



UNIVERSITI PUTRA MALAYSIA

**SYNTHESIS OF ACETYLATED GLUCOSE ESTERS OF PALM
FATTY ACIDS AND THEIR PROPERTIES AS SURFACTANTS**

OLOBO JONATHAN OBAJE

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By

OLOBO JONATHAN OBAJE

**Thesis Submitted in Fulfilment of the Requirements for the Degree of
Doctor of Philosophy in the Faculty of Science
Universiti Putra Malaysia**

March 2000



To my wife, 'Uchogwu

and Children,

Attah, Iduh and Olobo Jr.



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

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Chairman: Associate Professor Dzulkefly Kuang, Ph.D.

Faculty: Science and Environmental Studies

The present esterification methods for producing sugar-based fatty esters are faced with the problems of low yields, the use of toxic solvents and thermal instabilities of sugars. This project reviewed and modified a number of esterification methods with the view to overcome these problems. The methods studied include direct heating, acid anhydride, enzymatic, and chemical transesterification and interesterification methods. Subsequently, a solvent-free, low temperature chemical interesterification method, yielding up to 90% product (mainly mono- and di-fatty acid substituted acetylated glucopyranoses) was developed. The method involved the heating of glucose pentaacetate (GPA), with appropriate fatty acid methyl ester (FAME) in the presence of Na-metal catalyst, under reduced pressure. The optimal reaction conditions were found to be 1:3 mole ratio of GPA and FAME, respectively; 0.5% Na-metal catalyst; reaction temperatures of 85 – 90°C; reaction time of 5 to 6 hrs,



and at 20 mmHg pressure. Six acetylated glucose fatty esters (AGFE) were obtained as products using FAME of palm (PO) and palm kernel (PKO) fatty acids, C6 to C10 (C_{6/10}) mixed fatty acids, and oleic and stearic fatty acids. The reactions leading to the formation of the mono- and di-substituted acetylated glucose fatty esters were found to follow zero-order kinetics. Results from fatty-acyl group selectivity studies show that the longer-chain fatty acyl groups were generally preferred to the shorter chain-lengths.

A novel nuclear magnetic resonance (NMR) spectroscopic method for determining the molecular structures of polyacetylated glucose fatty esters was developed in the course of this work. Using heteronuclear multiple bond correlation (HMBC) technique and the fatty acyl substituent-induced changes (SCS) in the ¹³C-chemical shifts of the carbonyl-carbon atoms, the molecular structures of the mono- and di-substituted products were established as 1-O-fatty acyl 2,3,4,6-acetyl α-D-glucopyranose and 1, 6-O-fatty acyl 2,3,4-acetyl α-D-glucopyranose, respectively.

The surface activity properties of the products were studied. AGFE was not soluble in water due to the lack of free –OH groups in the molecular structure, thus limiting the hydrophilic character of the pyranosyl head groups. Hydrophile-lipophile balance (HLB) experiments showed that AGFE from stearic acid and PKO were potential water-in-oil (W/O) emulsifiers while AGFE from PKO, PO and oleic acid showed moderate oil-in-water (O/W) emulsion stabilization.

Cytotoxic experiments involving cancerous (HT-29 colon carcinoma and CEM-SS, T-cell lymphoblastic leukemia) and normal (3T3 normal mouse fibroblast) cell lines have shown that AGFE were non-cytotoxic towards cancer and normal cell lines.



The results of the antimicrobial experiments showed that the di-substituted PKO product (PKO-2) to have a moderate activity against *P. aeruginosa* with inhibition zone diameter of 14.0mm and weakly active against *S. auerus*, *B. subtilis* B28 and B29. AGFE from PKO (PKO-1) was weakly active against *P. aeruginosa* with inhibition zone diameter of 9.0mm. These compounds can thus perform the dual role as emulsifiers and preservatives.

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

**SINTESIS GLUKOSA ESTER TERASETAL DARI ASID LEMAK
KELAPA SAWIT DAN SIFATNYA SEBAGAI SURFAKTAN**

Oleh

OLOBO JONATHAN OBAJE

Mac 2000

Pengerusi: Professor Madya Dzulkefly Kuang, Ph.D.

Faculti: Sains dan Pengajian Alam Sekitar.

Kaedah-kaedah pengesteran menghasilkan ester lemak berasaskan gula pada masa kini menghadapi banyak masalah memberikan hasil yang rendah, penggunaan pelarut toksik, dan ketakstabilan terma gula. Dalam projek ini beberapa kaedah pengesteran telah dikaji dan diubahsuai untuk mengatasi masalah tersebut. Kaedah yang dikaji meliputi pemanasan terus, asid anhidrida, kaedah penginteresteran, pengtransesteran dan kaedah enzim. Akhirnya kaedah penginteresteran kimia pada suhu rendah tanpa pelarut yang menghasilkan sehingga 90% produk (hasil utama adalah glukopiranosida berasetil mono- dan dwi-tertukarganti asid lemak) telah dimajukan. Kaedah ini melibatkan pemanasan glukosa pentaasetat (GPA) dengan ester metil asid lemak (FAME) yang bersesuaian dengan kehadiran mangkin logam Na di bawah tekanan terturun. Keadaan tindak balas optimum didapati pada nisbah mol GPA dan FAME 1:3; mangkin logam Na 5%; suhu tindak balas diantara 85 – 90°C; masa tindak balas di antara 5 hingga 6 jam dan tekanan pada 20 mmHg. Enam ester lemak glukosa berasetil (AGFE) telah dihasilkan dari FAME asid lemak sawit (PO) dan isirong sawit (PKO), campuran asid lemak C6 hingga C10



(C_{6/10}), asid lemak oleik dan asid lemak stearik. Tindak balas pembentukan ester lemak glukosa berasetil mono- dan di-tertukarganti didapati memenuhi kinetik tertib sifar. Kajian kepilahan kumpulan asil lemak menunjukkan bahawa asid lemak rantai panjang lebih disukai dari asil lemak rantai pendek dalam proses penginteresteran tanpa pelarut.

Dalam kajian ini satu kaedah spektroskopi nukleus magnetik resonans (NMR) untuk menentukan struktur molekul ester lemak glukosa berpoliasetil telah dimajukan. Penggunaan teknik korelasi ikatan berganda heteronukleus (HMBC) dan perubahan teraruh tertukarganti (SCS) asil lemak ke atas anjakan kimia ¹³C bagi atom-atom karbon karbonil, struktur molekul produk mono- dan dwi-tertukarganti masing-masing ditentukan sebagai 1-O- asil lemak 2,3,4,6-asetil α -D-glukopiranosida dan 1, 6-O- asil lemak 2,3,4-asetil β -D-glukopiranosida.

Sifat aktiviti permukaan produk juga dikaji. AGFE adalah tidak larut di dalam air kerana kekurangan kumpulan -OH bebas dalam struktur molekulnya, yang menghadkan ciri hidrofilik kumpulan piranosil. Kajianimbangan hidrofil-lipofil (HLB) menunjukkan bahawa AGFE dari asid stearik dan PKO adalah berpotensi sebagai emulsifier air dalam minyak (W/O), manakala AGFE dari PKO, PO dan asid oleik menunjukkan kestabilan emulsi minyak dalam air (O/W) yang sederhana.

Kajian sitotoksik melibatkan sel kanser (HT-29 kolon karsinoma dan CEM-SS, sel T leukimia limfoblastik) dan sel normal (3T3 fibroblas tikus biasa) menunjukkan ketiadaan kesitotoksikan AGFE terhadap sel kanser dan sel normal.

Keputusan eksperimen anti bakteria menunjukkan bahawa produk PKO dwi-tertukarganti (PKO-2) mempunyai aktiviti sederhana terhadap *P. aeruginosa* dengan

garispusat zon perencatan 14.0mm dan mempunyai aktiviti lemah terhadap *S. auerus*, *B. subtilis* B28 dan B29. AGFE dari PKO mono-tertukarganti (PKO-1) mempunyai aktiviti lemah terhadap *P. aeruginosa* dengan garispusat zon perencatan 9.0mm. Sebatian-sebatian ini bertindak dengan dua peranan iaitu sebagai bahan pengemulsi dan pengawet.

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I certify that an Examination Committee met on 27 March 2000 to conduct the final examination of Mr. Olobo Jonathan Obaje on his Doctor of Philosophy thesis entitled "Synthesis of Acetylated Glucose Esters of Palm Fatty Acids and Their Properties as Surfactant " in accordance with the Universiti Pertanian Malaysia (Higher Degree) Act 1980 and the Universiti Pertanian (Higher Degree) Regulation 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Mawardi Rahmani, Ph.D.

Associate Professor/ Head of Chemistry Department,
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Chairman)

Dzulkefly Kuang Abdullah, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Member)

Anuar Kassim, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Member)

Zaizi Desa, Ph.D.

Associate Professor
Faculty of Science and Environmental Studies,
Universiti Putra Malaysia.
(Member)

Hamdan Suhaimi, Ph.D.

Professor / Deputy Dean,
Faculty of Science and Technology,
Kolej Universiti Terengganu.
(Member)

Badri Bin Muhammad Ph.D.

Professor
Palm Oil Research Institute of Malaysia (PORIM)
Bandar Baru Bangi, Selangor,
Malaysia.
(External Examiner)



MOHD GHAZALI MOHAYIDIN, Ph.D.
Professor/Deputy Dean of Graduate School,
Universiti Putra Malaysia.

Date: 18 APR 2000

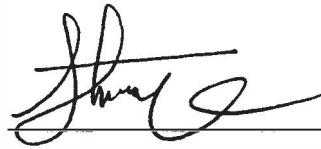
This thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.

Kamis Awang
Kamis Awang, Ph.D.
Associate Professor
Dean of Graduate School,
Universiti Putra Malaysia.

Date: 11 MAY 2000

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 at UPM or other institutions.



Olobo Jonathan Obaje

Date: 10 April 2000

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LIST OF ABBREVIATIONS

A	area
AE	alcohol ethoxylate
AGFE	acetylated glucose fatty ester
APE	alkyl phenol ethoxylate
APG	alkyl polyglucoside
AOCS	American Oil Chemist's Society
C6	hexanoic acid
C _{6/10}	C6 to C10 fatty acid mixture
C _{6/10-2}	1, 6-O-C _{6/10} -fatty acyl 2,3,4-acetyl α -D-glucopyranose
C8	caprylic acid
C10	capric acid
C12	lauric acid
C14	myristic acid
C16	palmitic acid
C18	stearic acid
C18:1	oleic acid
C18:2	linoleic acid
CMC	critical micelle concentration
DSC	differential scanning calorimetry
DMA	dimethyl acetamide
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
EDTA	ethylenediamine tetraacetic acid



FAME	fatty acid methyl ester
FCS	fetal calf serum
FTIR	Fourier transform infrared
g	gram
GA	alkyl glucamide
GC-MS	gas-chromatography mass-spectrometry
GPA	glucose pentaacetate
Hetcor	heteronuclear shift correlation
HDL	heavy duty liquid
HLB	hydrophile-lipophile balance
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
IRPA	intensified research in priority areas
LAB	linear alkylbenzene sulfonate
m	metre
mg	milligram
min	minute
mL	millilitre
mm	millimetre
MMT	million metric tones
NB	nutrient broth
NMR	nuclear magnetic resonance
°C	degree celcius
Oleic-1	1-O-oleoyl 2,3,4,6-acetyl α -D-glucopyranose
O/W	oil-in-water



PDA	potato dextrose agar
PKO	palm kernel oil
PKO-1	1-O-PKO-fatty acyl 2,3,4,6-acetyl α -D-glucopyranose
PKO-2	1, 6-O-PKO-fatty acyl 2,3,4-acetyl α -D-glucopyranose
PO	palm oil
PO-1	1-O-PO-fatty acyl 2,3,4,6-acetyl α -D-glucopyranose
PORIM	Palm Oil Research Institute of Malaysia
SCS	substituent-induced chemical shift changes
St-1	1-O-C18-fatty acyl 2,3,4,6-acetyl α -D-glucopyranose
TG	triglyceride
TLC	thin-layer chromatography
W/O	water-in-oil
w/w	weight/weight