UNIVERSITI PUTRA MALAYSIA

ENZYMATIC INTERESTERIFICATION OF PALMOLEIN WITH STEARIC ACID BY USING IMMOBILIZED PSEUDOMONASLIPASE

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ENZYMATIC INTERESTERIFICATION OF PALM OLEIN WITH STEARIC ACID BY USING IMMOBILIZED *PSEUDOMONAS* LIPASE

By

ATIF ABD ELMONEIM AHMED YASSIN

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Engineering Universiti Putra Malaysia

June 2001

Dedicated to

My parents, wife, brothers and sisters

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

INTERESTERIFICATION OF PALM OLEIN WITH STEARIC ACID BY IMMOBILISED *PSEUDOMONAS* LIPASE

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June 2001

Chairman: Associate Professor Mohd Nordin Ibrahim, Ph.D.

Faculty: Engineering

Interesterification of palm olein with stearic acid by immobilized *Pseudomonas* lipase in n-hexane was studied. The catalytic performance of the immobilized *Pseudomonas* was evaluated by determining the change in fatty acid composition and concentration using gas liquid chromatography (GLC), and the change in triglyceride composition and concentration using high performance liquid chromatography (HPLC).

Interesterification resulted in the formation of trisaturated triglyceride tripalmitin (PPP) and 1,2-dipalmitoyl-stearoyl glycerol (PPS), both of which were absent in the original oil. It also resulted in a decrease in the concentrations of the four main triglycerides in palm olein namely 1-palmitoyl-dioleoyl glycerol (POO), 1,3-dipalmitoyl-2-oleoyl glycerol (POP), 1-palmitoyl-2-oleoyl-linoleoyl glycerol (POL) and 1,3-dipalmitoyl-2-linoleoyl glycerol (PLP). On the other hand, interesterification

increased the concentrations of 1-stearoyl-dioleoyl glycerol (SOO), 1-palmitoyl-2-oleoyl-stearoyl glycerol (POS) and 1,3-distearoyl-2-oleoyl glycerol (SOS). Overall, the combined concentration of polyunsaturates, triunsaturates, and diunsaturates decreased, while that of the full saturates and monounsaturates increased, thus raising the slip melting point of the final product.

Interesterification caused substantial incorporation of stearic acid into the palm olein, with concomitant decreases in the palmitic and oleic acid contents. Immobilizing the *Pseudomonas* lipase resulted in a fast reaction, with the steady state reached after about 6 hours. Adding water to the reaction mixture increased the lipase activity.

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

INTERESTERIFIKASI MINYAK KELAPA SAWIT DENGAN ASID STEARIK OLEH LIPASE PSEUDOMONAS TERSEKAT-GERAK

Oleh

ATIF ABD ELMONEIM AHMD YASSIN

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Pengerusi : Profesor Madya Mohd. Nordin Ibrahim, Ph.D.

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Interesterifikasi minyak kelapa sawit dengan asid stearik di dalam n-heksana oleh Lipase Pseudomonas tersekat-gerak telah dikaji. Prestasi Lipase Pseudomonas tersekat-gerak sebagai enzim telah dinilai melalui penentuan perubahan di dalam komposisi dan kepekatan asid lemak dengan kromatografi cecair-gas (GLC), dan perubahan di dalam komposisi dan kepekatan trigliserida dengan menggunakan kromatografi cecair prestasi tinggi (HPLC).

Di dalam kesemua kes, proses interesterifikasi telah menghasilkan pembentukan trigliserida tritepu yang tidak wujud dalam minyak asal sebelum interesterifikasi, iaitu tripalmitin (PPP) dan 1,2-dipalmitol-stearol gliseraol (PPS). Pada masa yang sama, terdapat pengurangan di dalam kepekatan trigliserol-trigliserol utama dalam minyak sawit, iaitu 1-palmitol-dioleol gliserol (POO), 1,3-dipalmitol-2-oleol gliserol (POP), 1-

palmitol-2-oleol-linoleol gliserol (POL) dan 1,3-dipalmitol-2-linoleol gliserol (PLP). Secara keseluruhannya, gabungan kepekatan politaktepu, tri-tak-tepu dan di-tak-tepu telah menunjukkan pengurangan, manakala kepekatan tri-tepu dan mono-tak-tepu telah meningkat berbanding sebelum tindakbalas interesterifikasi. Keadaaan ini menyebabkan peningkatan dalam takat lebur produk yang telah dihasilkan.

Selepas tindakbalas interesterifikasi, terdapat pergabungan ketara di antara asid stearik ke dalam minyak sawit, dengan pengurangan serentak di dalam kandungan asid palmitik dan asid oleik. Lipase Pseudomonas tersekat-gerak telah mengakibatkan kadar tindakbalas interesterifikasi yang lebih pantas, di mana keadaan seimbang tercapai selepas 6 jam. Penambahan air kepada campuran tindakbalas telah meningkatkan aktiviti Lipase Pseudomonas tersekat-gerak tersebut.

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I certify that an Examination Committee met on 22nd June 2001 to conduct the final examination of Atif Abd Elmoneim Ahmed Yassin on his Master of Science thesis entitled "Enzymatic Interesterification of Palm Olein with Stearic Acid by Using Immobilized P*seudomonas* Lipase" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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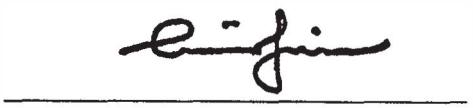
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.



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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.

ATIF ABD ELMONEIM AHMED YASSIN

Date: 16-7-2001

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LIST OF ABBREVIATIONS

FA	Fatty acid
FAC	Fatty acid composition
FFA	Free fatty acid
CB	Cocoa butter
CBS	Cocoa butter substitute
PO	Palm oil
POo	Palm olein
POs	Palm stearin
PKO	Palm kernel oil
PKOo	Palm kernel olein
CPO	Crude palm oil
PMF	Palm mid-fraction
RBD	Refined bleached deodorized
HPLC	High performance liquid chromatography
GLC	Gas liquid chromatography
SMP	Slip melting point
SFC	Solid fat content
U	Activity unit
POL	1-palmitoyl-2-oleoyl-linoleoyl glycerol
POP	1,3-dipalmitoyl-2-oleoyl glycerol
SOS	1,3-distearoyl-2-oleoyl glycerol

PLP	1,3-dipalmitoyl-2-linoleoyl glycerol
SOO	1-stearoyl-dioleoyl glycerol
POS	1-palmitoyl-2-oleoyl-stearoyl glycerol
PLL	1-palmitoyl-dilinoleoyl glycerol
OLL	1-oleoyl-dilinoleoyl glycerol
OOL	1,2-dioleoyl-linoleoyl glycerol
PPS	1,2-dipalmitoyl-stearoyl glycerol
OOO	Triolein
PPP	Tripalmitin
POO	1-palmitoyl-dioleoyl glycerol
MLP	1-myristoyl-2-linoleoyl-palmitoyl glycerol

CHAPTER 1

INTRODUCTION

It is a rare indeed natural oil/fat that fully meets the requirements for its intended use, and more often than not some modification is required, especially of its melting and crystallisation characteristics and nutritional properties. The ability to modify an oil/fat to its desired physical and chemical properties is therefore of great importance and interest industrially.

There are several methods of oil/fat modification, including hydrogenation and interesterification. Hydrogenation, unless carried to completion, converts the unsaturated *cis* fatty acids (FAs) to the *trans* form (Kenzo et al., 1982). This rather detracts from the process as *trans* FAs are increasingly implicated in several disease aetiology, including thrombogenesis which leads to coronary heart disease (Mensink and Katan, 1990; Zock and Katan, 1992; Willett et al., 1993). Interesterification modifies the properties of the triglyceride mixture in oils/fats (Macrae, 1983 a), and is traditionally carried out with chemical catalysts. However, it is difficult to introduce the desired acyl groups to specific positions on the glycerol (Tanaka et al., 1981) and the

modification may well lead to a deterioration in the oil/fat quality/characteristics (Lai et al., 1998). It is therefore not surprising that lipase interesterification is gaining interest. With lipases, interesterification can be carried out at lower temperature, and the reaction can be better controlled due to the high selectivity of enzymes for specific reactions (Rattray, 1984; Yamane, 1987; Mittelbach, 1990). The increasing popularity of lipase-catalysed processes for the modification of oil/fat is one of the major factors spurring interest in industrial lipases today (Bjorkling et al., 1991).

Interesterification takes several forms - the reaction of an ester with an acid (acidolysis), an ester with an alcohol (alcoholysis), an ester with another ester (ester interchange, or transesterification) and an ester with glycerol (glycerolysis) (Macrae, 1984; Wisdom et al., 1987). Sometimes, the term transesterification is used instead of interesterification to indicate that the process is a lipase-catalysed modification of the oil/fat (Adlercreeutz, 1994; Linko et al., 1994; Osterberg et al., 1989).

Cocoa butter (CB) is an important and expensive raw material used in the chocolate and related confectionery industries. It contains substantial quantities of the 2-oleoyl glycerides of palmitic and stearic acids, which confer the unique characteristics that give chocolate its sharp melting behaviour in the mouth (Macrae, 1985; Coleman and Macrae, 1980). As CB is expensive, a number of fats have been modified for use as substitutes in confectionery products. However, the ideal cocoa butter substitute (CBS) is yet to be found, and its quest, by enzymatic interesterification of oil/fat, has become popular biotechnological research.

Palm oil (PO), with its semi-solid characteristic, is an important raw material for the production of CBS (Coleman and Macrae, 1980; Bloomer, 1991), especially as the enzymatic interesterification of palm olein (POo) produces unique CB-like triglycerides (Chong et al., 1992). POo is the liquid fraction obtained by the fractionation of PO. It is fully liquid in warm climates, has a narrower range of triglycerides than PO and blends well with other seed oils (Pantzaris, 1987). Refined, bleached and deodorised POo (RBD POo), the major form of POo consumed and exported by Malaysia, contains 46% saturated FAs (mainly myristic, palmitic and stearic), 43% monounsaturated (oleic) and 11% polyunsaturated (linoleic) (Gunstone et al., 1986). POo is very popular in commercial and industrial uses because of its resistance to oxidation due to its low unsaturation and nutritionally good fatty acid composition (FAC) (no *trans* or *iso*-acids). One of its very best attributes is the lack of odour due to the absence of linolenic acid (Pantzaris, 1987).

Enzymatic modification of triglycerides is now very important in the food industry, and in the last decade interest in lipase interesterification has soared (Potts and Mukerheide, 1968; Lie and Molin, 1992; Anonymous, 1989). Lipases have been successfully applied to the synthesis of esters (Garcia et al., 1995; Okumura et al., 1979), hydrolysis of oils (Linfield et al, 1984) and several other interesterification reactions (Basheer et al., 1995; Macrae, 1983a).

In lipase interesterification, the equilibrium of a thermodynamically reversible chemical reaction can be manipulated (Martinek et al., 1981). A low water content is

used (Kyotani et al., 1988; Yamane et al., 1989) to veer the reaction towards interesterification from hydrolysis (Coleman and Macrae, 1980: Matsuo et al., 1981). By using organic solvents, the water content can be adjusted to control the final equilibrium of the reaction. However, the quest is still for the ideal solvent that will maximally solubilize the substrate and minimally affect the enzyme (Zaks and Klibanov, 1984; Deez and Rozzell, 1988).

Immobilisation refers to the localisation or confinement of an enzyme on a support base. This keeps the lipase physically apart from the reaction mixture so that it can be reused (Kilara and Shahani, 1977). Immobilization also improves the effectiveness of the lipase, and the enzyme can be recovered easily from the reaction mixture and recycled to reduce its cost (Messing, 1975). Besides, immobilization enhances the enzymatic properties, such as thermostability and activity, because of the increased enzyme/solid ratio (Yong and Al-Duri, 1996).

In this research, POo was interesterified with stearic acid in n-hexane, catalysed by immobilised *Pseudomonas* lipase. The performance of the immobilised lipase was evaluated by the change in triglyceride composition and concentration, determined by high performance liquid chromatography (HPLC). And the substrate conversion was determined by the amount of stearic acid incorporated in the triglycerides of PO, using gas liquid chromatography (GLC).

Objectives

The objectives of this study were to

1. Determine the FAC and FA concentrations in the modified POo.
2. Determine the triglyceride composition and concentrations in the modified POo.
3. Study the effect of water addition on the enzymatic interesterification reaction.
4. Study the change in the slip melting point (SMP) of the modified product.

CHAPTER II

LITERATURE REVIEW

Introduction

With the exception of tropical oils, the main source of solid fats is hydrogenated vegetable oils. However, there is at present concern over the health aspects of *trans* FAs formed during hydrogenation. This has generated a demand for non-hydrogenated, low (or even no) *trans* FAs solid fats, and spurred interest in alternative processing methods, such as interesterification, or the exchange of FAs between triglyceride molecules (Hernandez and Lusas, 1997).

Chemical interesterification of edible fats is usually done above 100^0C in a vacuum, or under a nitrogen blanket, to prevent oxidative degradation (Zainal and Yusoff, 1999), and is catalysed by a metal salt(s) (Hustedt, 1976; Sreenivasan, 1978). Interesterification can also be done using a lipase (Macrae, 1983a), with the advantages of requiring a lower temperature (25°C - 30°C) and producing more specific products.

However, lipase interesterification is still not widely used due to the high cost of enzyme and low yield. The introduction of reusable immobilized lipases may lower the cost and enhance its financial attraction (Goh et al., 1993). In this work enzymatic interesterification was carried out using immobilized *Pseudomonas* lipase.

The main problem with using a lipase is that it has to catalyze a reaction in a heterogeneous system containing a water insoluble substrate. In this system, satisfactory activity and solubility of the lipase are rather difficult to achieve. An immobilized enzyme overcomes the problem (Mojovic et al., 1994). Immobilization can also extend the enzyme life. As enzymes are not only expensive but also difficult to obtain in quantity, increasing the enzyme life would make the process simpler and cheaper.

As the reaction using an immobilized enzyme can be stopped rapidly by removing the enzyme, it is more controllable than using a solubilized enzyme. Also, the reaction mixture is not contaminated with the enzyme - an increasingly important point as stricter food laws are lowering the permissible level of enzymes in food (Messing, 1975).

Lipase interesterification is expected to become a parallel route for oils/fats modification, together with plant breeding and transformation to produce specific plants for specific oils. One example already in use is the production of CBS from cheaper feedstocks (Mojovic et al., 1993).