UNIVERSITI PUTRA MALAYSIA

LIPASE-CATALYSED SYNTHESIS OF FATTY AMINOESTERS FROM FATTY ACIDS AND TRIETHANOLAMINE

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by

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Chairman: Associate Prof. Dr Hjh. Mahiran Basri

Faculty: Science and Environmental Studies

Fatty aminoesters containing mixtures of mono-, di- and tri-aminoesters were prepared from the enzyme-catalysed esterification of fatty acids and triethanolamine in organic solvents. Screening of enzymes suitable for the reactions was carried out by conducting the esterification reaction in *n*-hexane catalysed by 5 types of enzymes. Lipozyme IM and Novozym 435 gave positive results indicated by the additional spots obtained on TLC analysis. FTIR analysis of the products indicated the presence ester linkage at wavenumber 1740 cm^{-1}. Reduction in transmittance of the hydroxyl group at 3500 cm^{-1} indicated the disappearance of the hydroxyl group which was

originally on the triethanolamine molecule. Effects of some reaction parameters such as time course, temperature, substrate mole ratio, substrate concentration, amount of enzyme and thermodynamic water activity were also investigated based on esterification of oleic acid and triethanolamine catalysed by Lipozyme IM and Novozym 435 in *n*-hexane.

Lipozyme IM gave maximum conversion of 43.9% in 24 hours while Novozym 435 gave a maximum conversion of 37.34% in 6 hours. Optimum activity was obtained when the lipases catalyse the reaction at 50°C, at substrate concentration of 1.0 M in *n*-hexane. The lipases, however, behaved differently when preequilibrated in salt hydrates of different thermodynamic water activity. Novozym 435 exhibited high esterification activity when preequilibrated in the salt hydrates of lowest a_w studied. On the other hand, Lipozyme IM exhibited optimum esterification activity when preequilibrated in salts hydrates having a_w value of 0.32. The activity of Lipozyme IM was drastically reduced when the mole of triethanolamine was increased while Novozym 435 gave optimum activity when the substrates were reacted at 1:1 mole ratio. In addition, when the oleic

acid was replaced by various type of fatty acids, Lipozyme IM exhibited affinity towards medium and long chain fatty acids while Novozym 435 catalysed the esterification reaction of all the fatty acids studied at the same rate. Their activity, however, decreased when the saturated fatty acid were replaced with unsaturated C18 fatty acids. The activity of the lipases also decreased significantly when triethanolamine was replaced with diethanolamine and ethanolamine.

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

SINTESIS AMINOESTER LEMAK DARI ASID LEMAK DAN TRIETANOLAMINA YANG DIMANGKINKAN OLEH ENZIM LIPASE

oleh

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Aminoester lemak yang terdiri dari campuran mono-, di- dan triaminoester telah disediakan melalui proses pengesteran antara asid lemak dan trietanolamina dalam pelarut organik menggunakan pemangkin enzim lipase. Penyaringan enzim yang sesuai untuk proses ini dilakukan dengan menjalankan proses pengesteran dalam pelarut *n*-hexana dan dimangkinkan oleh 5 jenis enzim. Enzim Lipozyme IM dan Novozym 435 memberi keputusan yang positif seperti yang ditunjukkan oleh tambahan tompokan pada plat TLC.

Analisa FTIR menunjukkan kehadiran molekul karbonil ester pada 1745 sm^{-1}. Kesan parameter-parameter seperti tempoh tindakbalas, suhu, nisbah bahan tindakbalas, kepekatan bahan-bahan tindakbalas dalam pelarut, kandungan enzim-enzim dan aktiviti termodinamik air telahpun dikaji berdasarkan proses pengesteran antara asid oleik dan trietanolamina yang dimangkinkan oleh enzim Lipozyme IM dan Novozym 435.

Enzim Lipozyme IM menunjukkan peratus tindakbalas maksimum sebanyak 43.9% dalam masa 24 jam manakala enzim Novozym 435 menunjukkan peratus tindakbalas maksimum sebanyak 37.34% hanya dalam masa 6 jam. Aktiviti optimum pula diperolehi apabila proses pengesteran dijalankan pada suhu 50°C dan pada kepekatan bahan-bahan tindakbalas 1.0M dalam pelarut organik. Walau bagaimanapun, enzim-enzim tersebut mempamerkan kelakuan yang berbeza apabila enzim-enzim dan bahan-bahan tindakbalas di simpan dalam bekas tertutup yang mengandungi garam terhidrat yang diketahui nilai aktiviti termodinamik air sebelum dicampurkan. Enzim Novozym 435 menunjukkan aktiviti pengesteran yang tinggi apabila bahan-bahan tindakbalas di simpan dalam garam terhidrat yang mempunyai nilai

termodinamik air terendah. Bagi enzim Lipozyme IM pula menunjukkan aktiviti optimum apabila bahan-bahan tindakbalas disimpan dalam garam terhidrat yang mempunyai nilai termodinamik air 0.32. Aktiviti enzim Lipozyme IM didapati menurun secara mendadak apabila nisbah mol trietanolamina dinaikkan manakala Novozym 435 pula menunjukkan aktiviti optimum apabila asid oleik bertindakbalas dengan trietanolamina pada nisbah 1:1. Apabila asid oleik digantikan dengan asid lemak-asid lemak lain, enzim Lipozyme IM menunjukkan aktiviti yang tinggi apabila asid lemak yang mempunyai rantaian karbon sederhana dan panjang digunakan manakala enzim Novozym 435 menunjukkan kadar aktiviti yang sama bagi semua asid lemak yang diuji. Kadar aktiviti kedua-dua enzim, walau bagaimanapun, menurun apabila asid lemak tepu digantikan dengan asid lemak tak tepu yang mempunyai rantaian karbon C18. Kadar aktiviti enzim juga menurun apabila trietanolamina diganti dengan dietanolamina and etanolamina.

CHAPTER 1

INTRODUCTION

The interest in the production of nitrogen-based surfactants having cationic functionality begin when Domagk discovered the importance of this class of compounds as bactericide during the World War 1 (Jungermann, 1969). The largest volume of nitrogen-based cationic surfactants produced are in the form of quaternary ammonium compounds, generally derived from the quaternization of a tertiary amines (Billenstein and Blaschke, 1984).

The largest market for this class of compounds is in fabric softeners followed by in manufacturing of organo-modified clays for drilling mud. On the other hand, the application of quaternary ammonium compounds in bactericidal or sanitizer products now became third in the list (Billenstein and Blaschke, 1984).

In fabric and textile softener industry alone, fatty quaternary ammonium compounds, had undergone rapid evolution due to

consumer demand for better performing products and stringent environmental regulation. Esterquats, quaternized triethanolamine esters, developed from the catalytic esterification of fatty acid and triethanolamine followed by quaternization of the fatty aminoester, was then introduced (Trius, 1991). Features offered by this compound are as listed below:

1. The presence of ester linkages connecting the core nitrogen atom and the hydrocarbon chain provide potential breaking points for easy biodegradation (Puchta *et al.*, 1993).

2. Cationic materials having two long alkyl chain used as active softening compounds are substantially water-insoluble. Conventionally, textile softening products contains 3-5% dispersion of di-stearyl di-methyl ammonium chloride as the softening compounds. It is generally not possible to prepare such aqueous dispersion with more than 10% of cationic materials without encountering problems such as viscosity and stability (Chang, 1991). With esterquats, it is possible to prepare stable and low viscosity dispersion containing up to 50% active softening compounds. This will reduce packaging and

transportation cost, reduce shelf space and utilise smaller and easier to handle containers (Keys, 1995)

Generally, industrial production of fatty amines and their derivatives involved classical methods (Billenstein and Blaschke, 1984). The fundamental intermediates for fatty amines are the fatty acid nitriles produced through reaction of fatty acid and ammonia at temperatures of between 280°C to 360°C at atmospheric pressure. Fatty amines were then obtained through catalytic hydrogenation of the fatty acid nitrile (Hui, 1996)

Howèver, rapid advances in biotechnology over the past decade have led to considerable interest in the development of biological methods for manufacturing industrial products. Consumers, now, are more aware of the environmental impact caused by the excessive use of energy and harsh conditions in production of these products via the classical methods. Now, clean and mild processes and biodegradability of particular products that they used have become as important as their functional performance. These changes in trends, as a result, imposed a greater challenge to the oleochemical-based surfactants manufacturers.

Biotechnology approach of producing industrial chemicals has gained considerable interest in the oleochemical industry. One aspect of biotechnology is "biotransformation" where a biocatalyst is used to convert a raw material into value-added product. The choice of biocatalyst is between isolated enzymes where the biotransformation is a one-step process or intact microbial cells where the biotransformation is a complex multistep process. The use of isolated or immobilised enzymes to catalyse reactions has opened up many new synthetic possibilities and applications. Enzymes which have the largest number of potential applications in the oleochemical industry are the extracellular microbial lipases.

This particular work was, therefore, forwarded with the following objectives:

1. To evaluate the feasibility of producing fatty aminoester, the intermediates for the production of esterquats, via enzymatic route.

2. To investigate the effects of various reaction conditions on the esterification of oleic acid and triethanolamine using commercially available immobilised lipase as biocatalyst.

3. To characterise the formation of fatty aminoester by thin layer chromatography, Fourier Transform Infrared Spectroscopy and acid value.

CHAPTER II

LITERATURE REVIEW

Synthesis of Oleochemicals

The production of oleochemicals is centred around the modification of natural triglycerides through reactions at the functional group or at the unsaturated bond (Figure 1). The five basic oleochemicals are fatty acids, fatty esters, nitrogen derivatives, fatty alcohols and glycerol. These basics oleochemicals are produced in different grades of quality depending on the application that it was meant for.

The application of oleochemicals and their derivatives cover a wide spectrum of industries ranging from industrial and household chemicals to fine ingredients for personal care and pharmaceuticals industries. Nevertheless, the search for better performing products and safe industrial processes to suit the environment and consumer requirements are never ending. New echnology innovations in the oleochemicals industry are necessary

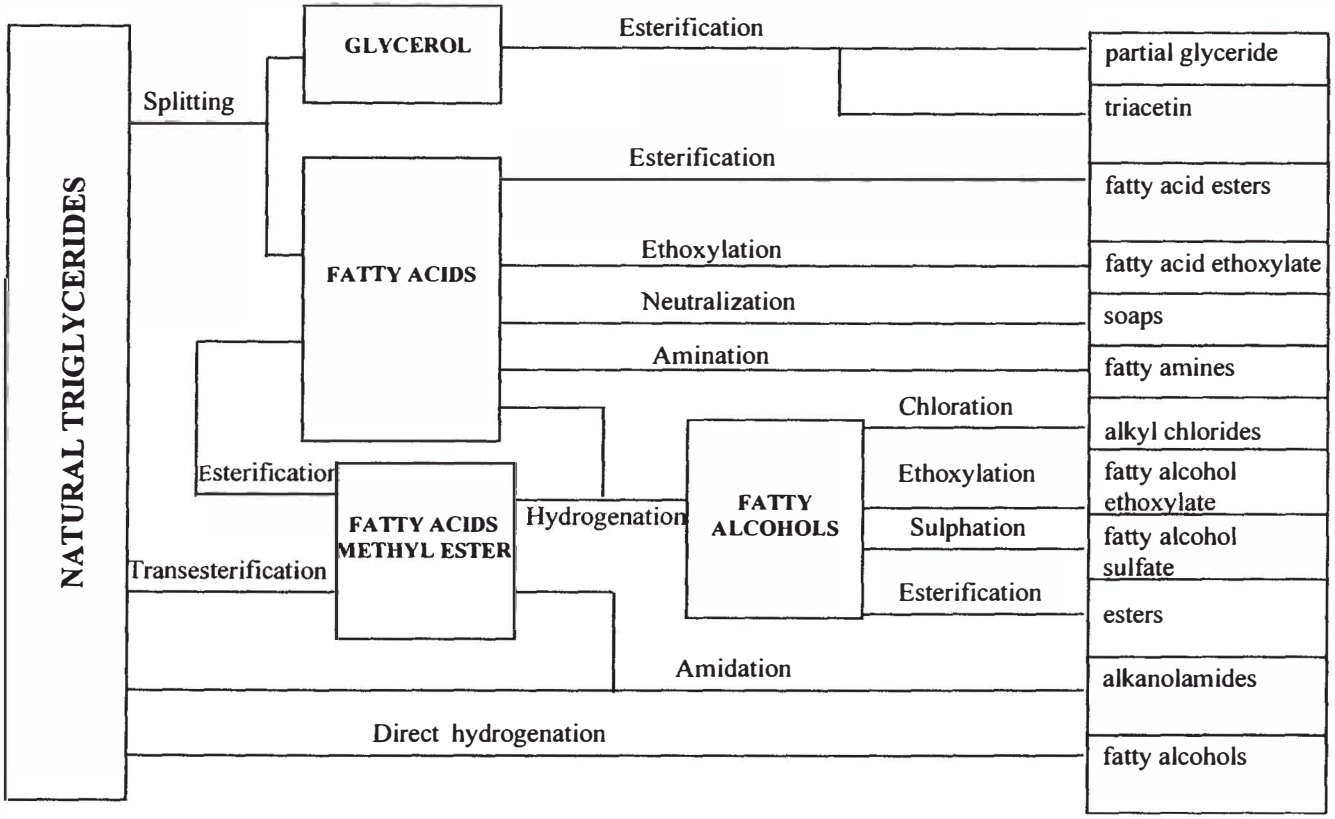


Figure 1: Possible Pathway for the Synthesis of Oleochemicals and Their Derivatives (Source: Henkel Oleochemicals Malaysia)

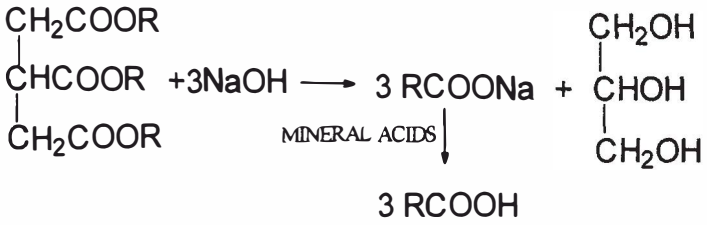
for the continuing supply of desired products as well as environment and energy conservation.

Production of Oleochemicals by Classical Methods

Fatty acids and Glycerine

Fatty acids, the most important oleochemicals have been industrially produced over a hundred years ago, can be derived from oils and fats through the following methods:

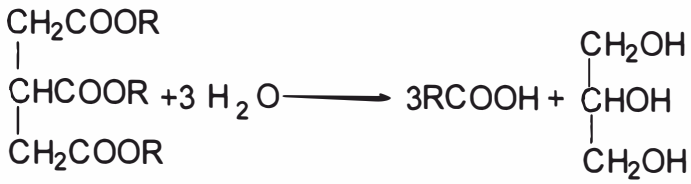
1. Via saponification process (Scheme 1). Through this method soap and glycerol are being liberated and the soap is further treated with mineral acid to give free fatty acids.



Scheme 1

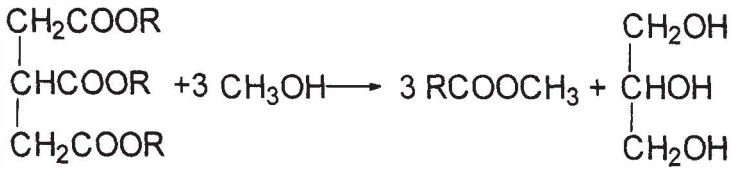
2. Via fat splitting process (Scheme 2). This process involved the hydrolysis of triglycerides to form free fatty acids and glycerol. At ambient temperature and atmospheric pressure, triglyceride or fat

do not mix with water, but through high pressure and temperature and with the help of suitable catalyst, these two reactants are brought together to a certain extent allowing hydrolysis to take place. Table 1 summarises the different types of fat splitting processes develop within 1942- 1984.



Scheme 2

3. Via alcoholysis (Scheme 3) to produce fatty ester followed by treatment with mineral acid to convert to free acids.



Scheme 3

Table 1: A Summary on The Different Methods and Conditions for Fat Splitting Processes.

Process	Conditions	Catalyst	Remarks
Twitchell Process	Three to four reboilings at atmospheric pressure (40 h) yield 95%	Twitchell reagents (sulfuric and sulphonic acids)	Small capacity Highly contaminated sweet water
Batch Splitting			
Medium or High Pressure Autoclave Splitting	Medium pressure (10 54 kg/cm²) Stage 1 period 6 - 8 h yield 85 - 90%	Zinc oxide (2-4%)	sweet water with 10 - 15% glycerol
	Stage 2 sweet water was replaced by fresh water) period 12 - 15 h yield 95 - 98%		
	High pressure (28 -32 kg/cm²) T°C 230 -240°C) period 6 - 8 h yield 92 -95%	No catalyst	Sweet water with 10 -15% glycerol Suitable for small batch production
Continuous Splitting			
Concurrent splitting	pressure 176 -246 kg/cm² T°C 315°C Yield 85 -90%	No catalyst	Sweet water contains 35% glycerol and a high percentage of free fatty acid This process has not reached commercial production
Countercurrent splitting	Pressure 30 -32 kg/cm² T°C 230°C or Pressure 51-56 kg/cm² T°C 260°C		The most favorite process Sweet water contains 15 - 20% glycerol

(Source Saran and Narula, 1995)