caproic acid). The screening process was performed and several parameters were varied to identify the optimum condition for xylose caproate ester synthesis by varying one parameter at a time. The screening process includes screening a variety of commercial lipases, different rotational speed, type of solvents, enzyme amount, reaction time, and sugar to fatty acid molar ratio. Characterization of the ester produced was then conducted.

1.2 Problem Statements

Synthesis of xylose ester is a challenging task. The presence of many hydroxyl groups in the cyclic ring structure together with insolubility towards organic solvents are two main challenges. The choices of solvent is important as a polar solvent tends to denature the enzyme. Possible formation of di- and tri-ester makes the choice of substrate molar ratio critical.

Recently, sugar ester has been stated in many published reports as a versatile non-ionic surfactant due to the wide variety of Hydrophilic Lipophilic Balance (HLB) value. The properties of sugar esters have made them suitable to be used as active ingredient in pharmaceutical, agriculture and cosmetics formulation, biotechnological industries and other industrial processes due to their low sensitivity towards water hardness and pH (Bizukojc *et al.,* 2005).

In 2005, researcher has studied a few selected anionic and non-ionic surfactant. Studies showed that anionic surfactants are harmful, while common non-ionic surfactants are toxic. The toxicity of surfactants were classified base on The European Community Legislation basis on LC 50. Polyoxyethylene alkyl ethers (AE) has been discovered as the most toxic surfactant to aquatic organism followed by polyoxylethylene alkyl phenyl ethers (APE) (Bizukojc *et al.,* 2005).

Normally, after the cleaning process, large detergent residues were released to the aquatic environment. 26 detergents and five softeners products has been found in Sweden as acutely toxic product (Bizukojc *et al.,* 2005). Therefore, the synthesis and production of xylose caproate ester as new non-ionic surfactant is necessary to replace the toxic surfactant which are currently in the market.

1.3 Objectives

This study embarked with the aim of synthesizing xylose ester via enzymatic catalysis. *Candida antartica* lipase immobilized on acrylic resin (Novozym 435) was selected as the main catalyst in this synthesis reaction due to its stability from previous reports. The objectives of our work are as follows:

1. To synthesize and characterize xylose caproate ester via lipase catalyzed esterification.
2. To optimize the reaction conditions using one variable at-a-time approach.
3. To investigate the physicochemical properties of the ester.

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