1 LITERATURE REVIEW

1.1 Introduction

Palm oil is an edible oil derived from the fruits of the oil palm *Elaeis guineensis* (Siew, 2002). Palm olein is one of the major palm oil products that domestically and industrially used as cooking/frying oil. The functions of frying oils are to transfer heat to cook foods and to produce characteristics of fried-food flavor.

The major advantage of palm olein is its high stability during frying that produced minimum amount of breakdown products in an acceptable level. Study conducted by Azmil and Siew (2008) shows that palm oil, single-fractionated palm olein and double-fractionated palm olein were more stable than high oleic sunflower oil after 80 hours of heating at 180 °C. These palm products also produced lower amount of free fatty acids, polar and polymer compounds, as well as preserved higher smoke points and tocols content. However, palm olein tends to crystallize at low temperature that limits its usage in temperate countries.

In spite of various nutritional studies, palm olein is not well considered as a recommended choice due to its higher saturation content. Against this factor, there is a need to reduce its saturation content, so as to enhance its versatility in applications for market penetration in cold countries as well as cater to market trends. Generally, the saturation content of palm olein can be reduced by multistage fractionation of palm olein. However removal of saturation in palm olein is difficult due to the difficulty in controlling the crystallization of palm olein (Gijs et al., 2007a). Other than that, blending palm olein with other soft vegetable oils such as canola oil, cottonseed oil, rice bran oil, sunflower oil, soybean oil etc

is implemented to reduce the saturation level of palm olein and for frying purposes in temperate countries (Razali and Nor'aini, 1994). In fact, blending of palm olein may also enhance the stability and frying performance of the oil.

In this study, palm olein is modified by enzymatic interesterification and dry fractionation to reduce the saturation content of the oil. Enzymatic interesterification enables interchange of acyl groups between and within triacylglycerols (TAGs) at specific positions to form new TAG species that have high melting TAGs, PPP and PPS. These saturated TAGs that causes the crystallization of palm olein, can be removed as stearin during fractionation. Two *sn*-1,3 specific immobilized lipases; Lipozyme® TL IM (*Thermomyces Lanuginosa*) and Lipozyme® RM IM (*Rhizomucor Miehei*) are selected as biocatalysts for interesterification in solvent-free system (Appendix A and B). Palm olein has been chosen as the feedstock due to its higher unsaturation content compared to palm oil. Two types of new palm oil products can be derived from this study; the low saturation palm liquid oils and the respective stearin fractions.

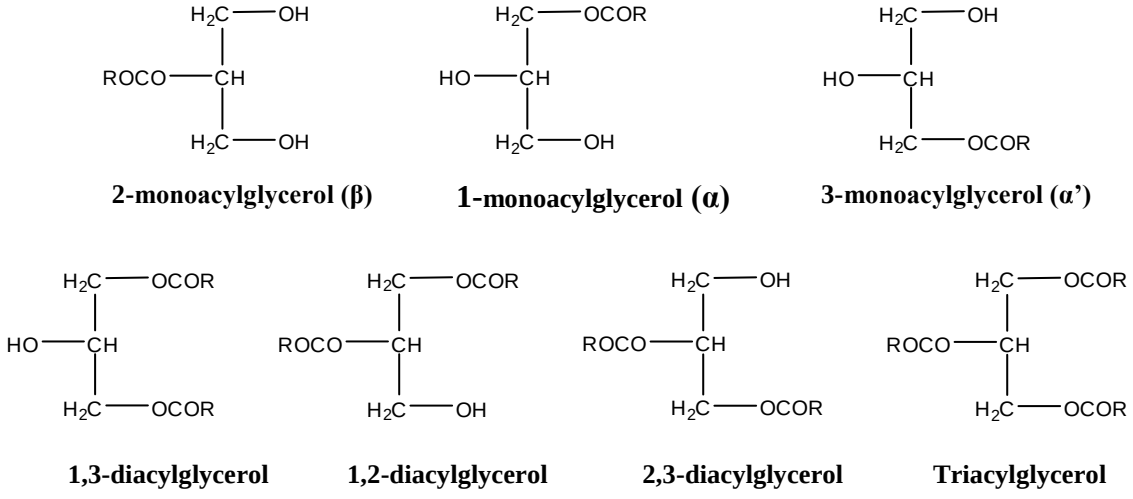
1.2 The Objectives of the Studies

The main objective of the studies was to prepare pure palm-based products with low saturation, via enzymatic interesterification of palm olein with iodine value (IV) of 62 follow by dry fractionation, as well as to characterize the physicochemical properties of the products. Besides, the efficiency of the lipases; *Rhizomucor Miehei* (Lipozyme® RM IM) and *Thermomyces Lanuginosa* (Lipozyme® TL IM) in the interesterification reaction will also be looked into. Optimization of the interesterification reactions and dry fractionation will also be carried out.

1.3 Chemical Properties of Palm Oil

Palm oil consists of mostly glyceridic materials with some non-glyceridic materials in trace amount (Chong, 1994). TAG is the most abundant glyceridic component in palm oil which comprises of triesters of high aliphatic acids or fatty acids, while monoacylglycerol (MAG) and diacylglycerol (DAG) are the minor glyceridic components in palm oil. The chemical structures of partial acylglycerols (MAG and DAG) and TAG were shown in Figure 1.1.

Figure 1.1
Partial acylglycerols and TAG molecules structures (Naudet, 1996)



TAGs are esters formed from glycerol acylation of three fatty chains, while acylation with one or two fatty chains formed partial acylglycerols (MAG and DAG). The hydrocarbon chains in the ester group, R could be varied in terms of carbon number and the chemical structure (bend structures for unsaturated fatty acids) (Chong, 1994). The physicochemical properties of the oil could be due to the types of fatty acid presence, and the manner in which fatty acids combine to form various TAG molecules (Naudet, 1996). In general, the hydrophobic nature of oil is due to the long fatty acid chains in the glyceridic materials.

The Fatty Acids Composition of Palm Oil

For palm oil, the fatty acids composition falls within a very narrow range from twelve to twenty carbon number, with a balanced fatty acids composition between saturation and unsaturation (Berger, 2001).

Table 1.1 shows the common name, systematic name, shorthand name of fatty acids presence in palm oil and its fatty acid composition. In most vegetable oils, the *sn*-2 position fatty acids of TAGs are preferentially occupied by unsaturated fatty acids such as oleic acid and linoleic acid. Saturated fatty acid (SFA) (e.g. palmitic acid) is found in the *sn*-2 position of animal fats TAGs for instance lard, tallow etc (Naudet, 1996)**.** Although palm oil contains high quantity of SFA, the *sn*-2 position fatty acids in the TAGs is preferably occupied by unsaturated fatty acids (mainly oleic acids) (Naudet, 1996**;** Nor Aini and Noor Lida, 2005).

Table 1.1

Common name, Systematic name, Shorthand name of fatty acids in palm oil and its fatty acid compositions (Sean, 2002; Siew, 2002)

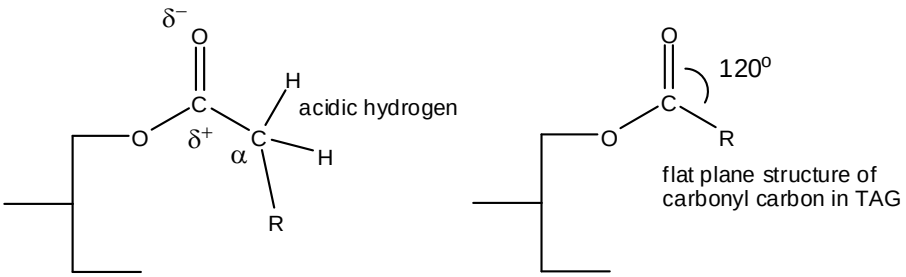
Common name	Systematic name	Shorthand	FAC
Lauric	Dodecanoic	12:0	0.1-0.4
Myristic	Tetradecanoic	14:0	1.0-1.4
Palmitic	Hexadecanoic	16:0	40.9-47.5
Palmitoleic	Cis-9-Hexadecenoic	16:1ω7	0-0.4
Stearic	Octadecanoic	18:0	3.8-4.8
Arachidic	Eicosanoic	20:0	36.4-41.2
Oleic	cis-9-Octadecenoic	18:1ω9	9.2-11.6
Linoleic	cis-9, cis-12, Octadecadienoic	18:2ω6	0-0.6
Linolenic	cis-9, cis-12, cis-15-Octadecatrienoic	18:3ω3	0-0.4

The Chemical Functions of Ester Groups in Oil Molecules

Glycerides or acylglycerols are made up of esters that attached to the glycerol backbone. In natural oils and fats, ester groups account for 90% to 96% of the overall molar mass of

TAGs (Naudet, 1996). The ester groups in TAG play an important role in the chemical and physical properties of the oil. For saturated TAG, the fatty acids have straight chains that do not contain any special chemical functional group. Only carboxylic group in TAG molecules can act as the functional group for chemical reactions. The carbonyl/ester group of the TAGs can take place in many chemical reactions by inducing a special reactivity at the α-carbon (Ucciani and Debal, 1996). Figure 1.2 shows the nucleophilic behavior of the carbonyl carbon and the acidity behavior of hydrogen at the α-carbon (Rousseau and Marangoni, 2002).

Figure 1.2
The chemical functional of acyl group in TAG



The electronegative oxygen pulls away electrons pair from the carbonyl carbon that lead to partial positive charge on the carbon. This partial positive charge carbon can easily attack by nucleophiles. In addition, the sp^2 orbital of carbonyl carbon with flat plane structure may permit easier access of nucleophiles to the carbonyl carbon. The electronegative behavior of oxygen that attached to the carbonyl carbon may also increase the acidity of the hydrogens that attached to the α-carbon (Rousseau and Marangoni, 2002).These ester groups in the TAGs are responsible for several chemical reactions during modification of oils and fats, including alcoholysis, interesterification, reduction (hydrogenolysis), hydrolysis and saponification (Ucciani and Debal, 1996).

1.4 Physical Properties of Palm Oil

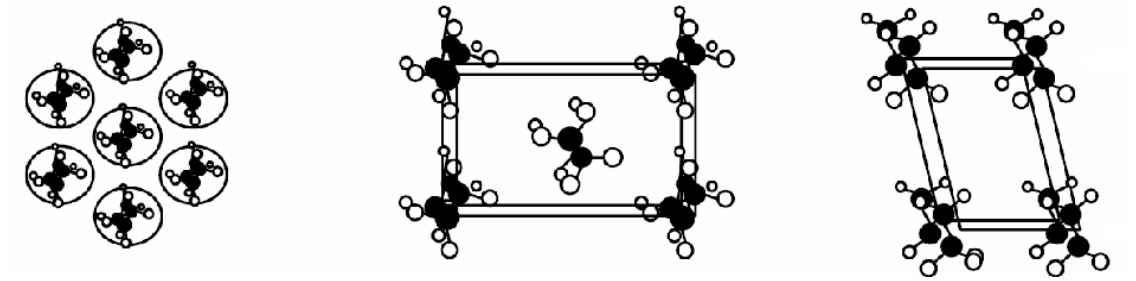
The physical properties of oils and fats are mainly referred to the melting and crystallization behavior with regards to the TAGs compositions. Melting and crystallization behavior of TAGs are very dependent on two factors; chemical structures and polymorphic behavior (Birker and Padley, 1987). The knowledge of palm oil physical properties is one of the key points for the development of palm oil fractionation technology, especially the crystallization selectivity (Kellens et al., 2007). Crystallization selectivity is referred to the degree of compatibility of the different TAGs in the solid state. Most of the studies for fractionation are mainly focus on the effects of cooling conditions that affecting the crystallization selectivity (Kellens et al., 2007).

Solid fat content (SFC), slip melting point (SMP), cloud point (CP), melting and crystallization properties by differential scanning calorimeter, crystals polymorphism studies by X-rays diffraction etc are common methods used to determine the physical properties of palm oil products.

1.4.1 Polymorphism of Fat Crystals

Crystallization can be studied by determined the crystal polymorphism and SFC of the fat blend. Combination of differential scanning calorimeter (DSC), X-ray diffraction is used to study the fat crystal polymorphism. There are total of seven crystals system in fat crystallization, including triclinic, orthorhombic, hexagonal, cubic, tetragonal, rhombohedral, and monoclinic; only three predominate in the crystalline TAGs (Figure 1.3).

Figure 1.3
Schematic representations of hexagonal, orthorhombic perpendicular and triclinic parallel subcells (Adapted from Lawler and Dimick, 2002)



Hexagonal **Orthorhombic perpendicular** **Triclinic parallel**

The most stable form of TAG crystals is triclinic subcell with parallel hydrocarbon-chain planes, followed by orthorhombic perpendicular-subcell with orthorhombic structure with perpendicular chain phases. Hexagonal is the subcell with no specific chain plane conformation, with the lowest stability and the highest Gibbs free energy (Lawler and Dimick, 2002).

Crystallization of palm oil is a complex process due to the existence of multiple polymorphic components including α, β' and β types of polymorphic crystals (Lawler and Dimick, 2002). The formation of different type polymorphic crystals is depended on the cooling rate during crystallization. Rapid cooling of the melt will result in α crystals formation (hexagonal structure) which is very fine and unstable (Lawler and Dimick, 2002). Slow crystallization with long induction time (thermocycling process) of palm oil formed β crystal (triclinic parallel structure) which is very stable (Lawler and Dimick, 2002). For good separation during fractionation, β' crystal (orthorhombic perpendicular structure) is preferred. The formation of stable β' crystal in palm oil has resulted in the addition of palm oil into shortening and margarine formulation (Lawler and Dimick, 2002).

1.5 Oils and Fats Modification

The natural oils and fats either from plants or animals may not necessary ideal for ultimate human used. Hence, the natural products may have to be modified. Oils and fats are normally modified to obtain a product with desired properties either nutritional or physical properties (Gunstone, 2001a). Procedures used for lipid modification are blending, distillation, fractionation, hydrogenation, and interesterification (by chemical catalyst or enzymic catalyst), as well as development of new oils and fats sources by biological approach (Gunstone, 2001b). Amongst these modification methods, blending, fractionation, hydrogenation and interesterification are the most common processes used in oils and fats industries. Blending and fractionation are physical processes that do not involve any chemical reactions. Whereas hydrogenation and interesterification involved chemical reactions in the fatty acids hydrocarbon chain and the carboxylic group in the TAGs, respectively.

Blending is one of the most important processes in the oils and fats industries to improve nutritional or physical properties. The process involved mixing of two or more oils in order to combine desirable nutritional and physical properties (Gunstone, 2001b). The concept of blending is applied in several commercial products such as Naturel® cooking oil from Lam Soon Sdn. Bhd. that involved blending of sunflower oil with canola oil. This product is available in Malaysia and Singapore markets.

Hydrogenation is used to harden liquid oils which contain high percentage of unsaturation fatty acids. In this process, double bonds are eliminated by addition of hydrogen in the presence of nickel or another metallic catalyst which results in a more saturated fat formation (Faul, 1996; Mohd Suria Affandi, 1996; Gunstone, 2001b). Partial hydrogenation

of liquid oil is potential to obtain a very specific melting profile product. The main disadvantage of partial hydrogenation is the formation of *trans* fats by isomerisation of *cis* unsaturated fatty acids. Such *trans* acids raise the melting point without any uptake of hydrogen or IV changed. Some nutritionists reported that *trans* fatty acids may bring negative impact to human health.

1.5.1 Interesterification

Interesterification is one of the most important processes commonly used for oil modification. Interesterification allows the modification of the physical properties (melting behaviors), so as the chemical and nutritional properties of the oils and fats **(**Ucciani and Debal, 1996; Lampert. 2000). Interesterificcation is one of the processes that involved chemical reaction of the carboxylic group in the TAG molecules. It is a reversible reaction which requires presence of a catalyst to reach the equilibrium condition **(**Ucciani and Debal, 1996). In a mixture of TAGs, the acyl group of the TAGs can be redistributed in two modes; the intramolecular mode and the intermolecular mode **(**Ucciani and Debal, 1996).

The intramolecular mode:

$$2R_1R_2R_3 \longrightarrow R_1R_3R_2 + R_3R_1R_2$$

The intermolecular mode:

$$R_1R_1R_1 + R_2R_2R_2 \longrightarrow R_1R_1R_2 + R_2R_2R_1$$

Interesterification can be defined as the rearrangement of acyl groups between esters at specific or non-specific positions of the glycerol backbone, without any changes in the fatty acids composition (Faur, 1996). Interesterification of food lipid can be divided into four classes of reactions which are acidolysis, alcoholysis, glycerolysis and transesterification

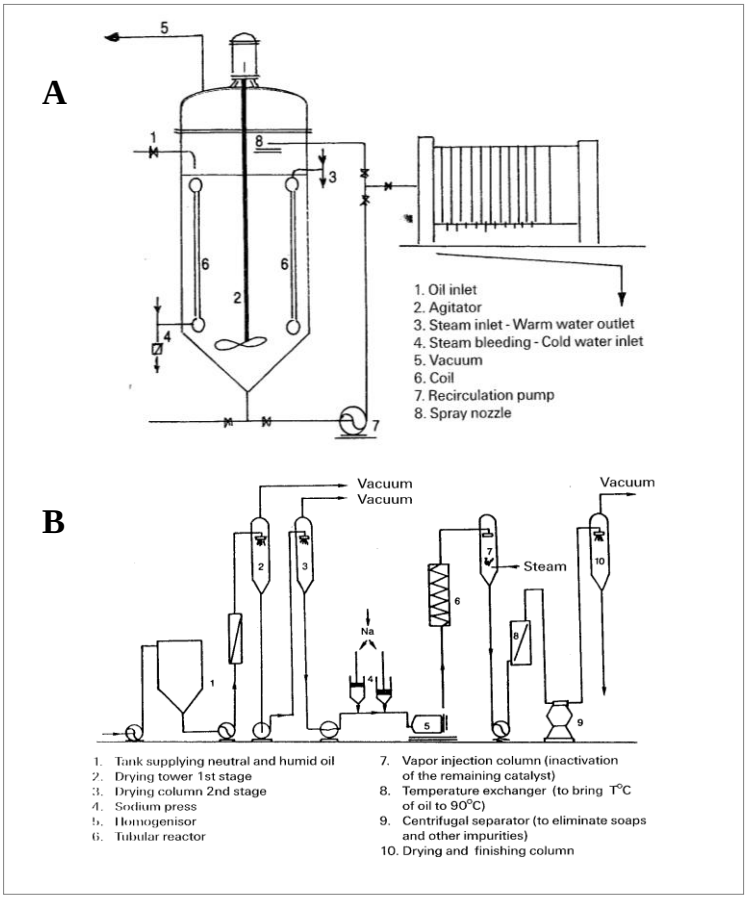
(ester-ester exchange) (Yang and Xu, 2001; Rousseau and Marangoni, 2002). Acidolysis involves the reaction between fatty acids and triacylgycerol; alcoholysis involves the reaction between alcohol and triacylglycerol; glycerolisis is an alcoholysis process that involves the reaction between glycerol and triacylglycerol in which glycerol act as an alcohol in the reaction; transesterification is an ester-ester exchange that is a reaction between an ester such as triacylglycerol or ethyl ester and another ester specifically or non-specifically (Yang and Xu, 2001; Willis and Marangoni, 2002). Interesterification can be achieved by mean of chemical catalysts or enzymes (lipases).

1.5.1.1 Chemical Interesterification

Chemical interesterification process involves a complete positional randomization of the acyl groups in the TAG (non-specifically) (Willis and Marangoni, 2002). This process is mainly used to alter the physical properties of the oil to produce hard base-stocks such as margarine and shortening (Sreenivasan, 1978; Lampert, 2000; Willis and Marangoni, 2002). Chemical interesterification has raised the interest of the nutritionists since it produce zero *trans* fatty acids. Therefore it is a potential reaction to replace partial hydrogenation process in preparation of hard-based stock (Gunstone, 2001b). Chemical interesterification is also advantage in terms of safety because it does not require the use of explosive gas (hydrogen), which is used in partial hydrogenation. In financial point of view, the processing cost of chemical interesterificaiton is about the same as partial hydrogenation (Gunstone, 2001b) that added the interest of the industries to use chemical interesterification.

A few commercial reactors that commonly used for chemical interesterification are discontinuous tank method (batch process) and continuous interesterification, as shown in the Figure 1.4 (Sreenivasan, 1978; Faul, 1996).

Figure 1.4
(A) Discontinuous tank method (batch); (B) Continuous interesterification reactor



In the industries, chemical interesterification is usually conducted by using chemical catalyst such as sodium/potassium alloys and the alkali methoxides/ethoxides (Naudet, 1996, Petrauskaité et al., 1998; Lampert, 2000). The amount of alkali metal usually used for the reaction is at level of 0.1 to 0.2 %, and 0.2 to 0.3% for sodium alcoholate (sodium methoxide) catalyst (Gunstone, 2001b; Ucciani and Debal, 1996). Heating at temperature of 80 to 130 °C is required for the reaction (Ucciani and Debal, 1996). The reaction rate of chemical interesterification is extremely fast, which requiring only a few minutes (Petrauskaité et al., 1998). Some literatures reported that 15 to 60 minutes is required to achieve equilibrium distribution in chemical interesterification (Ucciani and Debal, 1996; Petrauskaité et al., 1998). Therefore, the degree of interesterification cannot be controlled in

chemical interesterification (Petrauskaité et al., 1998). In other words, the reaction must to be completed to produce fully randomized products (Petrauskaité et al., 1998). For chemical interesterification, the distribution of fatty acids of TAGs products can be calculated statistically (Ucciani and Debal, 1996). About 2-4% of monoalcohol esters (e.g. fatty acids methyl ester) were formed in the reaction; depending on the quantity of catalyst used (Ucciani and Debal, 1996).

The effects of chemical interesterification on the physicochemical properties of oils and fats have been studied in many literatures (Sreenivasan, 1978; Zeitoun et al., 1993; David, 1998, Norrizah et al., 2003). These literatures reported that chemical interesterification can randomize the fatty acids distribution in the TAGs, increase the SFC, as well as change the crystal morphology and polymorphism behaviors of the oil.

1.5.1.1.1 Mechanism of Chemical Interesterification

There are two mechanisms of chemical interesterification; carbonyl addition and Claisen condensation, both of them have been discussed in the literature (Sreenivasan, 1978; Lampert, 2000; Rousseau and Marangoni, 2002). The chemical behavior of the carbonyl group of TAG is important in explaining the reaction mechanism as discuss in the section 1.3.

Carbonyl Addition

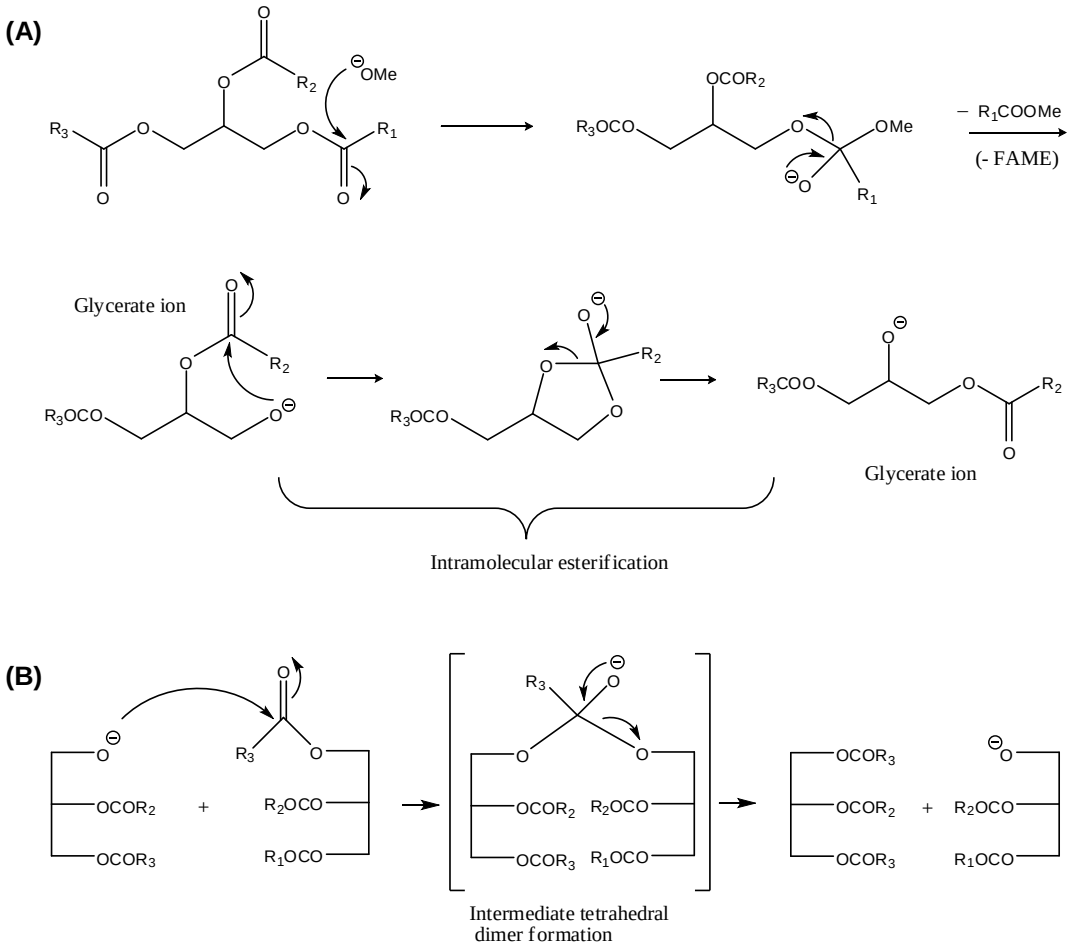
The principle behind carbonyl addition mechanism is based on the partial positive charge behavior of the carbonyl carbon that allowed the attack of methoxide anion. Carbonyl addition mechanism can be divided into a few stages. The first stage involved the formation of glycerate ion that also known as glycerylate anion (Rousseau and Marangoni, 2002).

According to Ucciani and Debal (1996), Rousseau and Marangoni (2002), glycerate ion or metal derivative of a DAG is the real catalyst in chemical interesterification rather than the alcoholate ion. This is because the alcoholate ion is continuously consumed during the reaction.

Initially alcoholate ion acts as a nucleophile to attack the partial positive charge carbonyl carbon and thus added into the carbonyl group in the TAGs (Ucciani and Debal, 1996; Lampert, 2000; Rousseau and Marangoni, 2002). A fatty acid methyl ester is released with every formation of glycerate ion. Intramolecular esterification (also known as intraesterification may take place in the glycerate ion molecule by formation of a cyclic intermediate compound (Ucciani and Debal, 1996). The new glycerate ion that has been formed will then participate in another ester-ester exchange reaction either by intramolecular or intermolecular way that contribute to the positional randomization of acyl groups in the TAGs. Figure 1.5 shows the relocation of the 2-position acyl group to the 3-position of the TAGs.

The intramolecular process involved formation of a tetrahedral dimer as the intermediate compound (Ucciani and Debal, 1996; Rousseau and Marangoni, 2002). This transition complex will decompose, either by regenerating the original species or to form a new TAG species together with a new glycerate ion (Rousseau and Marangoni, 2002). In other words, a new TAG species may not necessary to be formed. This process continues until all available fatty acids have exchanged positions to obtain an equilibrium composition (Rousseau and Marangoni, 2002).

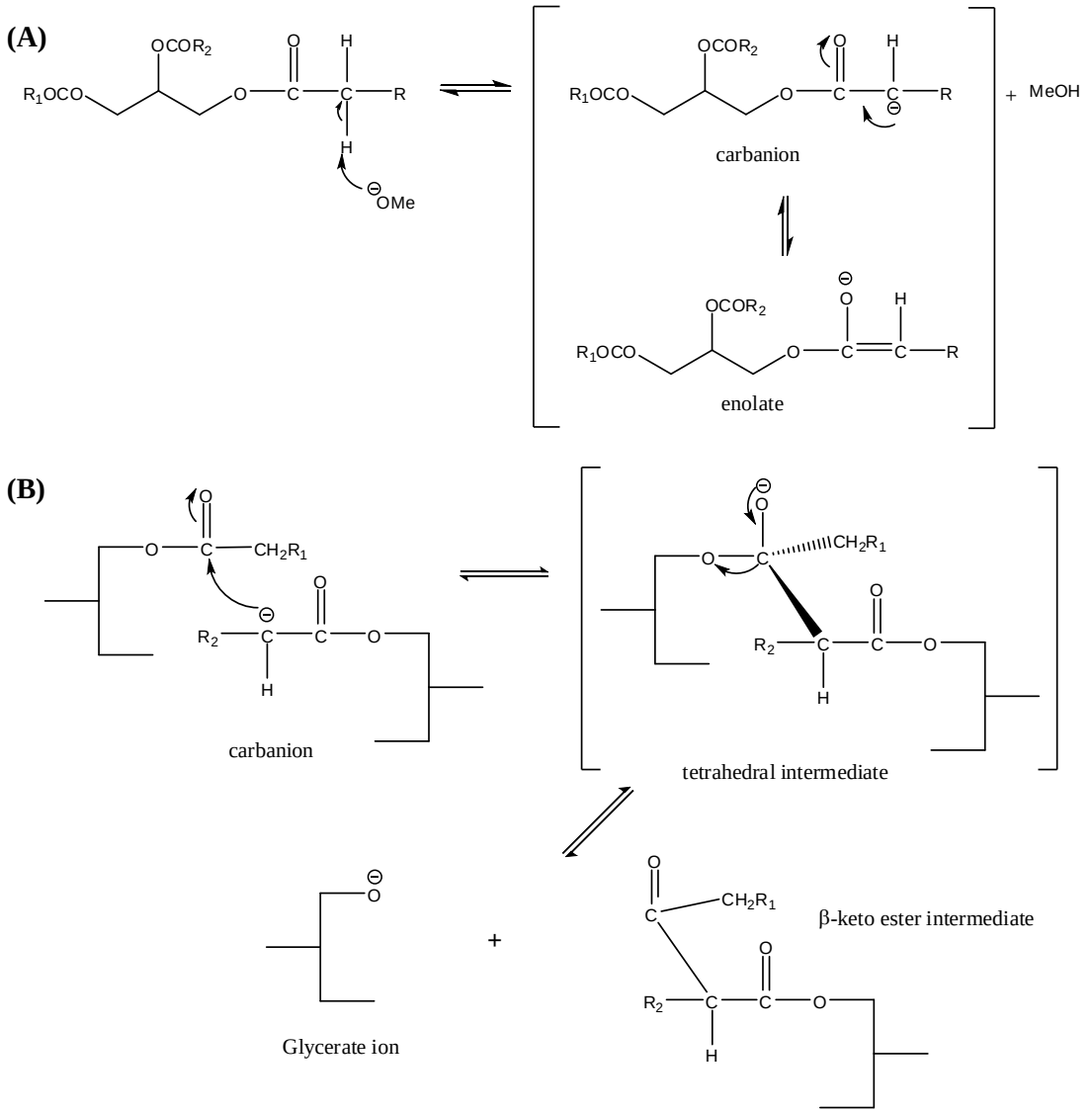
Figure 1.5
Mechanism of chemical interestericationvia carbonyl addition; (A) Intramoleculer esterification and (B) Interemoleculer esterification (Ucciani and Debal, 1996)



Claisen Condensation

Another mechanism of chemical interesterification is Claisen condensation (through enolate formation) (Rousseau and Marangoni, 2002) that shown in Figure 1.6. This reaction mechanism on oils and fats has been reviewed comprehensively in the literatures (Rousseau and Marangoni, 2002). Fundamental organic chemistry of Claisen condensation mechanismin of the carbonyl group has been found in some organic chemistry literatures such as (McMurry, 2004; Solomons, 2004a). The principle of Claisen condensation mechanism is based on the acidic behavior of the hydrogen that attached to α-carbon (section 1.3) (Lampert, 2000).

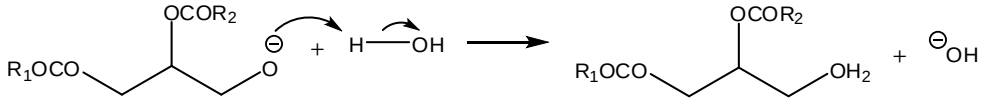
Figure 1.6
Mechanism of Claisen condensation: (A)Enolate formation, (B) Carbanion formation



The acidic hydrogen from the α-carbon has taken out by the methoxide anion and released as methanol. The carbanion formed can be transformed into a stable resonance structure known as enolate anion (Solomons, 2004a) to attack other carbonyl group. A β-keto ester intermediate and a glycerate ion are formed from a tetrahedral intermediate that combined two TAGs (Rousseau and Marangoni, 2002; Solomons, 2004a). Once the glycerate ion is formed, it is then free to attack other carbonyl carbon for ester-ester exchange either intermolecularly or intramolecularly (Rousseau and Marangoni, 2002).

Termination of Chemical Interesterification

Interesterification is stopped by additional of water or a dilute acid. Termination of the chemical interesterification reaction usually leads to the formation of MAG and DAG. This can explain the detection of higher amount of these partial glycerides in the product as compared to the feed oil (Ucciani and Debal, 1996).



The catalyst can be washed out by water to separate salt, or soap-rich aqueous phase. Phosphoric acid has also been used to form solid phosphoric salt that can be filtered out then. Both of these methods may lead to loss of fat products. Alternatively, carbon dioxide gas is added together with water to minimize the fat loss (Rousseau and Marangoni, 2002).

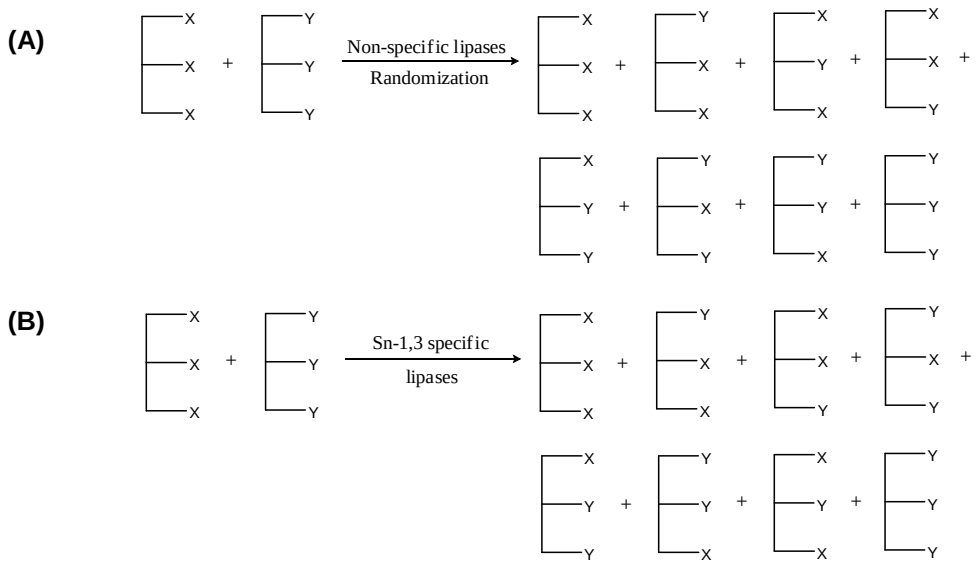
1.5.1.2 Enzymatic Interesterification

Interesterification can also be performed using lipase as catalyst that commonly known as enzymatic interesterification. Enzymatic interesterification has been known for many years as an efficient way of controlling the melting characteristics of oils and fats. The technology was not widely used until recently and this is due to the high cost of enzymes. In spite of this, enzymes are mainly used to obtain positional specificity of the interesterified products (Lampert, 2000). In general, enzymatic interesterification process can rearrange the fatty acids group at either non-specific distribution (randomization) or *sn*-1,3 specific distribution (Cheah and Augustine, 1987; Ghazali et al., 1995; Yang and Xu, 2001;).

Figure 1.7 illustrated both the non-specific and *sn*-1,3 specific enzymatic interesterification. Nonspecific enzymatic interesterification gives complete randomization of all fatty acids in

all positions and produces the same products as chemical interesterification. Therefore, these enzymes are not commonly used in interesterification due to the higher production cost compared to chemical intesterification. Examples of nonspecific lipases are lipases derived from *Candida cylindraceae*, *Corynebacterium acnes*, and *Stapylococcus aureus* (Willis and Marangoni, 2002).

Figure 1.7
Interesterification reaction schemes by (A)non-specific lipases and (B)*sn*-1,3 specific lipases (Yang and Xu, 2001)



1.5.1.2.1 The Catalytic Behavior of Lipase in Interesterification

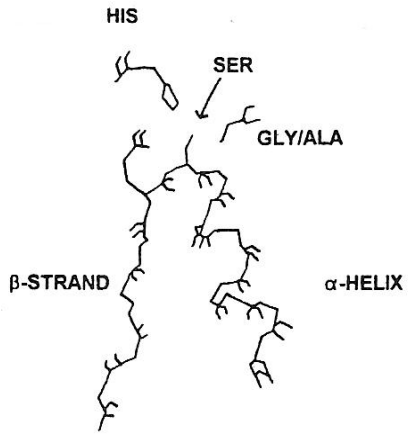
Enzyme/lipases can be derived from sources such as animal, bacterial and fungal. Almost all lipases have similar three-dimensional structures; yet lipases are different in the sequences of amino acids. Lipase can be defined as a polypeptide chain that folded into two domains; the C-terminal domain and the N-terminal domain (Willis and Marangoni, 2002). The polypeptide chain in the lipases is folded in similar ways to have similar active sites. The N-terminal domain in the lipase is responsible for the catalytic behavior of enzymes that contain active site with a hydrophobic tunnel. The hydrophobic active sites allowed the

attachment of long fatty acid chain onto it and that promised specificity during interesterification (Willis and Marangoni, 2002).

In the presence of lipids or organic solvents, the lid of lipase structure is opened, exposing the hydrophobic core that allowed reactions to take place. The lid differs for lipases in the number and position of the surface loops. The main component of active site is the α- or β-hydrolase fold that contains a core of mostly parallel β-sheets surrounded by α-helices (Willis and Marangoni, 2002). The α-helix structure in the lid is important for the lipase to bind to lipid at the interface. Enzyme activity will reduced when the amphiphilic properties of the loop are reduced.

Catalytic triad of the lipase are composed of serine (SER), histidine (HIS) and either glutamic (GLY) or aspartic (ASP) acid together with some oxyanion-stabilizing residues that generate the hydrophobic pocket of the lipase (Willis and Marangoni, 2002; Solomon, 2004b). The position of catalytic triad is determined by folding of the polypeptide chain of the lipase. Figure 1.8 shows the example of hydrophobic pocket of lipase derived from C*andida rugosa*.

Figure 1.8
Crystal structure and location of catalytic residues of the active site of *Candida rugosa* lipase (Willis and Marangoni, 2002)



The specificity behavior of lipases in catalyzing interesterification can be categorized into three main classes that are positional specificity (regiospecificity), fatty acids selectivity, and stereospecificity (Cheah and Augustine, 1987; Willis and Marangoni, 2002). Only a few enzymes in the nature that posses stereospecificity behavior, these lipases can differentiate *sn*-1 and *sn*-3 positions in which the reaction rate towards fatty acids at the position-1 and -3 are different (Cheah and Augustine, 1987; Willis and Marangoni, 2002). Lipases can only behave as a high specificity biocatalyst under specific reaction conditions. There are several factors that can affect the performance of enzyme such as the reaction system, reaction temperature, water level, enzyme dosage etc.

Table 1.2 shows the selectivity of some typical lipases in nature. Positional specificity in lipase-catalysed interesterification is due to steric hindrance of the *sn*-2 position fatty acids in TAGs (Willis and Marangoni, 2002). This steric hindrance effect prevents the fatty acid in the *sn*-2 position from entering the lipase active site. Examples of 1,3-specific lipases are those from *Aspergillus niger*, *Mucor miehei*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Pseudomonas sp.*, *Rhizopus oryzae* etc (Yang and Xu, 2001). Initially, 1,3-specific lipases will produce mixture of TAGs, 1,2-DAGs, and 1,3-DAGs without interfere the *sn*-2 position fatty acids. However after prolong reaction with the formation of 1,3-DAG, acyl migration will occur that permit some randomization of the 2-position fatty acids in the TAG backbone (Willis and Marangoni, 2002).

Enzymes/lipases Specificity

Table 1.2
Selectivity of typical lipase derived from various sources

Lipase sources	Substrate specificity	
	Position (*sn*-)	**Fatty acids**
Aspergillus niger	1,3 » 2	M › S › L
Aspergillus sp.	1, 2, 3	M, S, L
Candida rugosa	1, 2, 3	M, S, L
Candida antarctica	1, 2, 3	M, S, L
Candida lypolytica	1, 2 › 3	M, S › L
Candida parapsilosis	1, 2 › 3	M, S, L
Chromobaterium viscoum	1, 2 › 3	M, S, L
Geotrichum candidum	1, 2 › 3	M, S, L
Mucor javanicus	1, 3 › 2	M, S, L
Pancreatic (porcine)	1, 3	S › M, L
Papaya latex	1, 3 › 2	M, S, L
Pre-gastric esterase	1, 3	M, S » L
Penicillium sp.	1, 3 › 2	M, S, L
Penicillium camembertii	1, 3	MAG › DAG › TAG
Penicillium roquefortii	1, 3	S, M » L
Phycomyces nites	1, 3 › 2	S, M, L
Pseudomonas sp.	1, 3 › 2	S, M, L
Pseudomonas fluorescens	1, 3 › 2	M, L › S
Rhizomucor miehei *	1, 3 › 2	M, S, L
Rhizopus delemar	1,3 » 2	M, S » L
Rhizopus javanicus	1, 3 › 2	M, S › L
Rhizopus japonicus	1, 3 › 2	S, M, L
Rhizopus niveus	1, 3 › 2	M, L › S
Rhizopus oryzae	1,3 » 2	M, L › S
Rhizopus arrhizus	1, 3	S, M › L
Thermomyces lanuginose *	1,3 » 2	S, M, L

[Abbreviation: M = medium chain fatty acids, L=Long chain fatty acids, S=short chain fatty acids; * referred to the enzymes that have been selected for this project]
(Adapted from Yang and Xu, 2001)

The specificity performance of lipase is very dependent on the reaction conditions and system. Under specific reaction conditions, lipase can perform in an excellent specificity with minimize randomization (Quilan and Moore, 1993; Yang and Xu, 2001; Natália et al., 2006; Criado et al., 2007). Besides, there are some lipases that permit *sn*-2 specific reaction rather than *sn*-1,3 specific in catalyzing enzymatic processes. For example, lipase from *Candida parapsilosis*, *candida lypolytica*, and *Chromobaterium viscoun* can hydrolyzes *sn*-2 position fatty acids more rapidly than those at the 1- and 3- positions (Yang and Xu, 2001; Willis and Marangoni, 2002).

Fatty acids selectivity is also important in studying the lipases catalytic activity. In fact, some of the lipases are specific toward particular fatty acids substrates. The criterions for fatty acid selectivity can be the carbon chain length (short, medium or long chain), the unsaturation fatty acids (bend structure for unsaturation fatty acids) etc. For example, lipase derived from *Penicillium cyclopium* is specific toward long chain fatty acids, while porcine pancreatic lipase is specific toward shorter chain fatty acids (Willis and Marangoni, 2002). Lipase from *Mucor miehei* has strong affinity towards fatty acids that contained the first double bond form carboxyl end at an even-numbered carbon such as *cis*-4, *cis*-6, and *cis*-8. Therefore, the reaction rate towards other fatty acids will be slower (Willis and Marangoni, 2002). Hereby, we can see that fatty acids specificity is also an important criterion to be considered when selecting lipase for oils and fats modification process, instead of just the positional specificity, especially for production of specialty products with desired fatty acid chain length.

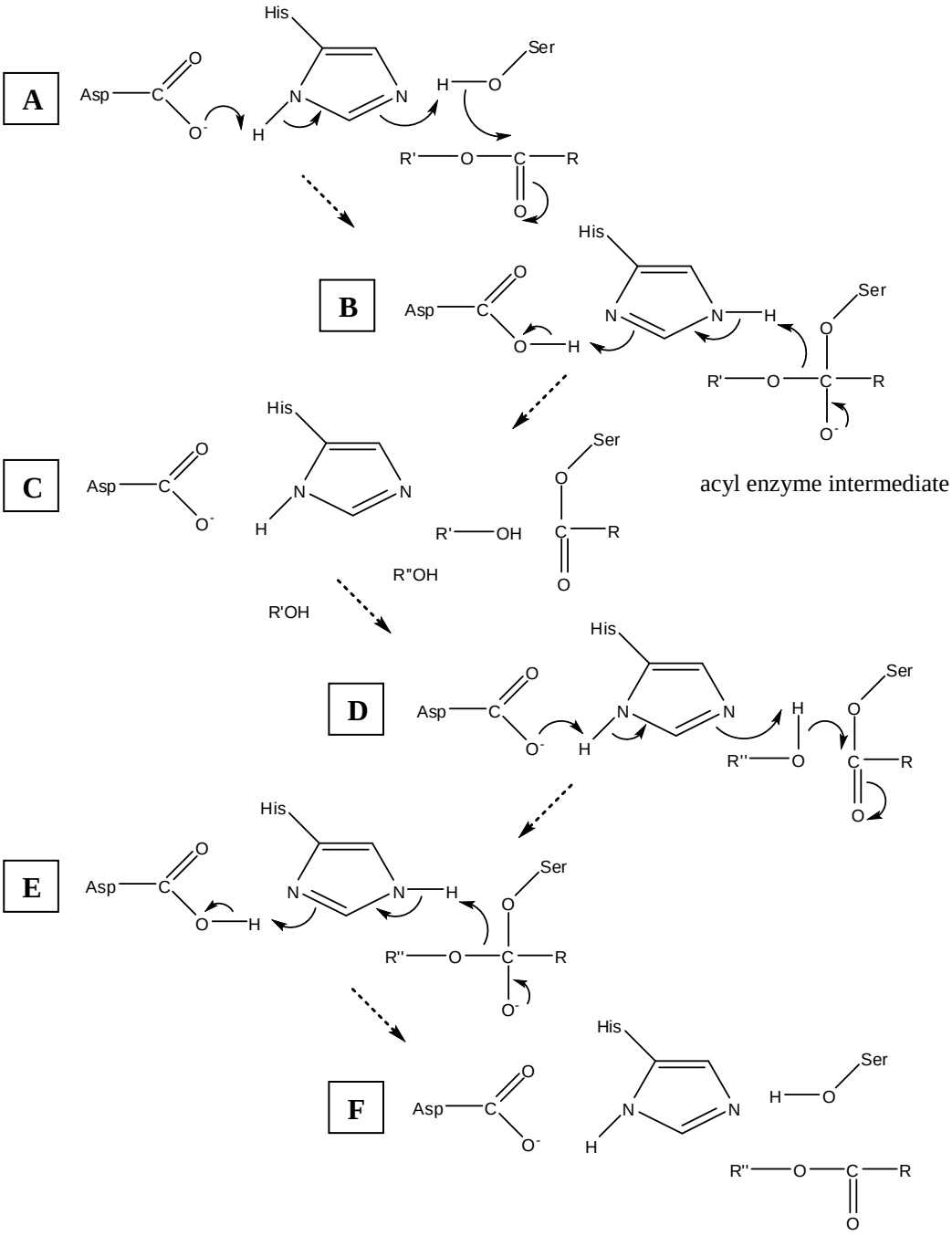
1.5.1.2.2 Mechanism of Enzymatic Interesterifcation

Enzymatic interesterification always begins with sequential hydrolysis followed by interesterification reaction (Willis and Marangoni, 2002). Hydrolysis of TAGs involved consumption of trace amount of water to produce free fatty acids and partial glycerides (MAG and DAG). The reaction will continue until equilibrium is established. When water level reduced, some lipase will continue to catalyze the reaction; at certain level, interesterification will dominate over hydrolysis (Quilan and Moore, 1993).

The catalytic site of lipase always plays an important role in selecting specific fatty acid either in terms of the fatty acid types or the position at TAG backbone to ensure specific interesterication. The catalytic triad of lipase is consists of serine (SER), hisidine (HIS) and aspartic (ASP) acid residues in particular position. With presence of HIS and ASP, SER acts as a strong nucleophile to attack the partial positive charge carbonyl carbon of the TAG substrate to form a tetrahedral acyl enzyme intermediate compound, as illustrated in Figure 1.9.

The formation of the enzyme-substrate intermediate compound often induces a conformational change in the enzyme that allows it to bind the substrate more effectively, that known as induced fit (Solomons, 2004b). An alcohol is released when the carbon-oxygen bond breaks. At this stage, other alcohol molecules will act as nucleophile to attack the acyl enzyme intermediate with assist of ASP and HIS acid residues and form a new TAG species. Due to steric hindrance effect of the *sn*-2 position fatty acids, SER can only attack the carbonyl groups at *sn*-1 and *sn*-3 position. This explains how the enzyme acts as a specific biocatalyst in interesterification reaction.

Figure 1.9
The reaction mechanism for enzymatic interesterification, with catalytic site containing Asp, His and Ser residues. (Adapted from Willis and Marangoni, 2002)



As compared to chemical interesterification, enzymatic interesterification is relatively slow and can be stopped at any point to obtain desired product. However, the reaction is normally allowed to achieve equilibrium compositions. Meanwhile, enzymatic interesterification is advantages in terms of specificities, mild reaction conditions, and less

by-product (Yang and Xu, 2001, Willis and Marangoni, 2002). Chemical interesterification required some purification processes such as washing, bleaching, deodorization which does not required for enzymatic interesterification (Yang and Xu, 2001). The use of chemical catalyst which is highly toxic and explosive also raised concerns from society regarding health and environmental issues.

1.5.1.2.3 The Studies and Applications of Enzymatic Interesterification

The enzymatic interesterification has been widely reviewed in Willis and Marangoni (2002), Yang and Xu (2001) and Xu et al. (2002) from various aspects including the immobilization process, the physicochemical properties of the interesteried products, enzyme specificity, and numbers of modification processes for production of specialty products and structured lipid etc. Besides, the comparison between chemical and enzymatic approaches in interesterification has been studied by Kowalski et al., 2004 etc.

Immobilized Enzymes/lipases

Lipases are commonly used as immobilized enzyme rather than the free enzymes. The advantages of using immobilized enzyme systems are included reusability, rapid reaction termination, lower cost, controlled product formation, and ease of separation of the enzymes from the oils (Cheah and Augustine, 1987; Willis and Marangoni, 2002). Enzyme immobilization is also known to improve the stability of enzyme (Cheah and Augustine, 1987). Beside, immobilized enzyme can also be easily and quickly loaded onto any packed-bed reactor that do not required filtration after interesterification (Cheah and Augustine, 1987; Vasudevan et al., 2004).

There are numbers of immobilized enzymes available commercially, such as Lipozyme® TL IM (*Thermomyces lanuginosa*), Lipozyme® RM IM (*Rhizomucor miehei*), and Novozyme® 435 (*Candida antartica*) from Novozyme Co.; PLC and PLG (*Alcaligenes sp.*) with different supporting materials from Meito Sangyo Co. ; PS-C 'Amano' II (*Pseudomonas cepacia*) from Amano Pharmaceutical Co. etc. The availability of immobilized enzymes from Meito Sanyo Co. and Amano Pharmaceutical Co. is very limited. Most of the products are free enzymes that required immobilization step before applied for modification process. Immobilization material is also known as filter aid that used for ease of separation (Ghazali et al., 1995). For example, Lipase D-200 (*Rhizopus delemar*: Amano Pharmaceutical Co.) was immobilized on Celite 535 before used in interesterification of canola and palm oils (Kurashige et al., 1993), while free lipases from *Candida rugosa*, *Pseudomonas sp*, *Rhizopus javanicus*, *Mucor javanicus*, *Aspergillus niger*, and R*h. niveus* that also can be obtained from Amano Pharmaceutical Co. were immobilized onto celite material for the study of transesterification of palm olein (Ghazali et al., 1995).

Lipozyme® TL IM is one of the most popular enzymes used for modification of oils and fats due to lower enzymes cost as compared to the others. Rønne et al., (2005) had indicated that Lipozyme® TL IM is nonselective toward neither different chain length fatty acids nor unsaturated fatty acids. There are several literatures reported on the operational stability of Lipozyme® TL IM during interesterification either in batch or continuous packed-bed processes. For batch process, Xu et al., (2002) reported that the enzyme was stable for at least 11 and 9 batches with 3 hours duration for each reaction, in the small and larger scale reactor, respectively. The authors also reported that the activity of the enzyme

can retain for two weeks for continuous packed-bed process without disturbing the water content of the system.

A study on operational stability of Lipozyme® TL IM of two blends; first involved 55: 25: 20 ratio of palm stearin: palm kernel oil: sunflower oil, and second involved 55: 35: 10 ratio of palm stearin: palm kernel oil: TAGs rich in n-3 polyunsaturated fatty acids (PUFA) was conducted in a continuous packed-bed reactor (Yamaguchi et al., 2004). This study indicated that the lipase activity decreased progressively along the operation period which is 580 hours and 390 hours, respectively. The authors also reported that higher PUFA level may lead to higher rate of oxidation and thus reduced the enzyme stability.

Lipozyme® RM IM is much more expensive in terms of enzyme cost as compared to Lipozyme® TL IM. There are also many enzymatic studies conducted using Lipozyme® RM IM. A study conducted by Criado et al. (2007) reported that in comparison to Novozyme® 435 (*Candida antarctica*) and Lipozyme® TL IM in a batch reaction system for interesterification of virgin olive oil with fully hydrogenated fat using orbital agitation, Lipozyme® RM IM required a longer time (> 8 hours) to achieve equilibrium stage, whereas Novozyme® 435 and Lipozyme® TL IM only took respectively 4 and 8 hours to achieve equilibrium stage. These studies also indicated that the differences in the *sn*-2 position fatty acids of these three products are negligible, besides oxidative stability of all interesterified products was lower as compared to the corresponding physical blends.

Studies of Enzymatic Interesterification for Commercial Applications

Enzymes have been used in many application either food or non-food area. Enzymatic reaction plays an important role especially in food applications. For example, production of

cocoa butter equivalent (CBE) in chocolate and related confectionery industries that usually involved the use of 1,3-specific lipases. In cocoa butter TAGs, oleic acid (O) is locate at the *sn*-2 position while palmitic acid (P) and stearic acid (S) at the *sn*-1,3 positions (Yang and Xu, 2001). The purpose of using enzymatic interesterification in the production of CBE is to retain O at the *sn*-2 position with P and S at the *sn*-1,3 positions. These symmetry structure TAGs with unique fatty acid composition and distribution in TAG backbone are responsible to the characteristic of chocolate (Yang and Xu, 2001). The production of cocoa butter equivalent by enzymatic approach has been reviewed by Quinlan and Moore (1993), and Yang and Xu (2001).

High POP vegetables oils such as palm mid fraction and material with stearic source such as SOS, SSS, and stearic acid, are the starting material that usually used for production of CBE that consists of a mixture of POS, SOS, and POP (Yang and Xu, 2001). For examples, production of cocoa butter-like fats was studied by Chang et al. (1990) via enzymatic interesterification of hydrogenated cottonseed oil and olive oil using immobilized *Mucor miehei*; enzymatic interesterification of palm oil and tristearin in supercritical fluid carbon dioxide (SC-CO_2) medium by Liu et al. (1997); and Bloomer et al. (1990) studied the production of CBE by immobilized enzyme interesterification of palm mid fraction and ethyl stearate. However, enzymatic process may produce small amount of DAG that will affect the formation of β crystals during chocolate tempering. Solvent is usually used for the removal of these DAG.

In addition, lipase-catalyzed interesterification reaction is also an important process for production of zero-*trans* hard-based stock to replace partial hydrogenation. For example, a study conducted by Zhang et al. (2000) involved producing of margarine fats by enzymatic

interesterification of palm stearin and coconut oil blend (75:25, w/w) with Lipozyme® IM (*Rhizomucor miehei* immobilized in an ion exchange resin) in 1 kg scale batch reactor. Due to the good characteristics of the margarine products, interesterification of the same oil blend was conducted by using Lipozyme® TL IM in a 300 kg pilot-scale batch reactor (Zhang et al. 2001). Both studies indicated that Lipozyme® TL IM and Lipozyme® IM had similar enzyme activity in for interesterification of the oil blend.

Besides, the production of zero-*trans* Iranian vanaspati was studied by Jamshid et al. (2006, 2007) using two oil blends. The first blend involved the use of palm olein, low-erucic acid rapeseed oil and sunflower oil via directed interesterification (Jamshid et al, 2006), while the second blend consists of fully hydrogenated soybean oil, rapeseed oil, and sunflower oil (Jamshid et al., 2007).

There are many specialty products of enzymatic interesterification that have commercially available. Table 1.3 shows some of the functional products, the trade name, and the invented company of the commercial products developed from enzymatic interesterification. Econa oil was officially withdrawn from the market on 16th September 2009 due to high levels of glycidol fatty acid esters in DAG oils (Tan, 2009).

Enzymatic interesterification also widely used as an effective approach to produce specific structured lipids that have been reviewed comprehensively by Xu (2000). In addition, enzymes also have been used to produce prodrugs, e.g. synthesis of 6-azauridine prodrugs by using Lipozyme® TL IM (Wang et al., 2009).

Table 1.3
The list of functional products, trade name and the invented company from enzymatic interesterification technology

Trade name	Functional products	Invented company
Betapol	Human milk fat substitute	Loders Croklaan
Econa	Diacylglycerols oil - anti-obesity	Kao Corporation, Japan
Enova	Diacylglycerols oil - anti-obesity	ADM Kao, USA
Resetta	Medium chain triacylglycerol oil - fast energy source - anti-obesity	Nisshin Oillio Group Ltd

In non-food area, applications of enzymes in production of biodiesel also have been widely studied in which enzymatic approach is said to be a greener way of produce biodiesel (Robles-Medina et al., 2009). Besides, enzymatic method also can overcome some problems encountered in chemical interesterification process, such as difficulty in the removal of alkaline catalyst from the product, recovering glycerol, treatment of alkaline waste water, and the interference of reaction by free acids and waters (Watanabe et al., 2000). Enzymes such as *Mucor miehei*, *Rhizopus oryzae*, *Candida antarctica*, *Thermomyces lanuginosa* and *Pseudomonas cepacia* have been used for biodiesel production that reported by Robles-Medina et al. (2009). Alcoholysis with methanol is the main reaction for the production of methyl esters from oils and fats. The strong inhibition behavior of methanol towards lipases activities is the problem identified in this process (Yang and Xu, 2001). Therefore, studies and development are still needed in order to commercialize this process so as to contribute to the biodiesel industry.

1.5.1.2.4 Reactors for Enzymatic Interesterification

Enzymatic modification has been radically developed from simple laboratory ideas at the beginning to industrial practices (Xu, 2003). According Willis and Marangoni (2002), there are five available reactors that have been used for enzymatic interesterification, including fixed bed reactor, stirred batch reactor, continuous stirred tank reactor, membrane reactor, and fluidized bed reactor.

Fixed bed reactor is one of the most common reactors used that basically based on continuous flow system, in which the substrate and the product are pumped in and out of the column packed with immobilized enzymes, at the same rate (Willis and Marangoni, 2002). The main reason of favorably application of fixed-bed reactor in the industries is due to the easy application to large-scale operation, with high efficiency, low cost and ease of separation. In addition, fixed bed reactor also provides more enzyme surface area that may ensure better contact of the substrates with the enzymes (Willis and Marangoni, 2002).

Stirred batch reactor is a simple process with an agitation tool attached to the tank. No addition and removal of substrates and products is performed during the reaction. The level of substrates in the reactor is reduced over time of reaction, in which conversion to products take place throughout the reaction. Free enzyme can be used in this reactor; however immobilized enzyme is still preferred due to the ease of separation and enzyme reusability (Willis and Marangoni, 2002).

The principle of continuous stirred tank reactor is based on the combination of both fixed-bed reactor and batch reactor. It is an agitation tank like stirred batch reactor, with same way of substrates loading and products removal like fixed bed reactor. In other words,

substrates and products are pumped in and out of the tank at the same rate (Willis and Marangoni, 2002). This design of reactor is disadvantaged due to the higher power consumption associated with continuous stirrer, and the possibility of breaking up of supporting material during agitation (Willis and Marangoni, 2002).

Membrane reactor involved immobilization of enzymes onto the surface of membrane (Xu, 2003) that involved two phase systems where interface of two phases is at a membrane (Willis and Marangoni, 2002). Materials used in membrane systems are polypropylene, nylon, acrylic resin, and polyvinyl chloride (Willis and Marangoni, 2002). The advantages of using membrane systems are lower pressure drops and fluid channeling with high diffusivity, chemical stability as well as higher membrane surface area to volume ratio (Willis and Marangoni, 2002). Membrane reactor is also suitable to be used in enzymatic hydrolysis, in which the reaction can take place on the membrane surface and the glycerol formed can be transported through the membrane to the water phase (Xu, 2003).

The other reactor that has been used for enzymatic interesterification is fluidized-bed reactor. In fluidized-bed reactor, the immobilized enzyme and support are kept suspended in the column by the upward flow of substrate at high flow rates (Willis and Marangoni, 2002). The fluidized bed reactor is advantages due to no channeling problems, less pressure changes at high flow rates and no separation of oils and particulates needed after the reaction. The major disadvantages of fluidized bed reactor is that only small amount of enzymes can be used since large void volume is needed to keep the substrates and immobilized enzyme suspended (Willis and Marangoni, 2002).

Amongst these reactors, the most common reactors are fixed bed reactor (packed-bed reactor) and stirred batch reactor (batch reactor). Both of these two reactors are selected in this study due to the simplicity of reactor design.

1.5.2 Fractionation

Fractionation can be defined as separation of a mixture into different fractions. Generally, the concept of physical separation process can be based on a few principles; the differences in solidification, solubility, and volatility of the different compounds. The techniques that usually used for fractionation are fractional crystallization, fractional distillation, short-path distillation, supercritical fluid extraction, liquid-liquid extraction, adsorption, complexation, membrane separation etc (Kellens et al., 2007).

In oils and fats industries, fractional crystallization is the process used for separating oils and fats into two or more components, which involved two steps; selective crystallization and filtration. The concept of fractionation is based on the difference in melting points of TAGs (difference in solidification) and the solubility of the solid TAGs in the liquid phase (Gunstone, 2001b). The difference in solubility is depending on the TAG molecular weight and degree of unsaturation that affects the ability of fats to produce crystals (Kellens et al., 2007). Fractional crystallization is a fully reversible process, which is basically a thermo-mechanical separation process (Kellens et al., 2007).

1.5.2.1 Fractionation of Palm Oil

Fractionation is an essential process for palm oil industries due to its fatty acid composition with 50% of saturated and 50% of unsaturated. The appearance of palm oil as semi-solid fat in tropical climate allows it to be separated into a low melting fraction-olein and a high

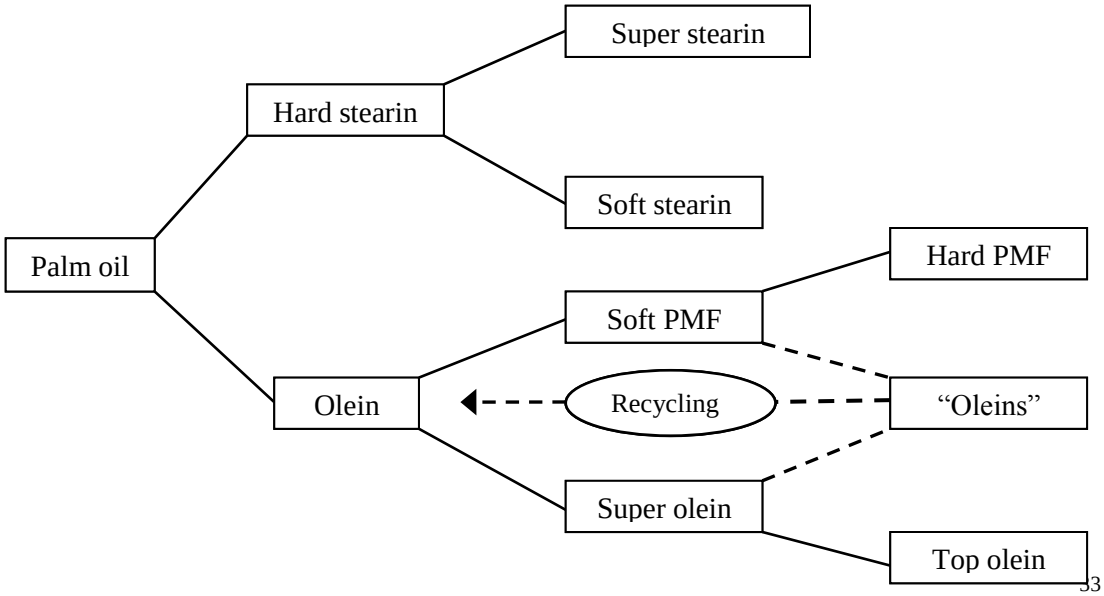
melting fraction-stearin (Deffense, 1985). In general, there are three fractionation processes used to fractionate palm oil; dry fractionation, detergent fractionation, and solvent fractionation.

Dry Fractionation

Dry fractionation is the simplest and cheapest process, which is available in most palm oil refinery factories. It is a dry process uses direct filtration of the TAG crystals after a controlled cooling program. This process is simple because it does not require the use of any chemicals with no effluent produced along the process. Hence, this process is also advantage in terms of minimum losses of the products (Kellens et al., 2007).

In dry fractionation, the oil is partially crystallized by controlled cooling of the melt to the desired fractionation temperature, leaving the substrate for crystals formation, followed by filtration by means of membrane filter press (Gunstone, 2001b). Figure 1.10 shows various palm oil products obtained from single, double and triple stage dry fractionation.

Figure 1.10
Dry fractionation of palm oil and its products by single, double and triple dry fractionation (Kellens, 2007)



Single stage dry fractionation of palm oil produces palm olein with IV of 56 and 62. The saturation content of palm olein can be further reduced by multiple stage fractionation in which double and triple fractionation produce oleins with IV of IV 65 (super olein) and IV 70 (top olein), respectively (Gijs et al., 2007; Kellens et al., 2007).

Detergent Fractionation

Detergent fractionation is first developed by Lanza that involved the addition of detergent as a wetting agent to improve the separation process of the crystals from the liquid phase (Deffense, 1985). Sodium lauryl sulfate is usually used as the wetting agents, in combination with magnesium sulfate as the electrolyte (Kellens and Hendrix, 2000). When the partially crystallized slurry is mixed with the detergent solution, the crystals are wetted by the detergent and easily suspended in the aqueous phase; the mixture is then separated by centrifugation (Kellens et al., 2007). The aqueous phase is then heated and the melted stearin is recovered through second centrifugation step. The olein and stearin fractions are washed with water and dried to remove the trace amount of detergent (Deffense, 1985). Detergent fractionation has lost its interest due to the contamination of the end products and the subsequently high production cost (Kellens et al., 2007).

Solvent Fractionation

Solvent fractionation is the most efficient fractionation process compared to other methods (Kellens and Hendrix, 2000). It is initially developed to overcome some bulk crystallization problems such as slow heat transfer, and limited nuclei movement. In solvent fractionation, the oil is diluted in organic solvent such as acetone and hexane in certain amount to reduce

its viscosity, which is different from bulk crystallization in dry and detergent fractionations (Kellens et al., 2007).

In dry fractionation, it is not possible to remove all liquid from the solid phase, in which part of the liquid will remain entrapped in the solid fraction. The addition of solvent will reduce the surface tension between the liquid and solid phases hence promises better separation. Solvent fractionation is more efficient in reducing the liquid oil entrapment, thus enhanced the liquid oil yield as well as increased the solid fraction purity. Similar to detergent fractionation, solvent fractionation has lost its interest due to the high production cost. Besides, higher risk to fire and human health hazards also lead to lesser implication of solvent fractionation in palm oil fractionation (Kellens et al., 2007).

1.5.2.1.1 Palm Fractionation Products

Palm olein can remains as clear liquid oil at temperatures above 18 °C, 20% of the compounds starts to form cloudy deposits below this temperature. Therefore, the usage of pure palm olein as liquid cooking oil is only limited in hot climates countries (Siew and Ng, 1996). In temperate countries, palm olein is used as blended oil in order to introduce more unsaturated components to the oil for better physical stability (Berger, 1981). The main applications of palm oil products are illustrated in Table 1.4.

Table 1.4
The main food applications of palm oil fractionation products (Berger, 1981)

Palm oil products	Food applications
Palm oil	vegetable ghee/vanaspati
Palm olein, superolein, top olein	cooking/frying oil
Palm stearin	bakery shortening, vegetable ghee, margarines,
Palm mid fraction	confectionery fats – cocoa butter equivalent

Palm stearin is one of the most popular materials used for productions of blended fat products such as margarines, shortenings, and vanaspati due to its lower cost and better health effects compared to partial hydrogenation soft oils (Berger, 1981). Other than that, palm oil and palm kernel oil are also the main ingredients for various types of margarines such as table margarines, pastry margarines, and bakery margarines (Berger, 1981). Palm mid fraction (PMF) is a valuable product from palm oil fractionation that widely used as confectionary fats. PMF is well compatible with cocoa butter due to its high POP content. PMF behaves like cocoa butter in any proportion without altering the physical properties such as melting, rheological and processing properties.

1.5.2.1.2 Principal of Fractionation: Crystallization

In general, crystallization of fats consists of a few stages; supercooling of the melt, nucleation, and crystal growth (Kellens et al., 2007).

Supercooling/supersaturated

Nucleation can be occurred when the melt becomes supercooled in which the temperature of the melt is much lower than the thermodynamic equilibrium temperature (ΔT) (Kellens et al., 2007). In this perspective of crystallization in a solution system, the TAGs that have to be crystallized are required to concentrate in the saturated-solution concentration, until achieved supersaturated condition. In other words, crystallization can only occur when solute concentration is greater than that in the saturated solution (Lawler and Dimick, 2002; Timms, 2005). The solvent for solution crystallization is not only referring to the organic solvents such as those added in the solvent fractionation. In bulk crystallization, the liquid

components in the TAG mixture can also performed as the solvent for the crystallization system.

Nucleation

At temperature much lower than the supercooling temperature (ΔT), nuclei can be formed in the system (Lawler and Dimick, 2002). Nuclei are defined as the smallest crystal that can exist in a solution at particular concentration and temperature (Timms, 2005). Generally, there are three types of nucleation phenomena that can be occur; the primary nucleation including homogeneous nucleation, heterogeneous nucleation and the secondary nucleation (Kellens et al., 2007). Homogeneous nucleation is nucleation that occurs in the bulk mother phase; heterogeneous refers to nucleation onto foreign substances in the crystallizer such as dirt, walls of crystallizers etc; secondary nucleation happens when tiny crystallites are removed from the surface of existing crystals which will then act as new nuclei in the crystallization system (Kellens et al., 2007), therefore secondary nucleation is undesirable in any fractionation process (Timms, 2005).

Crystal Growth

Once nuclei are formed, they will start to grow by incorporate with the TAG molecules from the adjacent liquid layer. Crystal growth can be affected by two factors; the internal and the external factors. External factors are including degree of supercooling, presence of inhibitors etc, whereas internal factors are polymorphic form, crystal morphology, crystal defects etc (Foubert et al., 2007).

According to Timms (2005) and Kellens et al. (2007), the growing rate is proportional to supercooling and inversely proportional to the viscosity of the system. When molecules

comes together to form crystal, two opposing forces will takes place simultaneously – the energy that release due to crystallization (heat of crystallization) and the surface tension between the crystals that leads to increase of viscosity. The increases of surface tension and viscosity are not only due to the increase of solid particles present in the liquid but also influenced by the crystals size distribution and the interactions between different crystals (Foubert et al. and Kellens, 2007).

To ensure a uniform crystals growth, the crystallization must be in a continuous system that allows homogeneous contacts of the nuclei and the surrounding supersaturated liquid (Timms, 2005). Therefore, a sufficient agitation rate with non-destructive feature is important to ensure a continuous and uniform crystallization (Kellens et al., 2007). A stable crystal will only form when the energy due to the heat of crystallization exceeds the energy that required to overcome the surface energy between the crystals (Timms, 2005).

1.5.2.1.3 Principal of Fractionation: Separation Step

To complete fractionation process, the solid phase needs to be separated from the liquid phase at the fractionation temperature. Efficient separation is essential to ensure production of purer stearin fraction with minimum entrainment of olein. There are a few separation methods used in fractionation plants, including centrifugation, vacuum filtration, and membrane filter press (Timms, 2005).

The principal of centrifugation is by density difference between solid and liquid phase of the slurry. It is only useful to separate slurry system with density difference of 10% between solid and liquid. Therefore this separation step is only applicable in detergent fractionation (Timms, 2005).

For dry fractionation, vacuum filtration and membrane filter press are used. The design of vacuum filtration is similar to laboratory vacuum filtration in which a vacuum is applied to suck the liquid oil through (Timms, 2005). There are two types of vacuum filtration methods used industrially, belt filter (Tirtiaux) and drum filter (Desmet) (Kellens and Hendrix, 2000). Belt filter performed better separation in terms of producing stearin with lower level of entrainment than the drum filter (Timms, 2005).

The most common filtration method used is membrane filter press. Initially, less than one bar pressure is applied to fill the filter press with the slurry. Higher pressure is applied to squeeze the entrained liquid oil. A standard filter press is normally applied at a pressure of 5-6 bar. When necessary, high-pressure membrane filter press that can operate up to 30 bar can be used (Kellens and Hendrix, 2000). In comparison, membrane press filtration is the most efficient separation method with the lowest liquid oil entrainment in stearin fraction, as well as it gives highest olein yield.

1.6 Dietary Fats and Coronary Heart Disease

1.6.1 Triacylglycerols Metabolism

There are a few enzyme lipases involved in the digestion of dietary fats in human body, such as lingual, gastric, pancreatic and co-pancreatic lipases that found in the mouth, stomach and small intestine, respectively (Voon and Kalanithi, 2008). In the stomach, about 10-30% of fats are pre-digested with presence of lingual and gastric lipases; bile salts will produce from the liver (Voon and Kalanithi, 2008).

In the intestine, dietary TAGs are dissolved with the help of bile salts, and some small quantities of fatty acids and MAGs (Ronald and Jogchum, 2002). About 70-90% of fats are digested with the presence of pancreatic lipase and co-pancreatic lipase particularly in the duodenum. Pancreatic lipase and co-pancreatic lipase hydrolyze *sn*-1 and *sn*-3 fatty acids of TAGs respectively, which resulted of the formation of 2-MAG, and *sn*-1(3) free fatty acids (Voon and Kalanithi, 2008).

Reesterification of the 2-MAG and free fatty acids in the intestine formed new TAGs that will incorporated into chylomicrons (C). The chylomicrons enter the lymphatic system for blood circulation in the subclavian vein. In the blood, the TAGs from the chylomicron core are hydrolyzed by lipoprotein lipase (LPL) and produced chylomicron remnants (CR), 2-MAG, and free fatty acids (Ronald and Jogchum, 2002; Voon and Kalanithi, 2008). Chlylomicrons remnants that carry the cholesterol ester and TAGs will transport back to the liver, while 2-MAG and free fatty acids will be used in TAGs synthesis in liver, or energy storage and supply (Voon and Kalanithi, 2008).

The *sn*-1(3) short and medium chain free fatty acids will be absorbed directly in the intestines after the hydrolysis, whereas long chain SFA will either be absorbed or mostly react with 2-MAG for resynthesis of new TAGs and chylomicron (Voon and Kalanithi, 2008). Predominantly, the long chain free fatty acids that hydrolyzed from *sn*-1 and *sn*-3 positions may not be absorbed or taken longer time for absorption due to their higher melting points as compared to human body temperature. In the intestine, long chain free fatty acids will tend to react with calcium ion, and thus excreted from the body as calcium soaps (Voon and Kalanithi, 2008).

The low activity of pancreatic lipase towards long chain polyunsaturated fatty acids (PUFA) has lead to low hydrolysis rate of PUFA at the *sn*-3 position in TAGs. Therefore long chain PUFA are mostly present in the form of 2,3-DAG instead of 3-MAG. These 2,3-DAG have to be hydrolyzed by hepatic lipase in the liver for production of 2-MAG and long chain PUFA. In other words, long chain PUFA from *sn*-3 position could not be directly absorbed by the body, and only those from *sn*-2 position can be directly absorbed in the form of 2-MAG (Voon and Kalanithi, 2008).

1.6.2 Impact of Dietary Fats towards Coronary Heart Disease

Coronary heart disease, also termed as cardiovascular disease is one of the major causes of morbidity and mortality in many countries especially prosperous western countries (Ronald and Jogchum, 2002). The major risk factors of coronary heart disease are cigarette smoking, elevated cholesterol level, elevated blood pressure, obesity and maleness (David, 2008). There are three preventable factors of coronary heart disease; the distribution of plasma cholesterol over low density lipoprotein (LDL) and high density lipoprotein (HDL), the oxidizability of LDL and hemostasis (Ronald and Jogchum, 2002). Therefore, the risk of coronary heart disease can be reduced by having a healthy lifestyle as well as maintaining a healthy diet as dietary fat is known to affect serum concentrations of total and lipoprotein cholesterol.

Generally, total cholesterol and low density LDL cholesterol are strongly related to coronary heart diseases. Reduction of LDL cholesterol levels is believed to be lower the risk of coronary heart diseases, whereas high HDL cholesterol levels are inversely related to the risk of coronary diseases (Krauss et al., 2000).

Saturated Fatty Acids (SFA)

There are three classes of SFA that show different effects towards coronary heart diseases i.e. medium chain fatty acids (MCFA), SFA with carbon chain length of C12:0-C16:0 and stearic acid (C18:0). MCFA slightly decreases LDL cholesterol concentrations as compared to palmitic acid (C16:0), while increases LDL cholesterol compared to oleic acid (C18:1) (Ronald and Jogchum, 2002). Lauric and myristic acis also raised total and LDL cholesterol relative to oleic acid, but it has lesser extent compared to palmitic acid. So, it can be concluded that SFA with carbon chain length of C12:0-C16:0 has raised the total and LDL cholesterol levels, which may increase the risk of coronary heart disease. On the other hand, C18:0 is neutral towards the total and LDL cholesterol levels (Penny et. al., 2001).

Monounsaturated Fatty Acids (MUFA)

Oleic acid is the major unsaturation fatty acids component in palm oil. Monounsaturated fatty acid (MUFA) is always known as neutral effects towards plasma total cholesterol levels, meaning that it has the same effect on plasma total cholesterol as compared with an isocaloric amount of carbohydrates. However, oleic acid has different effects on the distribution of cholesterol of the lipoproteins. Consumption of high amount of oleic acid may increase the good cholesterol lipoprotein, HDL relative to carbohydrates (Ronald and Jogchum, 2002).

Polyunsaturated Fatty Acids (PUFA)

PUFA intake is known to reduce the total and LDL cholesterol in the greatest effectiveness as compared to MUFA. PUFA can be divided into two classes, n-6 and n-3 PUFA (Ronald and Jogchum, 2002). In February 2009, American Heart Association (AHA) has published a scientific advisory to reduce the level of n-6 PUFA intake, as it specifically pertains to the

issue of CHD. Although consumption of n-6 PUFA can reduce the total and LDL cholesterol, but high dietary intakes of n-6 PUFA relative to n-3 PUFAs, would promote an overall pro-inflammatory and pro-thrombotic state due to over-production of arachidonic acid. This may lead to many diseases including CHD, cancer, arthritis and other autoimmune diseases (Lefevre, 2009). On the other hand, the downstream metabolites of n-3 PUFAs tend to be anti-inflammatory and anti-thrombotic that would reduce risk of coronary diseases. Besides, Long chain n-3 fatty acids from fish oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may decrease triglyceride levels, favorably affect platelet function, as well as decrease blood pressure in hypertensive individuals (Kris-Etherton, 2001).

Trans-fatty Acids

Trans-fatty acids generally found in partial hydrogenated fats, and some animal fats. Consumption of *trans*-fatty acids is known to be increased the level of bad-cholesterol (LDL), while reduced the good-cholesterol (HDL) (Krauss, 2000). Therefore, the effect of *trans*-fat on CVD is much serious as compared to SFA.

1.6.3 Global Dietary Recommendation

Table 1.5 below shows the summary of dietary fats recommendation for general adult population from World Health Organization (WHO) and AHA with objective of reducing the risk of coronary heart diseases. The recommendations are generally coupled with guidance on physical activity and weight maintenance and are distinct for individuals with specific metabolic profiles.

Table 1.5
Dietary fats recommendation of WHO and AHA for general adult population (WHO, 2003; Lichtenstein et al., 2006)

Dietary fats recommendation	Goal (% of total energy)	
	WHO	**AHA**
Total fat intake	15-30%	≤ 30%
Saturated fatty acids (SFA)	< 10%	< 7%
Total Polyunsaturated fatty acids (PUFA)	6-10%	≤ 10%
n-6 polyunsaturated fatty acids (PUFA)	5-8%	-
n-3 polyunsaturated fatty acids (PUFA)	1-2%	-
Trans fatty acids	< 1%	< 1%
Monounsaturated fatty acids (MUFA)	X%	X%
Cholesterol intake	< 300 mg per day	< 300 mg per day

[Abbreviation: MUFA intake, X (%) = total fat (%) – (SFA + PUFA + *trans* FA) (%)]

To reduce the risk of coronary heart disease (CHD) as well as other coronary diseases, AHA generally recommended three steps, 1) limiting intake of foods with high content of cholesterol-raising fatty acids such as SFA and *trans*-fatty acid, 2) limiting the intake of foods high in cholesterol, 3) to substitute grains and unsaturated fatty acids from fish, vegetables, legumes, and nuts (Krauss et al., 2000).

Although intake of high dietary cholesterol from foods may also contributed to higher LDL cholesterol level, but the effects is lesser as compared to the intake of SFA. Step 3 is necessary to maintain the total energy intake after reducing intake of SFA and *trans* fats, which is similar to the substitution of carbohydrate or unsaturated fat for SFA and *trans* fats (Krauss et al., 2000).

According to the scientific statement announced by AHA in February 2009, intake of 0.5-2.0% energy of n-6 PUFA (mainly linoleic acid) is sufficient for dairy dietary fats intake of an adult to maintain a healthy level of LDL cholesterol (Lefevre, 2009). Consumption of

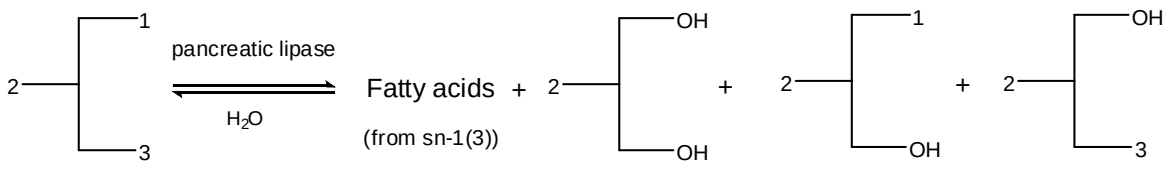
higher amount of n-3 PUFA (EPA and DHA) is rather important to prevent CHD, AHA generally recommended consumption of a fish meal or intake of approximately 850 mg of n-3 fatty acids supplement per day (Krauss, 2000).

1.7 Positional Fatty Acid Distribution of Triacylglycerols

Understanding of human body metabolism of dietary TAGs has raised the concern the oils scientists and nutritionists to determine the *sn*-2 position fatty acids in the TAGs of oils and fats. Basically, two methods: hydrolysis by pancreatic lipase and Grignard deacylation were commonly used to determine the regiospecificity of TAGs. Consequently, two approaches can be used: one involved analyzing of 2-MAGs that usually isolated by using thin layer chromatography followed by analyzing for their fatty acid composition (Becker et al., 1993; AOCS Official Method Ch 3-91, corrected 1996; Angers et al., 1998). The second approach involved analyzing of the DAGs and TAGs fatty acid composition to determine the *sn*-1(3) and *sn*-2 position fatty acids by calculation (Brockerhoff, 1971).

1.7.1 Pancreatic Lipase 1,3-specific Partial Hydrolysis

This method involved partial hydrolysis of fatty acids specifically at *sn*-1 and *sn*-2 position of TAGs by using pancreatic lipase to prepare a mixture of partial glycerides. The chemical equation below shows the possible products that can be derived from the reaction (Brockerhoff, 1960; 1971).



Theoretically, 2-MAG is the only monoacylglycerols produced in the reaction. However, Mattson and Volpenhein (1960) reported that the monoacylglycerols isolated from the digestion products are actually a mixture of 1- and 2-MAG. Longer digestion period will increase the proportions of 1-MAG in the mixture. The presence of 1-MAG is believed to be contributed by acyl migration of 2-MAG, indicating that the fatty acids attached to the 1-MAG is derived from 2-MAG. Therefore the fatty acids of 1-MAG may be considered representative of those in the *sn*-2 position of the original TAGs (Luddy et al., 1963). In other words, all fatty acids of MAGs are from the *sn*-2 position of TAGs. The assumption made for validity of this method is that the hydrolysis reaction is fully 1,3-specific, in which none of the non-preferential hydrolysis takes place in the reaction.

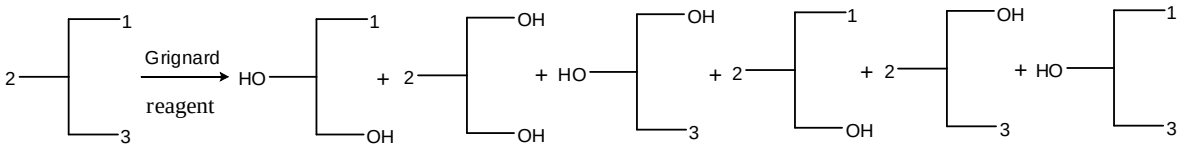
The catalytic behaviors of pancreatic lipase in terms of positional specificity and stereospecificity have been reviewed comprehensively by Desnuelle and Savary (1963). The paper reported that hydrolysis reaction does not occur in solution system indicating that the reaction probably take place at water-oil substrates. The rate and degree of hydrolysis is very dependent on some factors such as the surface area of oils substrate in the heterogeneous system that also depended on the agitation rate, the pH and temperature of the reaction system, and the presence of electrolytes, bile salts and calcium ions. The most favor temperature for the reaction is 40 °C.

The method is applicable for most of the oils and fats except for those containing substantial amounts of fatty acids with carbon number lower than twelve, fatty acid with highly unsaturated (more than four double bonds), very long chain fatty acids (fish and marine animal oils), fatty acids containing oxygenated groups, as well as those with other chemical functional group other than the acid group (AOCS Official Method Ch3-91, 1996).

In such cases, Grignard deacylation is a better way to analyze the positional distribution of fatty acids in the TAGs.

1.7.2 Grignard Deacylation

Unlike the pancreatic lipase hydrolysis, Grignard deacylation method involved a truly random degradation of TAGs that produced a mixture of partial glycerides together with the unreacted TAGs as illustrated in the equation below.



Turon et al., (2002) has conducted a study to compare the accuracy of both pancreatic lipase hydrolysis and Grignard deacylation methods in analyzing *sn*-2 position fatty acid of a high purity standard material, *sn*-1,3-dipalmitoleyl-*sn*-2-oleoyl-glycerol (POP) with 99%+ of O at the *sn*-2 position. The *sn*-2 position fatty acids were determined by analyzing the fatty acid composition of partial glycerides; 2-MAG, and 1,2-DAG. Instead of just 2-MAG and 1,2-DAG, 1-MAG was also analyzed for Grignard method. The *sn*-2 fatty acid composition is calculated according to the equations as follow.

$$sn\text{-}2 = (4 \times 1,2\text{-DAG}) - (3 \times \text{TAG}) \text{ ------ (1)}$$

$$sn\text{-}2 = (3 \times \text{TAG}) - (2 \times 1\text{-MAG}) \text{ ------ (2)}$$

In comparison to the direct analyzing of 2-MAG, Turon et al. (2002) found that calculated *sn*-2 position fatty acids from 1-MAG (equation 2) is closer to the actual *sn*-2 position fatty acid content of POP, with lower standard deviation as well. They also reported that

pancreatic lipase hydrolysis method is more accurate than the Grignard reaction in analyzing the *sn*-2 position fatty acids of POP.

This method is widely studied in many literatures. To date, there is still no standard method available for this analysis. Different Grignard reagents, reaction system, reaction termination agent, oil sample amount, as well as different reaction time were applied in many studies, as summarized in the Table 1.6.

Table 1.6
The Grignard reagents, the oil samples, and the reaction time taken for the deacylation reaction from the literatures

Reference	Oil sample	Grignard reagent	Termination agent	Time taken
Brockerhoff (1967)	1 g corn oil	2 mL of 3 M MMB	1 mL acetic acid	30 s
Becker et al. (1993)	6.5 mg SOS (99%+) & CPC	200 μL of 1 M AMB	acid buffer (0.27 M HCl in 0.4 M boric acid)	60 s
Ando et al. (1996)	0.5 mg bonito head oils (rich with C22:3ω6)	0.33 mL of 0.33 M EMB	acetic acid:diethyl ether (1:9 v/v)	15 s
Angers et al. (1998)	4 mg bovine milk, 10mg milk fat TAGs	42 μL 3.0 M EMB	10 μL glacial acetic acid; 300 μL of 10% boric acid	30 s
Angers and Arul (1999)	beef tallow, grapeseed oil, cottonseed oil	20 μL of 3.0 M EMB	glacial acetic acid; 300 μL of 10 % boric acid	30 s
Turon et al. (2002)	40 mg borage oil, tuna oil, milk fat, racemic synthetic POP (99%+)	670 μL EMB	300 μL glacial acetic acid; 5 mL of 0.4 M boric acid	15 s

[Abbreviation: MMB = methyl magnesium bromide EMB = ethyl magnesium bromide, AMB = Allyl magnesium bromide]

1.7.3 Separation of Acylglycerols by Thin-layer Chromatography (TLC)

After obtaining the acylglycerols mixture from hydrolysis using pancreatic lipase and Grignard reagent, TLC is often used for isolation of the products. In general, lipids can be resolved into monoglycerides, diglycerides and triglycerides, according to the number of

hydroxyl groups in the molecule, irrespective to the chain length and the degree of saturation, by using thin-layer chromatography. The method of pancreatic lipase hydrolysis does not involve separation of MAGs isomers; therefore the conventional TLC developing solvents can be used to resolve the acylglycerol compounds. Table 1.7 illustrated the formulas of TLC developing solvents that used for separation of MAGs, DAGs and TAGs.

Table 1.7
The TLC developing solvents used for separation of MAGs, DAGs, and TAGs

Reference	TLC Visualization	Proportion of TLC developing solvent, v/v/v			
		hexane	diethyl ether	formic acid	acetic acid
AOCS Official Method Ch 3-91 (1996)	DCF solution; UV	70	30	1	-
Dourtoglou et al. (2001)	Iodine vapour	80	20	-	2
Shimada et al. (2003)	H_2SO_4 in MeOH; heat at 150 °C	90	10	-	1

As Grignard deacylation implied random hydrolysis of TAGs that produced 1-MAG and 2-MAG, the TLC silica plate has to be impregnated with boric acid to prevent isomerization of the 2-MAG to 1-MAG. Thomas et al. (1965) reported that boric acid can act as an impregnated material by forming polyhydroxyl compounds with 2-MAG molecules. Moreover, the conventional TLC developing solvents is not appropriate for separation of the reaction products that required separation of 1-MAG and 2-MAG.

Table 1.8 shows the formulas of TLC developing solvents used for separation of 1-MAG and 2-MAG. Chloroform is often used as the major component for separation of 1-MAG and 2-MAG. The role of acetic acid is to enhance the migration rate of acylglycerols on the TLC, as well as to form a more compact spots for the isomeric compounds (Thomas et al. 1965).

Table 1.8
The TLC developing solvents used for separation of 1-MAG and 2-MAG

Reference	TLC Visualization	Proportion of TLC developing solvent, v/v/v			
		chloroform	acetone	methanol	acetic acid
Serdarevich and Carroll (1966)	50% H_2SO_4, charring	98	-	2	-
Becker et al. (1993), Ando et al. (1996)	DCF solution; UV	96	4	-	-
Angers et al. (1998)	DCF solution; UV	98	-	2	-
Turon et al. (2002)	DCF solution; UV	85	15	-	1
Shimada et al. (2003)	H_2SO_4 in MeOH; heat at 150 °C	96	4	-	1

The methods discussed above only enable us to determine the *sn*-1(3) and *sn*-2 positions fatty acids in the TAG molecules, without differentiation between the *sn*-1 and *sn*-3 fatty acid composition. Stereospecific analysis is conducted to differentiate *sn*-1 and *sn*-2 position, by using either pancreatic lipase hydrolysis or Grignard deacylation method to produce 1,2-DAG. The 1,2-DAGs are then converted synthetically to phospholipids and hydrolyzed by stereospecific phospholipase (Brockerhoff, 1971). By using this method, the exact fatty acid composition for *sn*-1, *sn*-2 and *sn*-3 positions can be established (Table 1.8).

Fatty acid composition of oils and fats is usually determined by preparation of methyl esters of fatty acids. These fatty acid methyl esters (FAME) that derived from fatty acids of acyglycerols are suitable for gas chromatography analysis. FAME can be prepared by a few methods, such as boron trifluoride-methanol method, alkaline methanolysis method, acidic methanolysis method, and rapid method.

2 EXPERIMENTAL

2.1 Materials

Refined, bleached and deodorized palm olein of IV 62 was purchased from Golden Jomalina Food Industries Sdn. Bhd (Sime Darby Plantation). The 1,3-specific immobilized lipases; Lipozyme® TL IM and Lipozyme® RM IM were obtained from Novozymes A/S. Chromatography grade solvents (n-hexane, heptane, acetone, chloroform and acetonitrile) and analytical grade 2-propanol were purchased from Fischer Scientific. The *sn*-2 analysis reagent, pancreatic lipase (from porcine source) and Allyl magnesium bromide solution (AMB) were obtained from Sigma-Aldrich. The laboratory reagents used were bile salt (Uni-chem), tris-hydroxymethylaminomethane (Fischer Scientific), phenolphathalein (APS Finechem, Australia), Alumina grade Super I (Sigma, Germany), silica gel 60G (Merck, Germany), Calcium Choride solutions (BDH Limited Poole England), sodium chloride; saturated aqueous solution (Prolabo France), sodium sulphate anhydrous (systerm), borontrifluoride; methanolic solution (Merck, Germany), hydrochloric acid, 36 % and acetic acid, 98 % (Systerm). In conducting the IV tests, the reagent used were Wijs reagent, potassium iodide, and starch powder from Merck, Germany, and sodium thiosulphate from J.T. Baker, USA. Standard material used for the TAG analysis by high-performance liquid chromatography (HPLC) was a secondary standard R.B.D palm oil purchased from Golden Jomalina Sdn. Bhd. while standard used in fatty acid composition and *sn*-2 positions determination by gas-chromatography was FAME mix RM6 obtained from Supelco.

2.2 Enzymatic Interesterification

2.2.1 Laboratory-scale (lab-scale) Batch Interesterification

The objective of this experiment is to study the efficiency of two commercial immobilized enzymes; Lipozyme® TL IM and Lipozyme® RM IM in catalyzing the interesterification

of palm olein IV 62. The optimum conditions were determined by the TAG composition of the reaction products. Two parameters; the enzyme to oil ratio and the reaction time were studied for optimization of the reaction conditions. In addition, the specificity of both enzymes in retaining the fatty acids at the 2-position was investigated.

The laboratory-scale (lab-scale) batch interesterification was conducted by using 65 °C water bath shaker. 2 %w/w (0.60 ± 0.01 g) of Lipozyme® TL IM was weighed separately into six 100 mL conical flask with stopper. 30.0 ± 0.02 g of palm olein with iodine value (IV) of 62 was added into each conical flask. The conical flasks were closed tightly with the stopper and wrapped with a few layers of parafilm followed by aluminum foil. The water bath shaker was filled with clean distilled water and set at 65 °C. As temperature achieved 65 °C, the six cornical flasks were fitted simultaneously into the water bath and shaken at 200 rpm. A conical flask was taken out for filtration at 1, 2, 3, 4, 8 and 24 hours of reaction. The experiment was conducted in duplicate. The same procedures were carried out for 5 %w/w (1.50 ± 0.01 g) and 10 %w/w (3.00 ± 0.01 g) enzyme ratio, for reactions catalyzed by Lipozyme® RM IM.

The entire products of the lab-scale interesterification were analyzed for their TAG compositions. Only the selected oil samples were analyzed for the *sn*-2 fatty acids by using Grignard degradation method.

2.2.2 Pilot-scale Interesterification

The batch interesterifications were conducted according to the optimum condition of the lab-scale interesterifications. On the other hand, the continuous interesterification was conducted by using 100 kg packed-bed reactor from Novozyme Sdn. Bhd. The reaction was

carried out by referring to the standard method provided by Novozyme Company. The large pore size of the reactor screen allows only the Lipozyme® TL IM to be used.

The pilot-scale interesterification products were analyzed for their TAG profiles, slip melting point (SMP), *sn*-2 fatty acids (by pancreatic lipase hydrolysis), thermal properties (by differential scanning calorimeter (DSC)), solid fat content (SFC), and cloud point (CP).

2.2.2.1 Batch Interesterification by Lipozyme® RM IM (Batch RMIM)

The pilot-scale batch interesterification by Lipozyme® RM IM was conducted according to the optimum condition obtained from the lab-scale interesterification. Two factors; the TAG composition and the enzyme cost were taken into consideration. Due to high cost of Lipozyme® RM IM, the reaction was conducted by using 2 %w/w enzyme rather than 5 % w/w or 10 % w/w. The reaction was carried out at High Oleic Pilot Plant in the Malaysian Palm Oil Board. This batch reactor with capacity of 100 kg is modified from the chemical interesterification reactor used in the pilot-plant.

Initially, the pre-heated 100 kg palm olein IV 62 was loaded into the batch reactor followed by addition of 2.0 kg Lipozyme® RM IM. The temperature of the reactor was adjusted to 65 ± 3 ºC by controlling of steam and cooling water. The reactor was blanketed with nitrogen gas to avoid the presence of moisture during the reaction. The reactor was equipped with an agitation tool. The reaction was conducted for 24 hours to obtain a product with desired high percentages of triunsaturated and trisaturated TAGs. The interesterified product was collected into two containers and stored in the cold room before used for dry fractionation.

2.2.2.2 Batch Interesterification by Lipozyme® TL IM (Batch TLIM)

The pilot-scale batch interesterification by Lipozyme® TL IM was designed according to the results of the lab-scale interesterification. The lab-scale results showed that 5 % w/w is the most suitable dosage used for the reaction due to shorter time needed in achieving equilibrium stage as compared to 2 %w/w. Moreover, the cost of Lipozyme® TL IM is three times cheaper than Lipozyme® RM IM. Therefore, 5 % w/w ratio to oil was chosen to implement in the reaction. The reaction was conducted by using the same reactor as the batch interesterification by Lipozyme® RM IM. In this experiment, the pre-heated 90 kg of palm olein IV 62 was loaded into the reactor and 4.5 kg of Lipozyme ® TL IM was added afterward. The temperature used in the reaction was 65 ± 3 ºC. The reaction was only conducted for eight hours which is the optimum time indicated in the lab-scale experiment. The interesterified products were collected into two containers and stored in the cold room.

2.2.2.3 Continuous Interesterification by Lipozyme® TL IM (packed-bed TLIM)

Interesterification reaction of palm olein by Lipozyme® TL IM was carried out by using 10 kg pilot-scale continuous enzyme bed reactor (Novozyme). Initially, 5 kg of Lipozyme® TL IM was fed into the clean column of the reactor. The condition of the reactor was monitored at 65 °C. Feed palm olein of IV 62 was loaded onto the column for the purpose of lipase conditioning. The flow rate of the feed was adjusted to 2 kg/hour for every kg of lipase by controlling the frequency of the pump. Oil samples were collected at every 15 minutes to determine the stage of conditioning. The interesterified product was collected once the FFA content was reduced to a constant value. The product was collected and stored in two containers.

2.3 Dry Fractionation

2.3.1 Lab-scale Fractionation

The pilot-scale interesterified products from batch interesterification by Lipozyme® TL IM and Lipozyme® RM IM, and the continuous interesterification by Lipozyme® TL IM were fractionated in lab-scale dry fractionation. The fractionation temperatures used for each interesterified products were slightly different due to the differences in the TAG composition and the physical properties.

The lab-scale olein fractions were analyzed for their IV, fatty acid composition, and TAG profiles.

2.3.1.1 Lab-scale Fractionation of Batch RMIM Products

100.0 g of oil sample was weighed into a stainless steel container. The container was fitted on a water bath that was connected to a temperature programmer. An agitation tool was attached to the device and set at 25-27 rpm. Temperature programmer was set through the water bath computer software. The temperature program included melting of the oil at 65 °C to eliminate any crystal present in the oil, followed by cooling in a controlled manner. The oils were cooled slowly to the fractionation temperatures and held at that particular temperature for 140 minutes. During the cooling process, supercooling of the oil occurred, resulting in nucleation and crystal growth (Zaliha et al., 2004; Kellens et al., 2007). Five different products at fractionation temperatures of 8 °C, 10 °C, 12 °C, 15 °C, and 18 °C were investigated. Each trial was carried out in duplicate. The oil and crystal phases appeared as semi-solid slurry after the crystal growth becomes stable. The slurry was then separated into olein and stearin fractions by vacuum filtration.

2.3.1.2 Lab-scale Fractionation of Batch TLIM Products

The batch interesterified product by Lipozyme® TL IM is harder in physical appearance as compared to the batch interesterified product by Lipozyme® RM IM. Therefore, the fractionation temperatures used in the lab-scale fractionation trials were 9 °C, 12 °C, 15 °C, 18 °C, and 21 °C which were slightly higher than the fractionation temperatures used for the product derived from Lipozyme® RM IM. In this experiment, the same procedures and instrument were applied as described in section 2.3.1.1.

2.3.1.3 Lab-scale Fractionation of Packed-bed TLIM Products

The fractionation temperatures used for fractionation of packed-bed TLIM product were the same as temperatures used in the fractionation of batch TLIM products, which were 9 °C, 12 °C, 15 °C, 18 °C, and 21 °C.

2.3.1.4 Cooling Programs for Lab-scale Dry Fractionation

The Cooling programs for lab-scale dry fractionation were illustrated in Table 2.1-2.7. The same cooling programs were used in fractionating all of the interesterified palm olein (Batch RMIM, Batch TLIM, and Packed-bed TLIM) for dry fractionation at 12 °C, 15 °C, and 18 °C as shown in Table 2.4, Table 2.5, and Table 2.6, respectively.

Table 2.1
The cooling programs for lab-sccale dry fractionation at 8 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	30
3	20	40
4	12	40
5	8	140

Table 2.2
The cooling programs for lab-scale dry fractionation at 9 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	20
3	23	30
4	16	40
5	13	40
6	9	140

Table 2.3
The cooling programs for lab-scale dry fractionation at 10 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	30
3	25	30
4	14	40
5	10	140

Table 2.4
The cooling programs for lab-scale dry fractionation at 12 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	25
3	23	25
4	16	70
5	12	140

Table 2.5
The cooling programs for lab-scale dry fractionation at 15 °C

Step	Temperature, °C	Duration (min)
1	65	60
2	35	30
3	25	30
4	18	60
5	15	140

Table 2.6
The cooling programs for lab-scale dry fractionation at 18 °C

Step	Temperature, °C	Duration (min)
1	65	60
2	35	30
3	25	30
4	21	60
5	18	140

Table 2.7
The cooling programs for lab-scale dry fractionation at 21 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	30
3	28	30
4	23	50
5	21	140

2.3.2 Pilot-scale Fractionations

10.0 kg of oil sample was fed into a 10 kg double-jacketed De Smet crystallizer attached with an agitation tool with rate of 12 rpm (Figure 2.1). The oil was first heated at 65 °C for one hour to destroy all crystal structures, followed by cooling in a controlled manner. The crystallization temperature was set at the T_{OP} for 160 minutes. T_{OP} is the optimum temperature determined from the lab-scale fractionation for each interesterified product. Crystal development of the oil during the crystallization period was studied by pulsed nuclear magnetic resonance (PNMR).

The solid and liquid phases of the slurry were separated by a filter press (Yabuta, Japan) (Figure 2.2). The filter press consists of filter membrane plates and filter chamber plates. To avoid the melting and deforming of the crystal during filtration, the filter press was chilled to similar temperature of the slurry before the filtration. The slurry was fed into the filter press with pressure rate of 0.5 bar min^{-1} and gradually increasing to a maximum pressure of 2.0 bars in 10 min.

Figure 2.1
10 kg pilot-scale Desmet Ballestra crystallizer for pilot-scale fractionation

Figure 2.2
10 kg pilot-scale membrane filter press (Yabuta) used for pilot-scale fractionation

Squeezing of the stearin cake inside the chamber was carried out by pumping chilled water into the filter membrane plates. Initially, the stearin cake was squeezed at 0.5 bar and slowly increased the pressure at rate of 0.5 bar min^{-1} in the first 10 min. The pressure rate was then increased to 1.0 bar min^{-1} to a maximum pressure of 15 bars. The filter press was opened and the stearin cake was collected in a hopper under the filter. The fractionation was conducted in duplicate. The oleins and stearins were weighed and analyzed.

The pilot-scale fractionated oleins and stearins were analyzed for their fatty acid composition, TAG profiles, SFC, SMP, thermal properties (by DSC) and IV. The CPs of oleins were determined.

2.3.2.1 Cooling Programs for Pilot-scale Dry Fractionation

The cooling programs used in pilot-scale dry fractionation of packed-bed TLIM product was shown in Table 2.8, whereas dry fractionation of batch TLIM and batch RMIM products were conducted using cooling program as illustrated in Table 2.9.

Table 2.8
The cooling program of pilot-scale dry fractionation of packed-bed TLIM product at 12 °C

Step	Temperature, °C	Duration (min)
1	65	60
2	35	30
3	23	30
4	16	100
5	12	180

Table 2.9
The cooling program of pilot-scale dry fractionation of batch RMIM and batch TLIM products at 8 °C

Step	Temperature, °C	Duration (min)
1	65	40
2	35	40
3	25	40
4	15	60
5	10	60
6	8	160

2.3.3 Dry Fractionation of Palm Olein IV 62

Lab-scale and pilot-scale fractionation of palm olein IV 62 was conducted at temperature of 5 °C and 2 °C, respectively. Cooling programs were illustrated in Table 2.10 and Table 2.11. Each trial was conducted in duplicate.

Table 2.10
The cooling program of lab-scale dry fractionation palm olein IV 62 at 5 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	35	30
3	18	40
4	12	40
5	8	40
6	5	120

Table 2.11
The cooling program of pilot-scale dry fractionation palm olein IV 62 at 2 °C

Step	Temperature, °C	Duration (min)
1	65	50
2	45	30
3	35	30
4	18	30
5	12	40
6	8	40
7	5	80
8	2	120

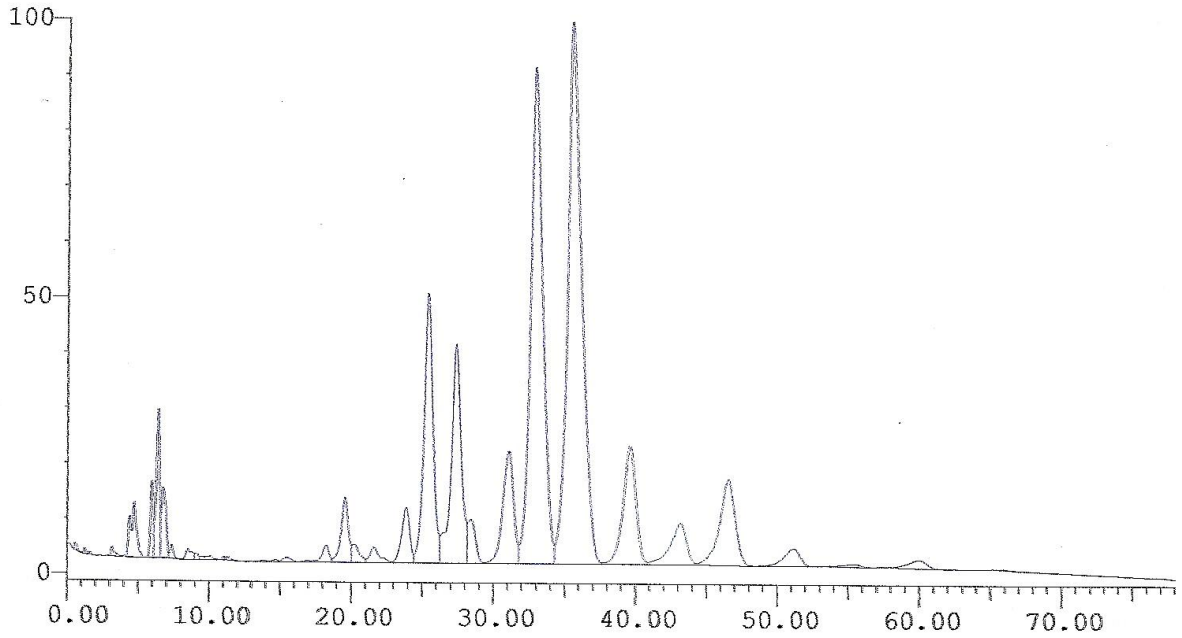
2.4 Analysis Methods

2.4.1 HPLC Separation of Triacylglycerols (TAGs)

TAG composition of the samples was determined by using reversed-phase high performance liquid chromatography (HPLC) from Gilson (France). The instrument was equipped with a refractive index detector from Waters 2410, USA, and Lichrospher®100 RP-18 column (250 cm) with 5 µm particle size. About 0.045 g of oil samples were weighed and diluted in acetone for injection into the HPLC column. The mobile phase used in the analysis was a mixture of equal volumes of acetone and acetonitrile at a flow rate of

1 mL/min. The TAG peaks identification was based on reference material – RBD palm oil and comparison with the literatures by Ghazali et al. (1995) and Swe et al. (1995). The most abundant TAGs in RBD palm oil are POO and POP, in which the term of POP refers to mixture of POP and very little amount of PPO.

Figure 2.3
The HPLC chromatogram of the reference material – RBD palm oil



Isomers of TAGs cannot be separated using this method. For example, POP is refers to the mixture of POP and PPO, with a very small proportion of PPO isomer. The chromatogram of TAG separation using HPLC was illustrated in Figure 2.3.

2.4.2 Analysis of the *sn*-2 Position Fatty Acids

2.4.2.1 Grignard Degradation Method

The regiospecific analysis by Grignard degradation method was conducted by referring to the literatures (Claus et al., 1993; Paul et al., 1998).

2.4.2.1.1 Preparation of Thin Layer Chromatography (TLC)

Five and a half clean glass plates (with same thickness) were placed onto the TLC spreader with the half plate on the left end. The plates were clean by wiping with acetone for a few times. 45 g of silica gel G (Fluka or Fischer) was weighed into a 500 mL conical flask. Distilled water (90 mL) was added into the flask and shook vigorously for exactly one minute. The silica gel mixture was spread smoothly from left to right onto the plates with a layer thickness of 0.5mm. The TLC plates were taken apart from each another immediately after spreading. After leaving in the room temperature for two hours, the plates were placed in the TLC plate holder and dried overnight at room temperature. The TLC plates were activated by heating at 110°C for one hour. After cooling to room temperature, the plates were sprayed with 0.4 M boric acid solution until fully saturated. The plates were dried overnight in the fume hood and then stored in the desiccators before used.

2.4.2.1.2 Purification of Oil Samples

The oil samples need to be purified to get rid of FFA, and the partial glycerides (monoacylglycerols and diacylglycerols) that may influence the degradation reaction. The oil is purified by conventional liquid chromatography. 200 g of silica gel 60G was weighed and transferred into a tray. The silica gel was heated for 5.5 hours and kept in desiccators for further usage.

60 g of the dry silica gel was weighed into a 500 mL conical flask and 5% water was added. The conical flask was shaken vigorously to homogenize the mixture and left for overnight. The “water treated” silica gel (20 g) was covered with petroleum spirit (petroleum ether) and stirred to remove air bubbles. About 10 mL of petroleum spirit was transferred into the column followed by the silica gel. Some anhydrous sodium sulphate was added onto the top

of the column. 5 g of oil samples was dissolved in 100 mL of a solvent mixture of petroleum ether and diethyl ether with ratio of 95: 5. The solution was transferred into the silica packed-column. The flow was adjusted to about 3-5 mL/min, and the effluent was collected in a 500 mL conical flask. Another 150 mL of the solvent mixture was added to flush the column.

2.4.2.1.3 Degradation Reaction

Four drops of oil sample (approximately 50-60 mg) was weighed into a clean 50 mL round bottom flask. Diethyl ether (10 mL) was added to dissolve the oil sample. The solution was stirred by using magnetic stirrer. 0.3 mL of AMB solution was withdrawn with a 1 mL glass syringe and added into the solution. The syringe has been flushed with nitrogen gas before used to eliminate the presence of air. The diethyl ether solution became opaque, indicating a spontaneous reaction. After exactly one minute, 8 mL of acidic buffer (1 mL of 37 % hydrochloric acid in 36 mL of 0.4 M boric acid) was added to neutralize the magnesium hydroxide which formed in the reaction mixture upon addition of water. The mixture was transferred into a test tube. The aqueous phase was removed by using a dropper. While the organic phase (diethyl ether phase) was washed twice with 8 mL of 0.4 M boric acid solution to remove the excess hydrochloric acid. The ether phase was dried briefly with some sodium sulphate anhydrous before transfer to a small vial. The ether phase was dried out by blowing with nitrogen gas. The acyglycerols mixture was re-dissolved in about 300 µL of diethyl ether.

2.4.2.1.4 TLC Separation of Acylglycerols

The sample was applied onto a boric acid impregnated TLC plate by using 0.1 mL syringe. The spot must be as small as possible to prevent tailing effect of the TLC bands. The TLC

plate was developed in a saturated chamber with the developing solvent (chloroform/acetone; 90:10). The saturation of the TLC chamber is indicated by the use of chromatography paper. The plate was taken out after 50 minutes and dried in the fume hood for about five minutes. The plate was placed into the saturated chamber once again for another 50 minutes. Then the plate was dried in fume hood, and sprayed with 2,7-dichlorofluoresceine. Visualization of the acylglycerols bands was done by using UV-lamp. The 2-monoacylglycerols (2-MAG) band (second band from the baseline) was scrapped into a vial. The 2-MAG was extracted into a 25 mL round bottom flask by adding about 3 mL of diethyl ether for three times. The filter paper was flushed for a few times to ensure complete extraction.

2.4.2.1.5 Preparation of FAME: Boron Trifluoride Methods

The solvent of the extract was evaporated by rotator evaporator. The methylation of the product (2-MAG) was conducted by using boron trifluoride method according to MPOB Test Methods P3.4-part 1 (2005). Initially, 4 mL of methanolic NaOH solution (0.4 M NaOH in methanol) and boiling aid were added into the flask with the test portion. The flask was fitted with the condenser. The test portion was boiled under reflux until fully dissolved (this usually takes 5 minutes). 5 mL of boron trifluoride solution was added from the graduated pipette through the top of the condenser to the boiling liquid. About 2 mL of heptane (chromatography grade) was added into the boiling mixture through the top of the condenser (the precise amount does not affect the reaction) and continue boiling for 1 min. Stop heating, the flask was cooled and removed from the condenser. A small portion of saturated sodium chloride solution was added to the flask and swirled gently for several times. Then, additional of more saturated sodium chloride solution to the flask in order to

bring up the level of liquid to the neck of the flask. The upper layer was transferred into a vial and sodium sulphate was added to remove any traces of water.

2.4.2.1.6 Gas-chromatography (GC) Injection

The fatty acid methyl esters was filtered into a 1.5 mL vial by syringe filter (pore size = 0.45 μm; diameter = 17 mm). Then, the test portion was concentrated and transferred into a GC-vial for analysis.

2.4.3 Thermal Analysis by Differential Scanning Calorimeter (DSC)

The thermal properties of the samples were measured using a Perkin Elmer differential scanning calorimeter. The instrument was calibrated using indium by a temperature programme of 120 °C to 180 °C at a rate of 5 °C/min. Volatile sample pans made of aluminium were used. The sample weights used were from 5-10 mg with an empty sample pan as the reference. The sample was heated from 55 °C to 80 °C and held at this temperature for 10 minutes to destroy the entire crystal structure. The sample was then cooled to -55 °C at a cooling rate of 5 °C/min for the recording of the cooling thermogram. The sample was held at this temperature for another 10 minutes. The melting properties of the samples were then investigated by heating the sample from -55 °C to 80 °C at a heating rate of 5 °C /min. The melting thermogram was recorded.

2.4.4 Determination of Solid Fat Content (SFC)

SFC of the interesterified oils and olein fractions were determined by pulsed nuclear magnetic resonance (PNMR) according to MPOB Test Methods P4.9-Section 1 (2005) (indirect method).

2.4.5 Determination of Cloud Point (CP)

CP of palm olein of IV 62, and the interesterified products were determined according to MPOB Test Methods P4.3 (2005).

2.4.6 Determination of Fatty Acids Composition

Preparations of fatty acid methyl ester (FAME) for the fatty acid composition analyses were conducted according to MPOB Test Methods P3.4-Part 4 (2005). FAME was investigated by using Agilent 5890N series II GC. 1 µL of the sample was injected into SGE BPX70 column with dimension of 60 m × 0.25 µm × 0.25 µm. The instrument was fitted with a Flame ionization detector (FID). The carrier gases used were hydrogen with ratio of 1: 100 with air and a flow rate of 0.8 mL/min. Detector and injector temperature were both set at 240 ºC. The separation of FAME was performed under isothermal condition with temperature of 185 ºC. Standard material used for the fatty acid identification was FAME mix RM6 purchased from Supelco.

2.4.7 Determination of Slip Melting Point (SMP)

SMP of the interesterified oils and feeds were determined by the MPOB Test Methods P4.2 (2005).

2.4.8 Determination of Iodine Value (IV)

IV of the olein fractions were determined by the MPOB p3.2 test method (MPOB Test Methods P4.2, 2005).

3 RESULTS AND DISCUSSION

The main objective of this project was to produce low saturation palm oil products due to the concern of consumers on the negative impact of saturated fatty acid (SFA) on human health. To date, World Health Organization (WHO) recommended intake of 15 to 30% energy of total fat intake, with maximum intake of 10% energy of SFA (WHO, 2003), while American Heart Association (AHA) recommended fat intake of less than 7% energy SFA out of total 30% energy fat intake per day in order to reduce the risk of coronary heart disease (Lichtenstein et al., 2006). In other words, the saturation content for ideal oil for good health should not exceed one third or 33.3% of the total fat intake.

Palm olein is somehow not considered as a recommended choice because of its relatively higher SFA concentration compared to other vegetable oils. The SFA content in palm oil is about 50%, 41.9-50.1% in palm olein with IV less than 60, and 34.4-43.3% in superolein with IV greater than 60 (Siew, 2002). For high temperature cooking purposes, presence of some saturation is necessary for better oil stability.

Although dry fractionation can significantly reduce the saturation content in palm oil, the saturation level achieved is still considered high according to the dietary guidelines recommended by AHA and WHO (WHO, 2003; Lichtenstein et al., 2006). Further reduction of SFA in palm olein by dry fractionation requires some very specific conditions in both crystallization and filtration steps. Low concentration of trisaturated TAGs causes difficultly in controlling the crystallization of palm olein (Calliauw et al., 2007a). Therefore, an alternative approach is proposed in this study to reduce the saturation content in palm olein, which is via combination of enzymatic interesterification and dry fractionation.

Focus of the Study

The study was mainly focus on enzymatic interesterification using two enzymes; Lipozyme® TL IM and Lipozyme® RM IM. The behaviors of the enzymes in catalyzing interesterification had been looked into; including the effects of enzyme dosage in batch interesterification, the *sn*-1 and *sn*-3 selectivity of the enzymes in catalyzing the interesterification reactions, and the changes in physicochemical properties of palm olein after the enzymatic interesterification reactions.

Lab-scale batch interesterification was conducted to study the equilibrium stage of batch interesterification reactions based on TAGs conversion and the changes in solid fat content (SFC). The main objective of conducting pilot-scale interesterifications was to obtained sufficient amount of interesterified products for dry fractionation. The physicochemical changes of palm olein were studied to evaluate the performance of the enzymes in each pilot-scale reaction, as well as to select the suitable temperature range for dry fractionation.

Dry fractionations were conducted to remove the saturation content in the interesterified products. Lab-scale fractionation of each interesterified product involved crystallization at five fractionation temperatures that selected according to their physicochemical properties. The purpose of conducting lab-scale fractionation was to optimize the dry fractionation process, in order to obtain olein fractions with high quality and yield. Evaluation of the olein fractions were based on some major analyses such as fatty acid composition, TAG profiles, IV, SFC and thermal properties. Due to the inefficiency of lab-scale filtration step – vacuum filtration, in separating the olein and stearin fractions, pilot-scale fractionations were carried out to obtain the exact olein and stearin yields.

The final products derived from pilot-scale fractionations were fully evaluated. Single-step dry fractionation of the feed olein – palm olein of IV 62 was conducted in both lab-scale and pilot-scale. The process of single-step dry fractionation and enzymatic route process were evaluated based on the final products quality and yield, as well as the ease of dry fractionation process.

3.1 Enzymatic Interesterification

Generally, *sn*-1,3 specific enzymatic interesterification reaction allowed the rearrangements of the fatty acids between and within the TAG molecules at the *sn*-1 and *sn*-3 positions of the TAG backbone (Willis and Marangoni, 2002). This rearrangement of fatty acids will lead to production of trisaturation TAGs that could be easily removed as solid fraction during dry fractionation. Lipozyme® TL IM (*Thermomyces lanuginose*) and Lipozyme® RM IM (*Rhizomucor miehei*) were used as 1,3-specific lipase to catalyze the reaction by release of fatty acids specifically from the outer 1- and 3- positions of TAGs.

3.1.1 Equilibrium Stage for Batch Reactions

Lab-scale interesterification was conducted in batch reaction to study the effects of enzyme dosage to the reaction rate, as well as to determine the equilibrium stage for each reaction. Two parameters, including the changes of TAG composition and SFC over 24 hours of reaction were studied to determine the equilibrium stage for each reaction. For both lab-scale batch interesterification reactions, enzyme dosage of 2, 5, and 10 %w/w were used. All reactions were conducted at 65 °C.

3.1.1.1 Equilibrium Stage for Batch TLIM

The enzymatic interesterification reaction allowed rearrangement of fatty acids that will lead to formation of new TAGs, which would alter the chemical properties of the oil and therefore the physical properties. In comparison, the reaction rate for enzymatic interesterification was relatively slower than chemical interesterification (Novozyme, 2004). The reaction can be terminated at any time by separating the enzymes from the oil. Hence, determination of the physicochemical properties of the samples at particular reaction time can be used to determine the time needed to achieve equilibrium stage for each reaction.

Figure 3.1 showed the changes of some TAG species in palm olein IV 62 over 24 hours of reaction. In overall, trisaturated TAGs (PPP and PPS) and triunsaturated TAGs (OLL, OLO and OOO) increased, while diunsaturated TAGs (PLL, PLO, POO and SOO) and disaturated TAGs (PLP) decreased for all the lab-scale batch TLIM interesterification reactions. The raw data for TAG composition of lab-scale batch TLIM products were illustrated in Appendix C-E.

Figure 3.1

Batch TLIM: changes of TAG composition of palm olein IV 62 during 24 hours reaction; (A) PPP content, (B) OOO content, (C) POO content and (D) PLP content

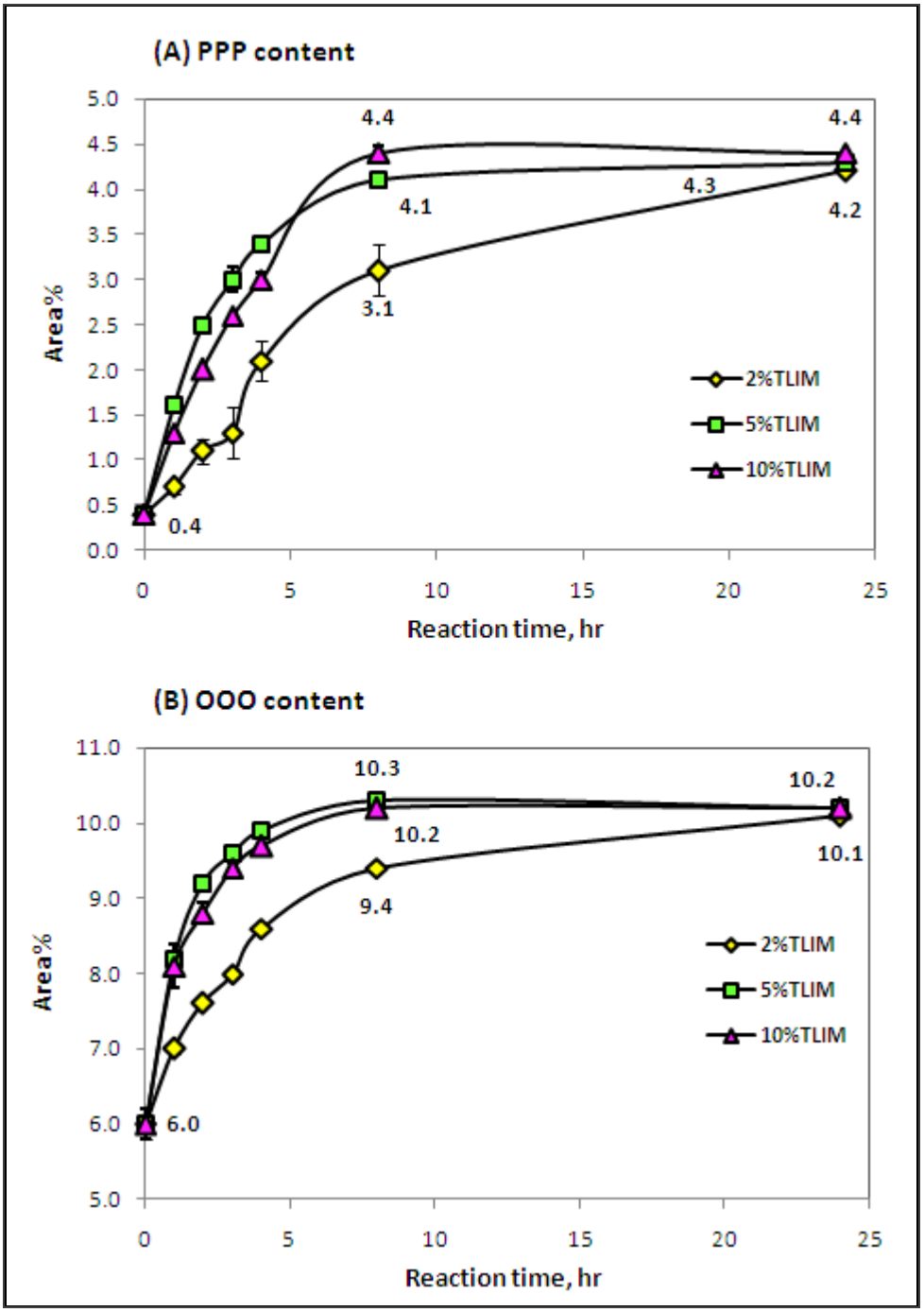
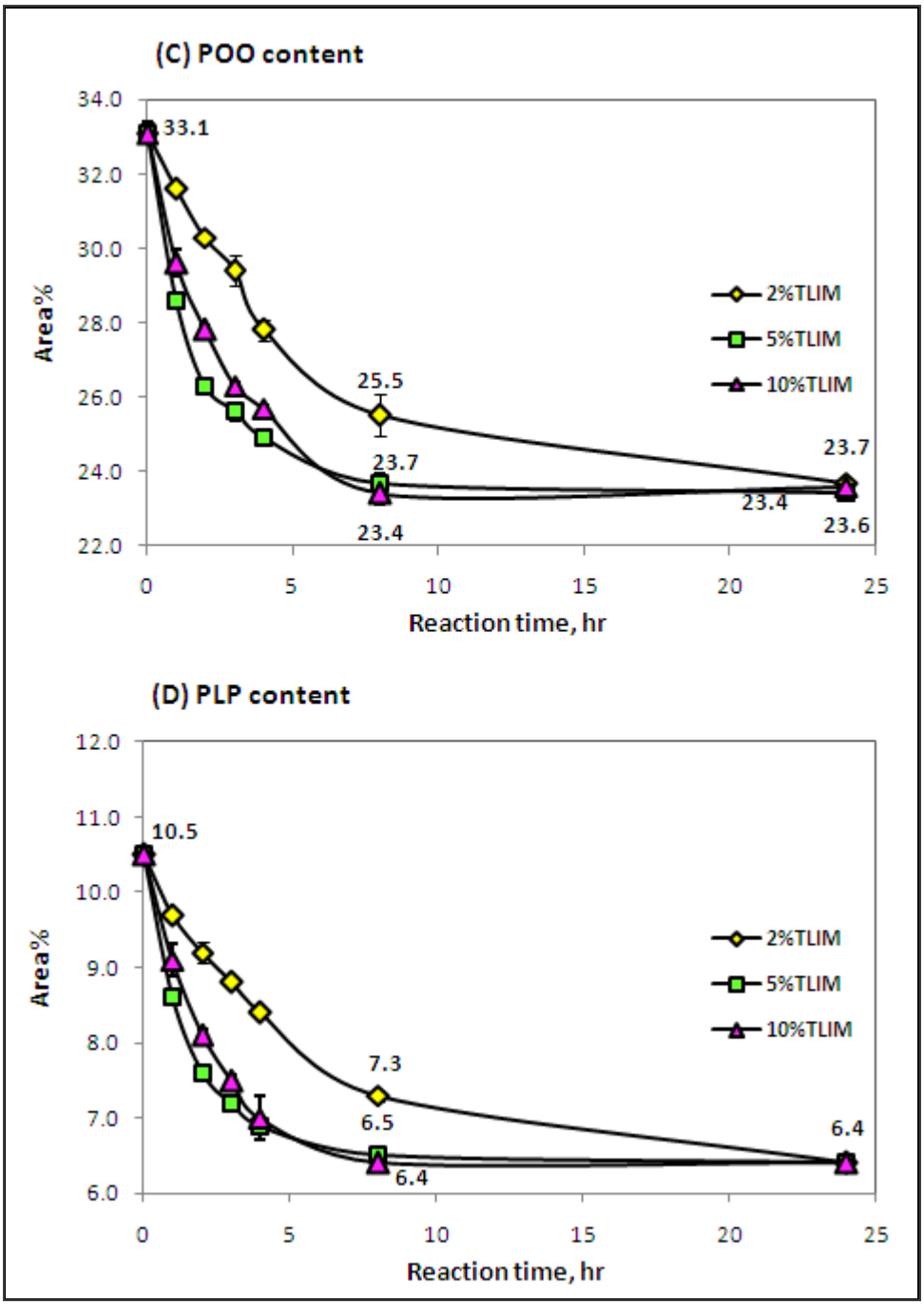


Figure 3.1, continued



PPP increased from 0.4% to about 4% for 2, 5 and 10 %w/w enzyme dosage after 24 hours of reaction, which means all reactions have achieved equilibrium at 24 hours reaction.

Figure 3.1 (A) and (B) showed that the rate of increasing of PPP and OOO for 5 %w/w and 10 %w/w were about the same. At the beginning of the reaction, the rate of TAGs conversion for 5 %w/w enzyme ratio was slightly higher than 10 %w/w reaction. At 8 hours reaction, the amount of PPP for 10 %w/w reaction had overtaken 5 %w/w reaction and achieved optimum PPP which was 4.4%.

The formation of trisaturated TAGs during 1,3-specific enzymatic interesterification was because of the presence of a small amount of TAGs with palmitic acid at the *sn*-2 position, for example, PPO, OPO, PPL, OPL, and so on (Tan, 1979; Gee, 2007). These TAGs were thus used as backbone for production of trisaturated TAGs such as PPP and PPS.

Figure 3.2 showed the SFC of the lab-scale batch TLIM interesterified products at ambient temperature (25 °C). This analysis could be a quick indicator to study the reaction as it could reflect the amount of trisaturated TAGs such as PPP and PPS produced in the reaction. This had been proven as the SFC was increased with increased of trisaturated TAGs, such as PPP (Figure 3.1 (A)). The higher formation of OOO TAG appears not sufficient to suppress the effect of the higher melting PPP. The highest SFC achieved was 12% with 10 %w/w enzyme dosage, and slightly lower SFC was observed for 5 %w/w and 2 %w/w reactions. The formation of new TAGs such as PPP and PPS which has lower solubility in the liquid phase, would improve its ability to produce crystals and thus the higher SFC observed (Gunstone, 2001b).

Figure 3.2
SFC (%) of the interesterified products of 2, 5, and 10%w/w Lipozyme® TL IM at 25 °C

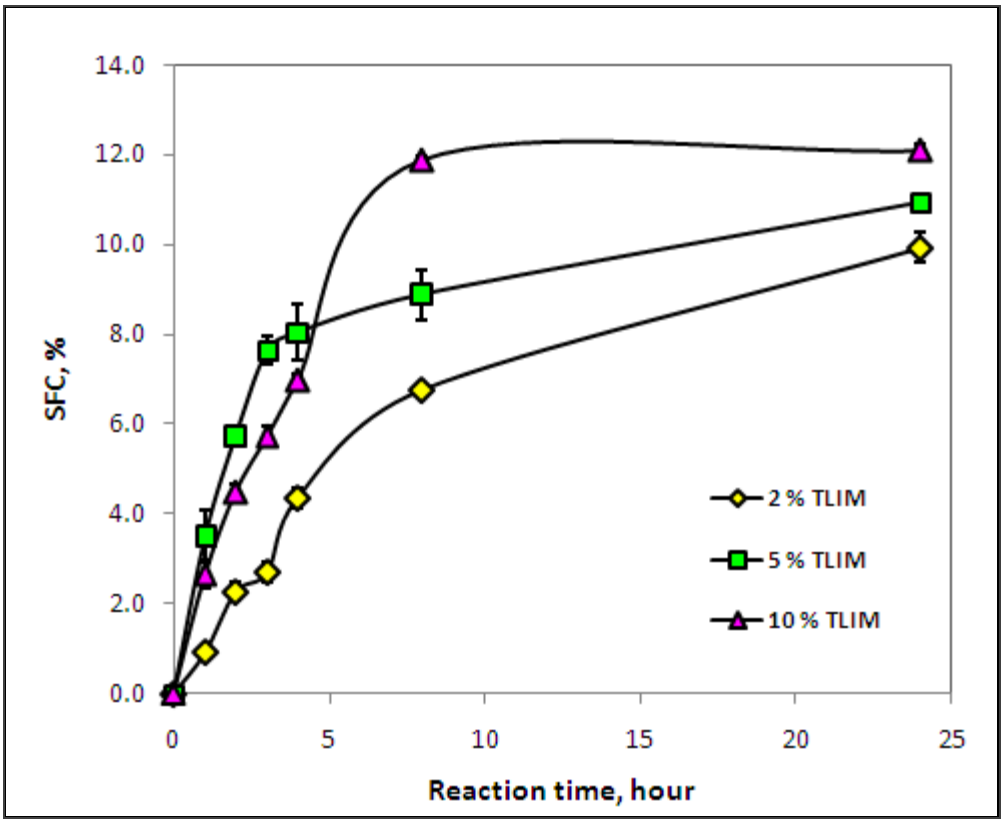


Figure 3.1 (B) showed important information on investigating the reaction rate and the equilibrium stage of the batch TLIM reaction. OOO content was increased to 10.2% concentration for both 5 and 10%w/w reactions. Both reactions reached equilibrium OOO content at 8 hours of reaction. The same phenomenon was observed in Figure 3.1 (C) and (D), in which POO and PLP reduced at the almost same rate and achieved equilibrium composition at 8 hours of reaction. In comparison, the rate of TAGs conversion for 2 %w/w was relatively much slower compared to both of the higher dosage reactions.

For industrial processes, cost of production, yield and quality of the products are the important parameters for profit optimization (Xu, 2003). In this lab-scale batch TLIM study,

it was clear that 5 %w/w was the optimum enzyme dosage, as it promised adequately high conversion rate which was comparable to 10 %w/w enzyme dosage. The reaction time required for this reaction was 8 hours. Although 2 %w/w is cheaper in term of the enzyme cost, much longer time (3 fold) was required for it to achieve equilibrium. This would increase the production cost as long reaction time will lead to higher energy consumption plus more manpower needed for conducting the reaction.

3.1.1.2 Equilibrium Stage for Batch RMIM

Figure 3.3 showed the changes of some TAG species in palm olein IV 62 over 24 hours of reaction with Lipozyme® RM IM in lab-scale batch reactor. Similar to lab-scale batch TLIM reactions, the lab-scale batch RMIM reaction also resulted in an increase of trisaturated TAGs (PPP and PPS) and triunsaturated TAGs (OLL, OLO and OOO), and a reduction of diunsaturated TAGs (PLL, PLO, POO and SOO) and disaturated TAGs (PLP). Some TAG species remain unchanged, including MLP, POP, POS and SOS. The data for TAG composition were illustrated in Appendix F-H.

The rate of TAGs conversion was higher for higher enzyme dosage reactions (Figure 3.3). Unlike the lab-scale batch TLIM reactions, different dosage of enzymes for the lab-scale batch RMIM reactions gave significantly different TAG composition at the equilibrium stage. The changes in TAG composition were increased sequentially with high enzyme dosage. The highest changes achieved in 10 %w/w, followed by 5 %w/w and 2 %w/w reactions. Figure 3.3 showed that PPP, OOO, POO, and PLP for 2 and 5 %w/w Lipozyme®

RM IM were about the same at 24 hours of reaction, indicating that the percentage of conversion for 2 %w/w reaction was comparable to 5 %w/w reaction.

Figure 3.3
Batch RMIM: changes of TAG composition of palm olein IV 62 during 24 hours reaction; (A) PPP content, (B) OOO content, (C) POO content and (D) PLP content

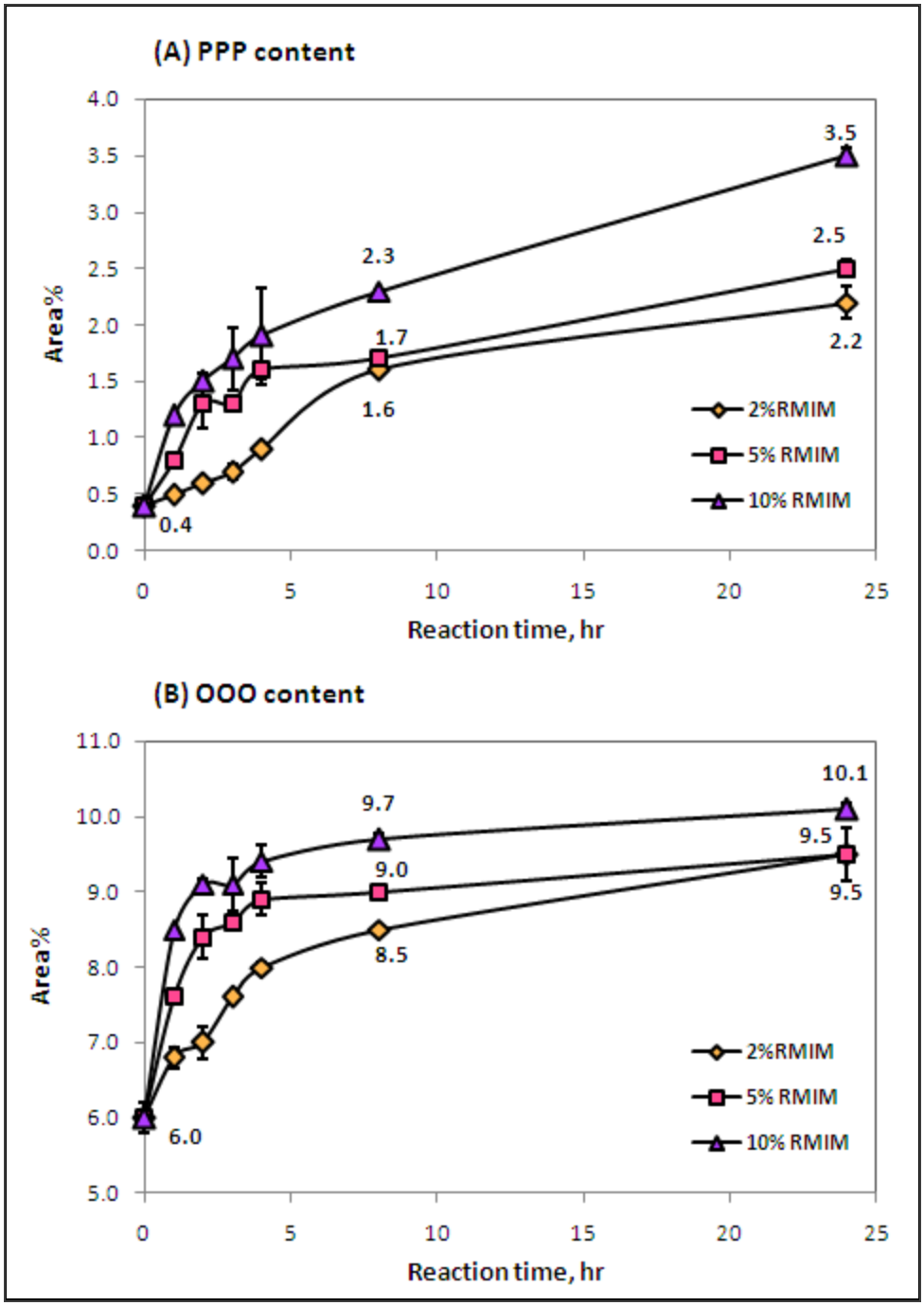


Figure 3.3, continued

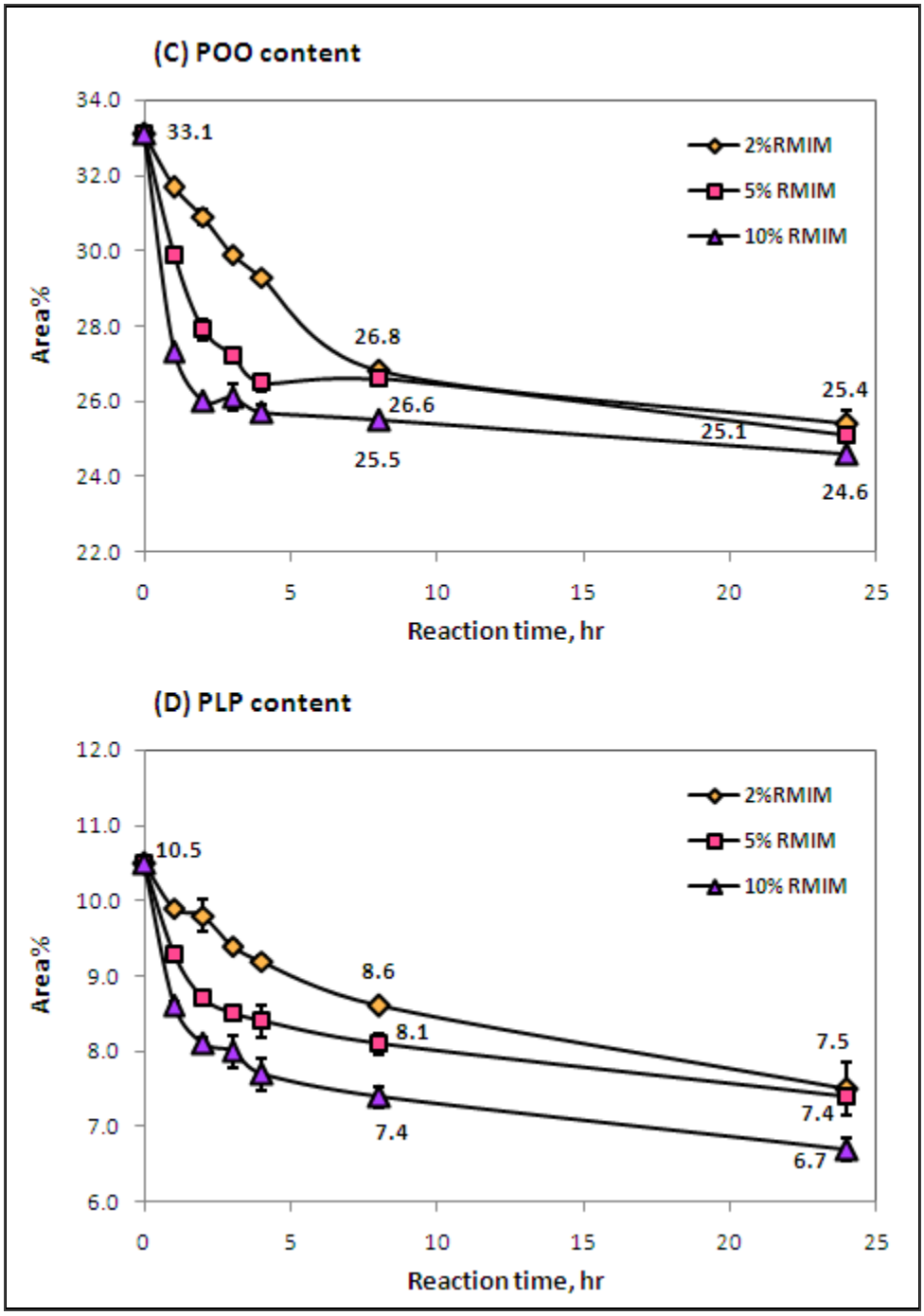
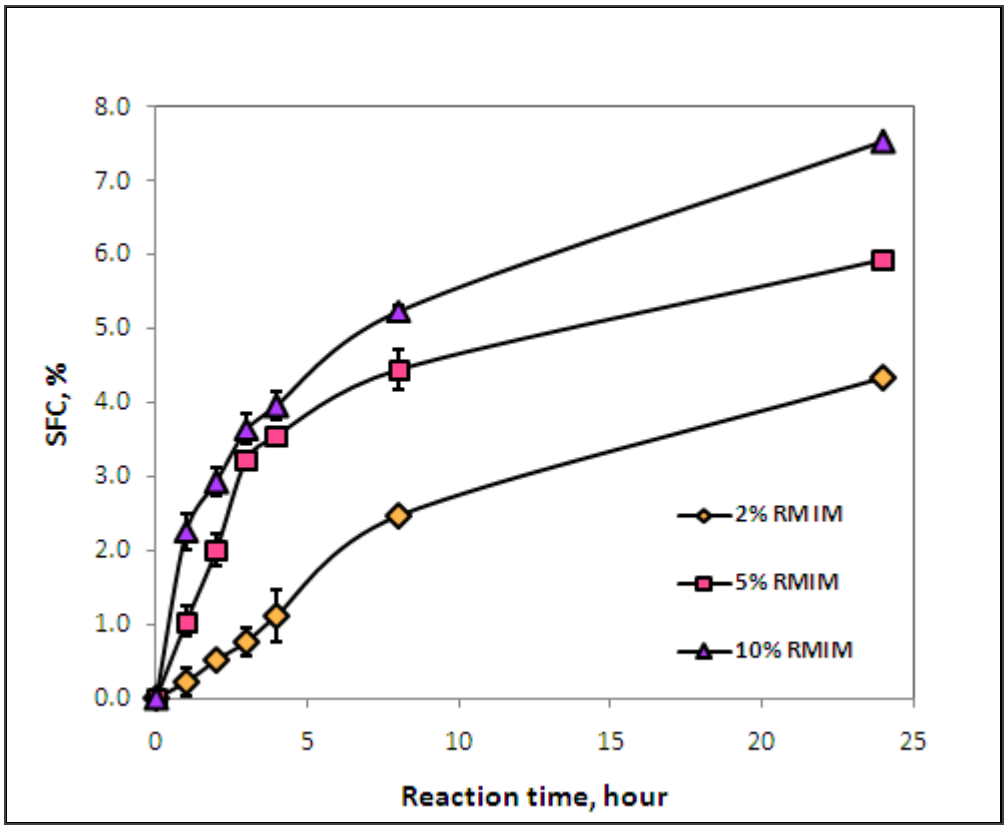


Figure 3.4 showed the SFC of the interesterified products for 2, 5, and 10 %w/w Lipozyme® RM IM reactions at ambient temperatures (25 °C). SFC had increased over time of reactions, as more trisaturated TAGs were produced in the reaction. The trend of SFC was proportional to the formation of trisaturated TAGs. This figure showed that 10 %w/w achieved the highest SFC (about 7.5%) due to production of the greatest amount of trisaturated TAGs (3.5% PPP) in the reaction, compared to the lower enzyme dosage reactions (Figure 3.3 (A)).

Figure 3.4
SFC (%) of the interesterified products of 2, 5, and 10%w/w Lipozyme® RM IM at 25 °C



As mentioned before, cost of production is also a very important criterion for profit optimization. The cost of Lipozyme® RM IM was at least 4 times more expensive than Lipozyme® TL IM. Although 10 %w/w enzyme dosage gave the highest TAGs conversion and adequately high reaction rate, lower enzyme dosage was preferred for this reaction. In terms of the TAGs conversion, there was no significant difference in TAG composition for interesterified products of 2 and 5 %w/w Lipozyme® RM IM. Hence, 2 %w/w was chosen as the enzyme dosage to be used in the 100 kg scale-up enzymatic interesterification process. The reaction had to be carried out for 24 hours in order to maximize the TAGs conversion.

3.1.2 Characterization of the Pilot-scale Interesterification Products

The main objective of conducting pilot-scale interesterifications was to produce sufficient amount of products for dry fractionation. Three pilot-scale (100 kg) enzymatic interesterification processes were carried out. For pilot-scale batch interesterification, the reactions were conducted according to the optimum enzyme dosage and reaction time obtained in the lab-scale experiments, which was 5 %w/w for Lipozyme® TL IM and 2 %w/w for Lipozyme® RM IM. An additional packed-bed reaction was conducted by using the 10 kg packed-bed reactor provided by Novozyme Co. The packed-bed reactor was designed specifically for Lipozyme® TL IM with specific pore size. Thus, this reactor cannot be used for Lipozyme® RM IM due to its smaller particle size. The flow rate used in the packed-bed reaction was based on recommendation of Novozyme Co., in which the flow rate used can ensure a complete interesterification reaction (Novozyme, 2004).

The physicochemical characteristics of the pilot-scale interesterified products were used to verify the suitable temperature range to be used in dry fractionation in order to separate the saturation components.

3.1.2.1 Chemical Properties of the Pilot-scale Interesterified Products

Triacylglycerols Composition

TAG composition of the pilot-scale interesterified products was shown in Table 3.1. Pilot-scale interesterification increased the content of FFA & MAG, DAG and some TAG species. In overall, the total triunsaturated and trisaturated TAGs were increased, while total diunsaturated and disaturated TAGs were reduced after the enzymatic interesterification reaction.

Table 3.1
Chemical properties: acylglycerols composition of the feed olein (POo IV62), and the pilot-scale enzymatic interesterified products

Triacylglycerols	POo IV 62	Packed-bed TL IM	Batch TL IM	Batch RM IM
FFA & MAG	**0.2±0.0**	**2.6±0.1**	**3.0±0.1**	**1.0±0.2**
DAG	**6.2±0.0**	**5.9±0.0**	**11.2±0.1**	**8.0±0.0**
Total triunsaturated	**8.9±0.2**	**18.4±0.0**	**18.2±0.0**	**14.9±0.0**
OLL	0.6±0.0	2.3±0.1	2.3±0.0	1.6±0.0
OLO	2.6±0.0	7.5±0.1	7.1±0.1	5.6±0.0
OOO	5.7±0.1	8.6±0.0	8.8±0.1	7.7±0.0
Total diunsaturated	**53.7±0.2**	**40.0±0.1**	**38.2±0.1**	**43.9±0.0**
PLL	3.3±0.1	2.7±0.0	2.8±0.1	3.0±0.1
PLO	15.0±0.0	13.3±0.0	12.8±0.0	14.1±0.0
POO	31.9±0.2	21.8±0.1	20.5±0.0	24.3±0.1
SOO	3.5±0.1	2.2±0.0	2.1±0.0	2.6±0.0
Total disaturated	**30.9±0.0**	**27.8±0.1**	**25.1±0.1**	**30.4±0.1**
MLP	0.4±0.1	0.7±0.1	0.8±0.1	0.8±0.1
PLP	9.5±0.0	6.1±0.1	5.6±0.1	7.7±0.0
POP	17.4±0.0	17.7±0.1	15.3±0.0	17.9±0.0
POS	3.2±0.1	3.1±0.1	3.0±0.0	3.6±0.1
SOS	0.4±0.0	0.4±0.0	0.4±0.0	0.5±0.0
Total trisaturated	**0.1±0.0**	**5.5±0.0**	**4.5±0.0**	**1.9±0.0**
PPP	0.1±0.0	4.3±0.0	3.5±0.0	1.5±0.0
PPS	0.0±0.0	1.2±0.0	1.0±0.0	0.4±0.0
Total TAG	**93.6±0.0**	**91.7±0.1**	**86.0±0.2**	**91.1±0.2**

Initially, palm olein contained 8.9% of triunsaturated TAGs with 5.7% of OOO content. After the reaction, the amount of OOO increased from 5.7% to 8.6% and 8.8% for reaction using Lipozyme® TL IM in packed-bed and batch reactor, respectively. In comparison, the total triunsaturated TAGs conversion for batch RMIM was much lower, which was at least 3% lower than those reactions using Lipozyme® TL IM.

Palm olein of IV 62 only contained trace amount of trisaturated TAGs. After the enzymatic interesterification, packed-bed TLIM produced the greatest amount of total trisaturated TAGs compared to other reactions, which was 5.5% with 4.3% of PPP and 1.2% of PPS. Slightly lower amount of total trisaturated TAGs were formed in batch TLIM reaction (4.5%). Batch RMIM produced least amount of total trisaturated TAGs (1.9%) that was less than half of that produced in packed-bed TLIM and batch TLIM reactions.

Total diunsaturated TAGs of palm olein IV 62 was reduced 9.8%, 13.7% and 15.5% in pilot-scale interesterification of batch RMIM, packed-bed TLIM and batch TLIM, respectively. The most significant reduction in diunsaturated TAGs was POO, where 7.6%, 10.1% and 11.4% reduction of POO was observed in batch RMIM, packed-bed TLIM, and batch TLIM, respectively. For disaturated TAGs, only PLP dropped in significant amount, the rest were maintained at about the same level as the feed olein.

In overall, the TAGs which had reduced significantly in the reaction were POO, PLP, POP, and SOS. These TAGs had undergone interesterification in the presence of biocatalyst to form new TAGs. The isomers of these TAGs such as PPO, OPO, PPL and OPL were also presence in a very small proportion (Tan, 1979; Gee, 2007). The glycerol backbones of these TAGs were used for formation of new TAGs such as OOO, OLO, PPP, PPS etc.

Generally, polypeptide chain in the enzymes is folded in a similar way to have active sites with a specific behavior, including fatty acids selectivity, and positional specificity (Willis and Marangoni, 2002). For different enzymes species, the polypeptide chain is folded in different way to have different active sites that responsible for the specificity and catalytic

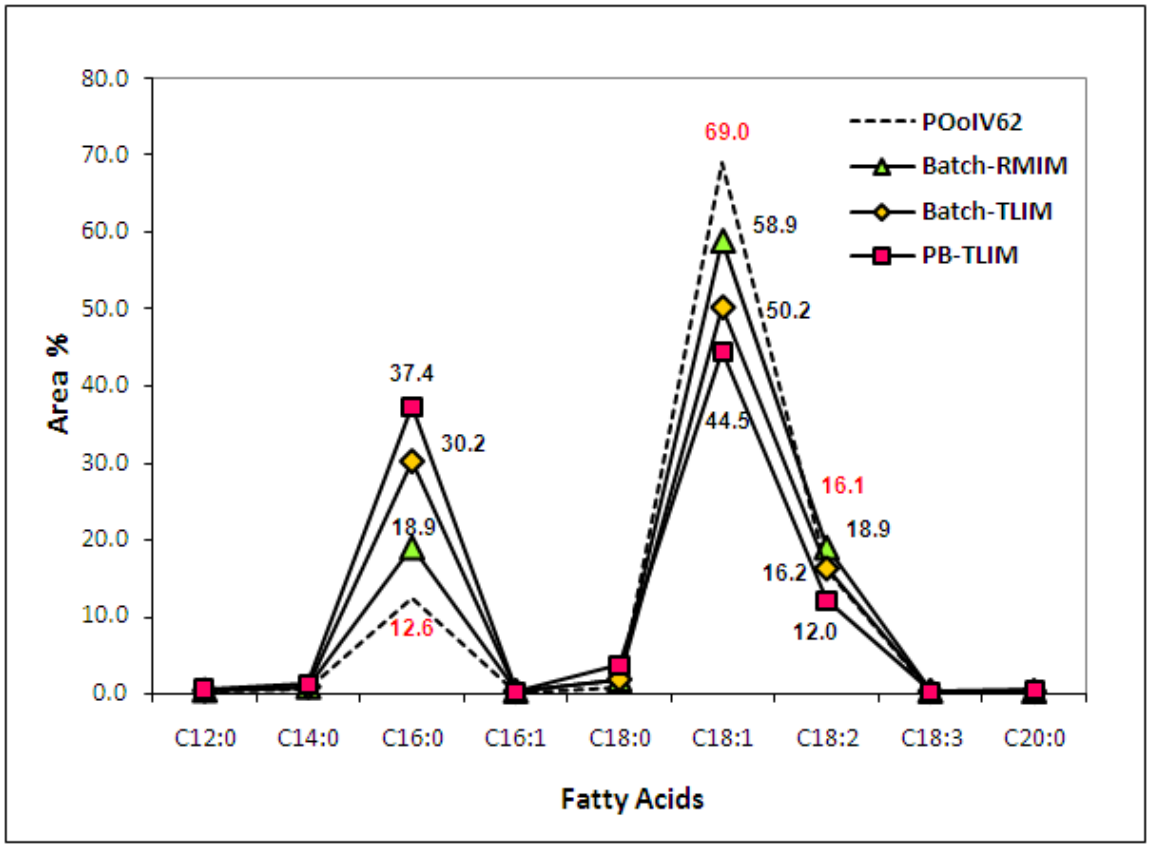
activity in the reaction (Willis and Marangoni, 2002). Therefore, the interesterified products from interesterification using Lipozyme® TL IM in packed-bed and batch reactor had similar TAG composition, which was very different from the interesterified product using Lipozyme® RMIM. The slight different of TAG composition between packed-bed and batch TLIM may be due to the difference in the reactor type, reaction condition and reaction system (Quilan and Moore, 1993).

Positional Analysis

Both Lipozyme® TL IM (*Thermomyces lanuginose*) and Lipozyme® RM IM (*Rhizomucor miehei*) are known as 1,3-specific lipases in which these lipases may retain the *sn*-2 position fatty acids in the glycerol backbone (Yang and Xu, 2001). The specificity performance of enzyme is very dependent on the reaction system and conditions. In other words, they can only perform their specificity under particular conditions (Natália et al., 2006; Criado et al., 2007). Therefore, positional analysis was conducted to study the specificity of the enzymes in catalyzing the interesterification reactions.

Figure 3.5 showed the *sn*-2 position fatty acids of the feed oil and the interesterified products. Theoretically, the *sn*-2 position fatty acids should remain unchanged after 1,3-specific interesterification. However, some positional randomization of fatty acid residues in TAGs had occurred during these three enzymatic interesterifications as indicated in Figure 3.5. In other words, the reaction had changed the fatty acid at the *sn*-2 position of the TAGs.

Figure 3.5
Chemical properties: *sn*-2 positional fatty acids composition feed olein and the pilot-scale batch and packed-bed enzymatic interesterified products



The most obvious changes were the percentage of oleic acid (C18:1) and palmitic acid (C16:0) at the 2-position; percentage of C18:1 was decreased while percentage of C16:0 was increased significantly in the pilot-scale interesterification. These changes may be due to acyl migration. The factors of acyl migration were reaction time, reaction temperature, presence of water, reactor type and reaction system (Xu et al., 2001). In comparison, batch RMIM was more specific than packed-bed and batch TLIM, where we can see that its *sn*-2 position fatty acids composition was closer to the feed palm olein. Yet, TAGs conversion of batch RMIM was lesser compared to the interesterification using Lipozyme® TL IM.

It can be concluded that under these reaction conditions, Lipozyme® TL IM in packed-bed and batch reaction did not permit a perfectly 1,3-specific interesterification which will retain the *sn*-2 position fatty acids. Besides, the *sn*-1,3 specificity performance of Lipozyme® TL IM were different in two different reactor; packed-bed and batch reactor, although the reactions were conducted at same temperature (65 °C). Batch TLIM was more specific than packed-bed TLIM. In this case, the difference in the specificity might be due to different in the reactor type or different in moisture content in the reaction system.

Although the enzymatic interesterifications did not perform perfect specificity in retaining the fatty acids at the sn-2 position, the reactions were much better than chemical interesterification that involves fully randomization of fatty acids. Moreover, enzymatic interesterification is a greener technology without involving the use of any chemicals. To date, the cost of enzymatic interesterification using packed-bed Lipozyme® TL IM is apparently comparable with chemical interesterification (Novozyme, 2004).

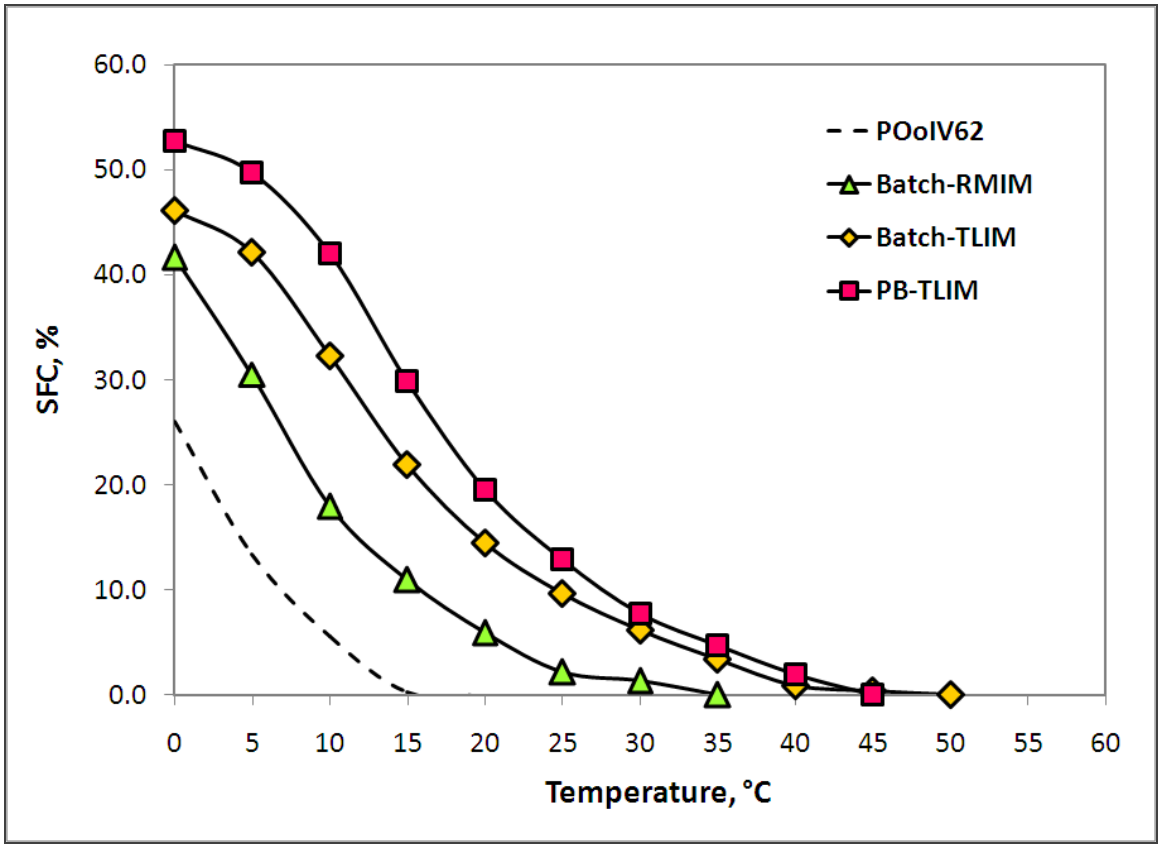
3.1.2.2 Physical Properties of the Pilot-scale Interesterified Products

Solid Fat Content (SFC)

The SFC of the palm olein of IV 62 and the pilot-scale interesterified products were measured as a function of temperature as illustrated in Figure 3.6. From the results, it was found that the interesterified products had much higher SFC than the feed palm olein. At 0 °C, the interesterified product from packed-bed TLIM contained about 53% of solid fat which was doubled of that in the feed olein (about 26%). The interesterified products from batch TLIM and batch RMIM contained similar solid fat which was about 46% and 42%,

respectively at 0 °C. At 5 °C, SFC of batch RMIM dropped drastically to about 30%, while batch TLIM still retained at about 41% of SFC.

Figure 3.6
Physical properties: SFC of the feed palm olein and the pilot-scale interesterified products



Apparently, SFC is very dependent on the TAG composition of the oil. The SFC of the feed olein was the lowest as it did not contain any trisaturated TAGs. The solid fats/crystals appeared in the feed olein was mainly contributed by disaturated TAGs which consists of mainly POP.

The interesterified products from packed-bed and batch TLIM contained higher percentage of trisaturated TAGs than batch RMIM as both of the products of Lipozyme® TL IM contained higher trisaturated TAGs (5.5% and 4.5%, respectively) as compared to batch

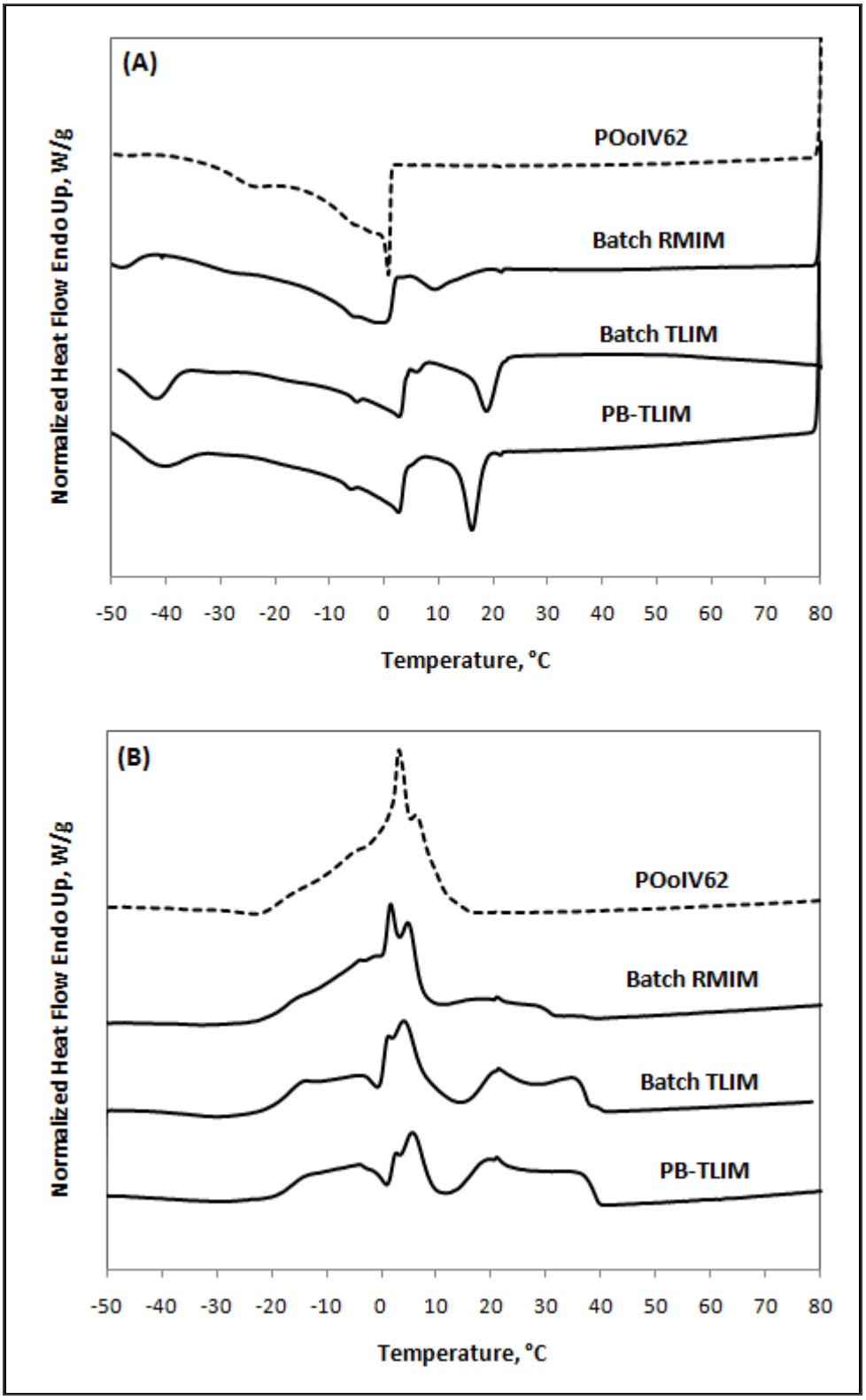
RMIM (1.9%). The presence of trisaturated TAGs which was high in melting points will promote crystallization, thus increased the SFC (Gunstone, 2001b).

Thermal Properties by Differential Scanning Calorimeter

DSC curves reveal the transition temperatures and heat of fusion or crystallization (Zaliha et al., 2004). Figure 3.7 showed the thermal properties of the pilot-scale interesterified products as compared to the feed palm olein. From the melting thermograms (Figure 3.7 B), palm olein showed a sharp endothermic peak at about 3 °C, while the interesterified products displayed broad peaks and shoulders at high temperature ranging from -20 °C to 40 °C. Both interesterified products from batch TLIM and packed-bed TLIM displayed a broad shoulder at high temperature that ended at about 40 °C, whereas the broader shoulder in melting curve of batch RMIM ended at about 30 °C.

In general, the melting and crystallization properties of the oil are dependent on the chemical structure and polymorphic behavior of TAG mixture (Kellens et al., 2007). From the crystallization thermograms (Figure 3.7 A), it was observed that the reaction products started to crystallize at higher temperature as compared to the feed olein. A sharp peak was observed in the crystallization thermogram of palm olein at 0 °C, meaning that the TAGs in the feed olein was crystallized mostly at 0 °C. For the interesterified product from batch RMIM, two broad exothermic peaks were observed at about 0 °C and 8 °C, indicating some conversion of the low melting TAGs into new TAGs with higher melting properties.

Figure 3.7
Thermal properties: (A) crystallization, and (B) melting properties of the feed palm olein and the pilot-scale interesterified products



The peak value of crystallization thermogram of the interesterified products from batch TLIM and packed-bed TLIM was about 20 °C and 17 °C, respectively. These sharp exothermic peaks were not present in the thermograms of feed palm olein and batch RMIM products. This indicating the presence of significant amount of trisaturated TAGs in both batch TLIM and packed-bed TLIM products, which was absent in the feed olein, and only present in small quantity in batch RMIM product.

The melting thermogram of the feed olein did not show broad shoulders at high temperature, indicating that all TAGs in palm olein had melted at lower temperature. The thermograms of batch TLIM and packed-bed TLIM reactions indicated that three major components were present in the oil, and could be categorized by the melting points ranging from -20 to 0 °C, 0 to 10 °C, and 10 to 40 °C. For melting thermogram of batch RMIM, the curve was generally similar to the feed olein at low temperature (from -20 to 10 °C), the broader shoulder observed at 10 to 30 °C indicating the production of a smaller quantity of high melting TAGs (mainly trisaturated TAGs) in the reaction, as compared to batch TLIM and packed-bed TLIM.

Cloud Point and Slip Melting Point

The interesterification reactions produced harder fats without altering its IV. This had been further confirmed in the cloud point (CP) and slip melting point (SMP) determination. The pilot-scale interesterification reactions had increased the CP and SMP of the palm olein. CP of palm olein of IV 62 was increased drastically from 3.7 °C to 23.2 °C in packed-bed TLIM reaction, whereas SMP increased from 17.9 °C to 39.4 °C (Table 3.2). In comparison,

the product of batch TLIM had slightly lower SMP (35.7 °C), followed by batch RMIM product (29.9).

Table 3.2
Cloud point and slip melting point of the pilot-scale intesterified products and the feed olein

Sample	Cloud point, °C	Slip melting point, °C
Feed POo IV 62	3.7 ± 0.3	17.9 ± 0.2
Batch RMIM	18.7 ± 0.4	29.9 ± 0.1
Batch TL IM	17.5 ±0.2	35.7 ± 0.2
Packed-bed TLIM	23.2 ± 0.2	39.4 ± 0.2

The physical properties of the modified oils were important for determination of suitable temperatures for fractionation. In the crystallization diagrams (Figure 3.7 A), it was observed that the high melting components in the batch TLIM and packed-bed TLIM products were quiet significant, and could be easily separated at temperature in between 10 °C to 20 °C. Hence, 9, 12, 15, 18, and 21 °C were selected for lab-scale dry fractionation of both batch TLIM and packed-bed TLIM.

The crystallization peak in batch RMIM thermogram at about 5 °C indicated that lower temperature might be required for the removal of the high melting components. As crystallization at too low temperature is not sensible due to the high processing cost, the lowest temperature used was 8 °C. Therefore, 8, 10, 12, 15 and 18 °C were selected for fractionation of batch RMIM product.

3.2 Fractionation

The objective of conducting fractionation of the interesterified products was to remove some of the saturated TAGs that were formed during the interesterification reaction, in

order to lower the saturation content in the oil. In the lab-scale study, the temperatures used to fractionate batch TLIM and packed-bed TLIM products were 9, 12, 15, 18, and 21 °C, while 8, 10, 12, 15 and 18 °C were used for batch RMIM product.

Lab-scale fractionations were conducted to study the effectiveness of fractionation in removing saturated TAGs, under various cooling programs. The filtration step in the lab-scale fractionation involved the use of vacuum filter, without pressing of the stearin cake. Therefore, pilot-scale fractionations were conducted based on the optimum temperature obtained in the lab-scale fractionation, with the objective of determining the exact olein yield which could not be obtained in the lab-scale fractionation.

3.2.1 Fractionation of Batch RMIM

Table 3.3 showed the yield and acyglycerols composition of the lab-scale and pilot-scale olein fractions. The olein yields achieved in all the fractionation reactions were very high, ranging from 71.2 to 84.2% due to the low concentration of saturated components in the interesterified product. The TAGs and fatty acid composition of the corresponding stearin fractions were showed in Appendix I and J, respectively. The results showed that the trends of the stearin composition were not as expected due to the inaccuracy in vacuum filtration.

In comparison to the feed olein, the olein fractions contained higher amount of total triunsaturated TAGs, in which OLL and OLO involved increment of more than one fold. OOO content increased about 3% to the range of 8.2-8.7% after the modification processes. Similar to the feed olein, the olein from fractionation at 8 °C still retained some trace

amount of trisaturated TAGs. Initially, the interesterified palm olein from batch RMIM contained 1.9% of trisaturated TAGs (Table 3.1). The lab-scale fractionation had removed majority of the trisaturated TAGs. Fractionation at higher temperatures; 18 ℃ retained the trisaturated TAGs at 0.4%, indicating that 18 ℃ was not sufficiently low enough to remove all trisaturated components.

Table 3.3
Yield and acylglycerols composition of the olein fractions from lab-scale and pilot-scale fractionation of batch RMIM [PL8=pilot-scale olein at 8°C; PS8=pilot-scale stearin at 8°C]

Yield/ Acylglycerols	POoIV62	Oln8°C	Oln10°C	Oln12°C	Oln15°C	Oln18°C	PL 8	PS 8
Yield (%)	-	71.2±0.1	71.3±0.1	76.8±1.0	78.7±2.1	84.2±0.7	74.8±0.3	25.2±0.3
Total DAG	**6.2±0.0**	**7.4±0.8**	**8.0±0.4**	**8.0±0.2**	**7.8±0.2**	**7.9±0.4**	**7.6±0.0**	**10.3±0.2**
OLL	0.6±0.0	1.5±0.1	1.4±0.0	1.4±0.0	1.4±0.0	1.4±0.1	1.5±0.0	0.8±0.0
OLO	2.6±0.0	5.9±0.0	6.0±0.1	5.8±0.1	5.8±0.1	5.6±0.0	6.0±0.0	3.6±0.0
OOO	5.7±0.1	8.6±0.1	8.7±0.0	8.5±0.1	8.3±0.0	8.2±0.0	8.5±0.1	5.7±0.0
Total triunsaturated	**8.9±0.2**	**15.9±0.1**	**16.1±0.1**	**15.7±0.3**	**15.5±0.1**	**15.2±0.1**	**16.0±0.2**	**10.1±0.1**
PLL	3.3±0.1	3.4±0.1	3.3±0.1	3.2±0.0	3.3±0.1	3.2±0.2	3.5±0.0	2.0±0.0
PLO	15.0±0.0	15.8±0.2	15.8±0.1	15.6±0.4	15.2±0.1	14.8±0.1	15.7±0.1	9.1±0.0
POO	31.9±0.2	27.0±0.1	27.0±0.4	26.6±0.6	25.8±0.1	25.2±0.1	26.3±0.1	17.5±0.0
SOO	3.5±0.1	2.9±0.1	3.0±0.0	2.9±0.0	2.8±0.0	2.8±0.1	2.8±0.0	1.7±0.0
Total diunsaturated	**53.7±0.2**	**48.9±0.3**	**49.1±0.4**	**48.3±0.9**	**47.0±0.1**	**46.0±0.4**	**48.2±0.3**	**30.3±0.0**
MLP	0.4±0.1	0.6±0.1	0.6±0.1	0.6±0.1	0.8±0.1	0.8±0.1	0.8±0.1	0.5±0.0
PLP	9.5±0.0	7.3±0.3	7.3±0.2	7.4±0.2	7.7±0.0	7.7±0.1	7.6±0.1	7.8±0.0
POP	17.4±0.0	15.7±0.1	15.0±0.4	15.5±0.4	16.6±0.1	17.1±0.0	15.4±0.3	26.0±0.1
POS	3.2±0.1	2.9±0.1	2.8±0.0	3.0±0.1	3.2±0.0	3.3±0.1	2.9±0.1	5.2±0.0
SOS	0.4±0.0	0.4±0.0	0.3±0.0	0.4±0.0	0.5±0.1	0.5±0.1	0.4±0.1	0.7±0.0
Total disaturated	**30.9±0.0**	**26.8±0.4**	**25.9±0.2**	**26.9±0.9**	**28.7±0.1**	**29.4±0.1**	**27.1±0.4**	**40.3±0.1**
PPP	0.1±0.0	0.1±0.0	0.2±0.2	0.2±0.1	0.2±0.1	0.4±0.1	0.0±0.0	6.0±0.1
PPS	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	01±0.0	0.0±0.0	1.5±0.1
Total trisaturated	**0.1±0.0**	**0.1±0.0**	**0.2±0.2**	**0.2±0.1**	**0.2±0.1**	**0.4±0.1**	**0.0±0.0**	**7.5±0.2**

For diunsaturated TAGs, PLL and PLO content of the olein fractions were similar to that in the feed olein. The content of POO was consequently reduced with increasing fractionation temperature, POO content was slightly reduced from 31.9% to 27.0% in both olein 8 °C and olein 10 °C. Whereas for disaturated TAGs, PLP and POP content were reduced, while the other disaturated TAGs such as MLP, POS and SOS remained unchanged.

The fatty acids composition and IV of the olein fractions and the feed oil were shown in Table 3.4. The unsaturation content of the olein fractions had been improved as compared to the starting material (palm olein of IV 62). As fractionation temperature reduced, greater amount of high melting TAGs were removed, which resulted in reduction of the SFA content and increment of the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Table 3.4

Fatty acid composition and iodine value (IV) of the feed olein, the olein fractions from lab-scale and pilot-scale fractionation of batch RMIM

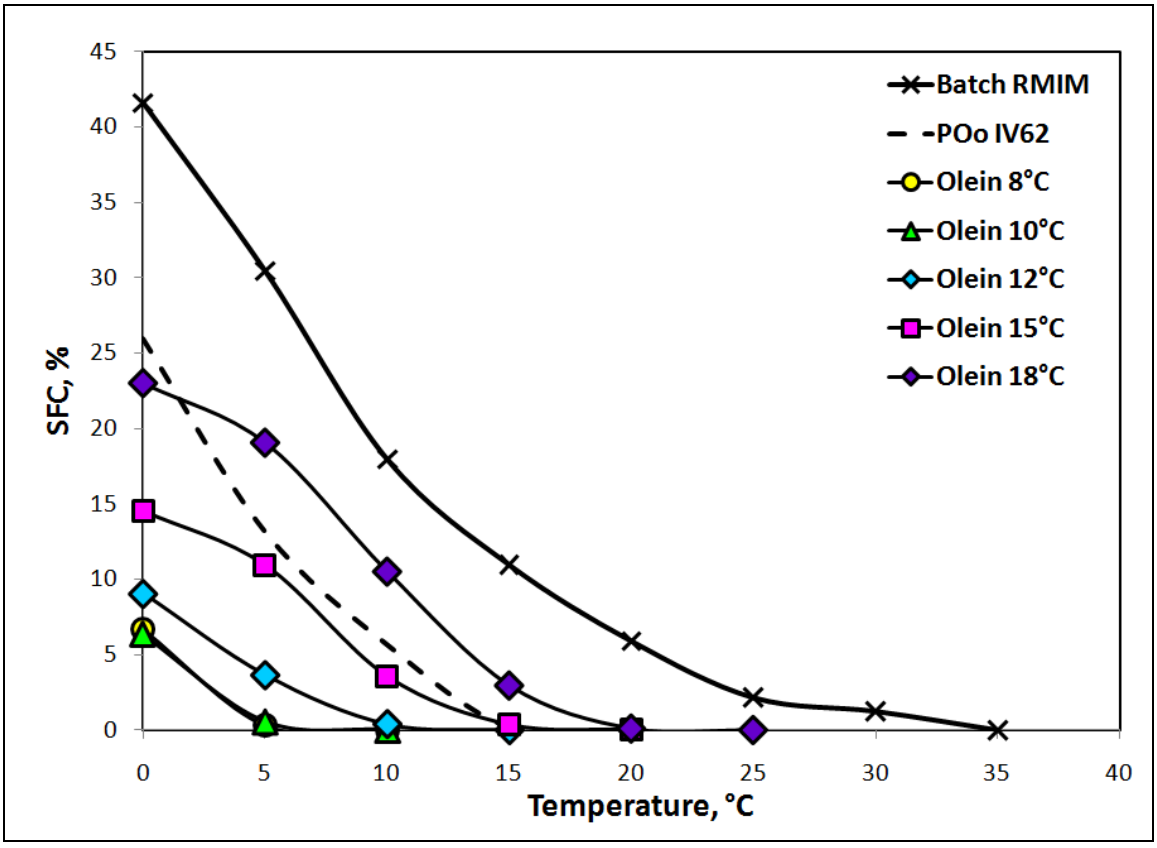
Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
POo IV 62	0.3±0.0	1.1±0.0	36.3±0.1	3.6±0.0	0.4±0.0	**41.7±0.1**	0.2±0.0	44.9±0.1	**45.1±0.1**	13.0±0.0	0.2±0.0	**13.2±0.0**	62.0±0.0
Olein 8 °C	0.3±0.0	1.0±0.0	30.9±0.1	3.4±0.0	0.4±0.0	**36.0±0.1**	0.3±0.1	49.3±0.0	**49.5±0.1**	14.3±0.1	0.2±0.0	**14.5±0.1**	67.6±0.1
Olein 10 °C	0.3±0.0	1.1±0.0	30.7±0.2	3.4±0.1	0.4±0.0	**35.8±0.3**	0.3±0.1	49.5±0.2	**49.7±0.3**	14.3±0.0	0.2±0.0	**14.5±0.0**	67.8±0.2
Olein 12 °C	0.3±0.0	1.0±0.0	31.6±0.4	3.4±0.0	0.4±0.0	**36.7±0.4**	0.3±0.1	48.8±0.4	**49.1±0.5**	14.0±0.0	0.2±0.0	**14.2±0.0**	66.8±0.4
Olein 15 °C	0.3±0.0	1.1±0.1	31.8±0.0	3.5±0.0	0.4±0.0	**37.1±0.1**	0.3±0.1	48.6±0.0	**48.9±0.0**	13.9±0.1	0.2±0.0	**14.1±0.1**	66.4±0.2
Olein 18 °C	0.3±0.0	1.0±0.0	33.6±0.0	3.6±0.0	0.4±0.0	**38.9±0.0**	0.2±0.0	47.3±0.1	**47.5±0.1**	13.5±0.1	0.2±0.0	**13.7±0.1**	64.5±0.1
PL 8	0.2±0.0	1.0±0.0	31.6±0.0	3.3±0.0	0.3±0.0	**36.5±0.0**	0.3±0.0	48.6±0.0	**48.9±0.0**	14.4±0.1	0.2±0.0	**14.6±0.1**	67.4±0.2
PS 8	0.2±0.0	1.2±0.0	47.2±0.0	4.9±0.0	0.4±0.0	**53.9±0.0**	0.2±0.1	36.0±0.0	**36.2±0.1**	9.5±0.2	0.1±0.0	**9.6±0.2**	47.6±0.3

However, the modification processes did not significantly reduce the SFA content in the feed oil. SFA only reduced about 6% with increasing of IV from 62.0 to about 68 at fractionation temperature of 8 and 10 °C. This was basically due to the formation of too low amount of trisaturated TAGs in the batch RMIM reaction that resulted in difficulty in the formation of hard crystals during crystallization.

Overall, the TAG composition and fatty acid composition of the pilot-scale olein was comparable to the lab-scale olein fractionated at the same temperature. Pilot-scale fractionation conducted at 8 °C has further increased the olein yield to 74.8% as compared to the lab-scale olein yield, which was 71.2% (Table 3.3). This is due to the application of high pressure in the membrane filter press which effectively squeezed out some entrapped olein from the stearin cake. However, some fine crystals may also squeezed out under high pressure during pressing of the stearin cake. Therefore, SFA was slightly increased in the pilot-scale fractionation as compared to the lab-scale (Table 3.4).

The SFC of the olein fractions from lab-scale and pilot-scale fractionation of batch RMIM interesterified product were compared to the feed palm olein as illustrated in Figure 3.8. In fact, SFC was always depended on the TAGs distribution in the oil. It was basically reliant on the intersolubility of the TAGs in the TAG mixture, depending on their chemical structures or their polymorphism form. Some TAGs will be very soluble when mixed; some will crystallize separately and being immisible in the solid state that giving rise to form an eutectic interactions (Gibon, 2006).

Figure 3.8
Comparison of the SFC of feed palm olein, the interesterified products of batch RMIM and the lab-scale olein fractions fractionated at 8, 10, 12, 15, and 18 °C

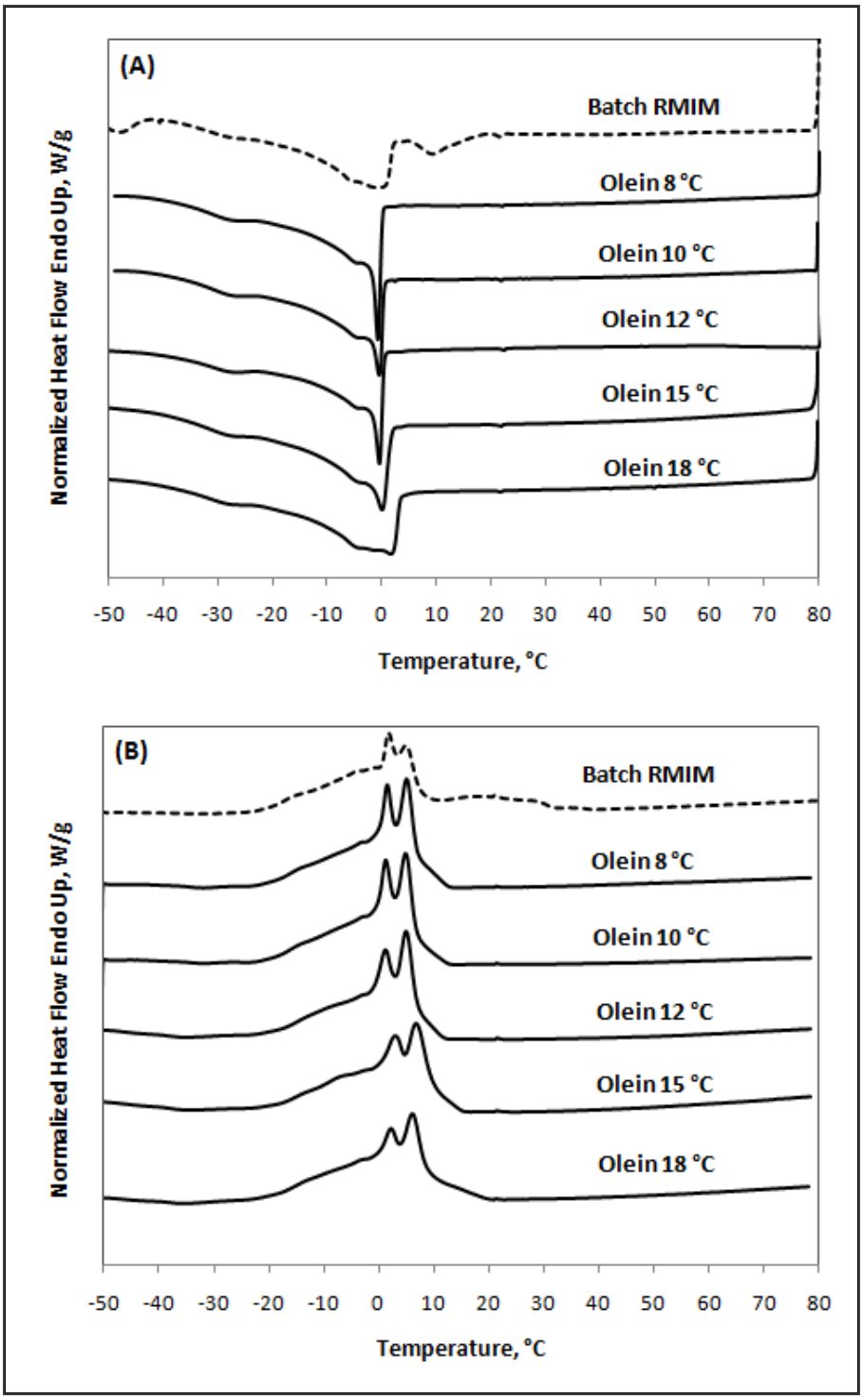


The olein products contained lower solid fat than the feed olein at temperature of 0 °C. At temperature above 2 °C, olein fractionated at 18 °C contained significantly higher amount of SFC than the feed olein. Although olein 18 °C contained higher triunsaturated TAGs with higher IV as compared to the feed olein, the diunsaturated content was significantly lower, and thus resulted in greater SFC at temperature above 2 °C (Table 3.3). When fractionation temperature was reduced, the TAG distributions in olein fractions gradually moved towards lower trisaturated and disaturated TAGs, with higher triunsaturated and diunsaturated TAGs. This had caused a significant reduction in SFC when lower temperature was applied.

Similar trends were observed in the crystallization and melting properties of the olein fractions (Figure 3.9). The oleins fractionated at lower temperature generally started to crystallize at lower temperature, and melt at lower temperature. For olein 15 ℃ and 18 ℃, the crystallization peaks and melting peaks were broader due to the TAG composition with wider range of melting points.

Sharper peaks in low temperature fractionated olein were due to TAG components with narrower crystallization and melting temperatures. The figure also shows visible removal of high melting components during the dry fractionation, whereby the high temperature peaks in both crystallization and melting thermograms of batch RMIM product had disappeared in all the olein fractions.

Figure 3.9
Comparison of (A) crystallization, and (B) melting properties of feed palm olein, and the lab-scale olein fractions from fractionation of batch RMIM at 8, 10, 12, 15, and 18 °C



3.2.2 Fractionation of Batch TLIM

Table 3.5 shows the yield and acylglycerols composition of lab-scale olein fractions, and pilot-scale fractionation products (PL9 and PS9). Olein yields from fractionation of batch TLIM were adequately lower as compared to batch RMIM. Lowering the fractionation temperature resulted in reduction of the olein yield due to the formation of greater amount of crystals under cooler crystallization conditions.

Table 3.5 showed that the oleins derived from dry fractionation of batch TLIM product contained significantly higher amount of total diacylglycerols compared to the feed olein. This was due to the formation of high quantity of partial glycerides during the batch interesterification reaction as shown in Table 3.1. The presence of DAG further complicates the crystallization and melting behavior, as DAG can significantly affect the physical properties of the oil, such as melting properties, polymorphism behavior, rate of crystallization, crystals size and habits (Siew, 2001).

When lower fractionation temperature was applied, the content of triunsaturated and diunsaturated TAGs gradually increased, with reduction in the disaturated TAGs content. At higher fractionation temperature, majority of trisaturated TAGs were removed together with minor amount of disaturated TAGs. For example, at fractionation temperature of 21 °C, 3.7% of trisaturated TAGs were removed with only 1% reduction in disaturated TAGs. Reducing the fractionation temperature has further removed the disaturated TAGs from 25.1% to 21.1% at 9 °C. The removal of disaturated and trisaturated TAGs had resulted in the increment of triunsaturated and diunsaturated TAGs significantly in the oil.

Table 3.5

Yield and acylglycerols composition of the olein fractions from lab-scale and pilot-scale fractionation of batch TLIM [PL9=pilot-scale olein at 9°C; PS9=pilot-scale stearin at 9°C]

Yield/ Acylglycerols	POoIV62	Oln9°C	Oln12°C	Oln15°C	Oln18°C	Oln21°C	PL 9	PS 9
Yield (%)	-	57.4±0.3	62.6±1.0	64.4±0.6	64.9±0.4	68.4±0.5	61.0±0.2	39.0±0.2
Total DAG	**6.2±0.0**	**11.2±0.1**	**11.7±0.1**	**11.2±0.1**	**11.5±0.0**	**11.5±0.1**	**11.4±0.1**	**12.6±0.1**
OLL	0.6±0.0	2.2±0.0	2.2±0.1	2.2±0.1	2.1±0.0	2.1±0.0	2.2±0.0	1.4±0.0
OLO	2.6±0.0	8.1±0.1	7.9±0.0	7.7±0.0	7.6±0.0	7.5±0.0	8.0±0.0	5.1±0.0
OOO	5.7±0.1	10.0±0.1	9.8±0.1	9.7±0.1	9.5±0.1	9.3±0.0	9.9±0.0	6.9±0.0
Total triunsaturated	**8.9±0.2**	**20.2±0.1**	**19.8±0.0**	**19.5±0.1**	**19.2±0.1**	**18.9±0.0**	**20.1±0.0**	**13.4±0.0**
PLL	3.3±0.1	3.3±0.1	3.2±0.1	3.2±0.1	3.2±0.1	3.1±0.0	3.3±0.0	2.0±0.0
PLO	15.0±0.0	14.6±0.1	14.4±0.1	14.1±0.1	13.7±0.0	13.6±0.1	14.6±0.0	9.2±0.0
POO	31.9±0.2	23.5±0.1	23.4±0.2	22.8±0.0	22.3±0.1	22.0±0.1	23.5±0.0	15.5±0.0
SOO	3.5±0.1	2.6±0.1	2.5±0.0	2.5±0.0	2.5±0.0	2.4±0.0	2.5±0.0	1.5±0.0
Total diunsaturated	**53.7±0.2**	**43.8±0.1**	**43.4±0.2**	**42.5±0.1**	**41.6±0.1**	**41.1±0.2**	**43.9±0.1**	**28.3±0.0**
MLP	0.4±0.1	0.7±0.0	0.6±0.1	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.5±0.0
PLP	9.5±0.0	5.1±0.0	5.1±0.1	5.4±0.1	5.5±0.1	5.5±0.0	5.2±0.0	5.7±0.0
POP	17.4±0.0	12.6±0.1	13.1±0.1	13.8±0.1	14.3±0.1	14.6±0.0	12.7±0.0	19.5±0.0
POS	3.2±0.1	2.3±0.0	2.5±0.1	2.6±0.0	2.8±0.1	2.8±0.0	2.4±0.0	4.1±0.0
SOS	0.4±0.0	0.4±0.0	0.4±0.1	0.5±0.1	0.5±0.0	0.5±0.0	0.4±0.0	0.7±0.1
Total disaturated	**30.9±0.0**	**21.1±0.1**	**21.5±0.3**	**22.9±0.1**	**23.7±0.1**	**24.1±0.0**	**21.2±0.0**	**30.5±0.1**
PPP	0.1±0.0	0.3±0.0	0.3±0.1	0.4±0.0	0.5±0.0	0.7±0.0	0.2 ±0.1	9.1±0.1
PPS	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.0±0.0	2.7±0.1
Total trisaturated	**0.1±0.0**	**0.3±0.0**	**0.3±0.1**	**0.4±0.0**	**0.6±0.0**	**0.8±0.0**	**0.2±0.0**	**11.8±0.1**

Overall, the modification process via batch TLIM had significantly increased the triunsaturated TAGs and reduced the diunsaturated and disaturated TAGs, with no significant changes in trisaturated TAGs composition in the new oleins. The highest amount of triunsaturated TAGs was achieved in olein fractionated at 9 ℃ with over one fold increment as compared to the feed olein. The high increment of triunsaturated TAGs was mainly contributed by OLO and OOO which involved 5.5% and 4.3% increment, respectively. OLO increased in the highest rate with increment of more than two fold compared to the feed olein. The diunsaturated and disaturated TAGs of the olein reduced to 43.8 and 21.1%, respectively.

Table 3.6 shows the fatty acids composition and IV of the olein fractions and the feed oil. As fractionation temperature was reduced, MUFA and PUFA increased with decreased of SFA. The trend of fatty acid composition was particularly related to the performance of dry fractionation in the effectiveness of separating different melting points TAGs. For fractionation at 21 ℃, SFA reduced from 41.7% to 35.8%. The SFA content gradually decreased to 33.1% as fractionation temperature was reduced to 9 ℃. The trend of reduction in SFA was believed to be predominantly contributed by the removal of disaturated TAGs (Table 3.5). As compared to the feed olein, it was found that the modification processes had generally improved the unsaturation content of palm olein, whereby MUFA and PUFA increased 6.6% and 2.0%, respectively. IV was improved from 62.0 to 70.7 after batch TLIM interesterification and fractionation at 9 ℃.

Table 3.6
Fatty acid composition and iodine value (IV) of the olein fractions from lab-scale fractionation of batch TLIM

Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
POo IV 62	0.3±0.0	1.1±0.0	36.3±0.1	3.6±0.0	0.4±0.0	**41.7±0.1**	0.2±0.0	44.9±0.1	**45.1±0.1**	13.0±0.0	0.2±0.0	**13.2±0.0**	62.0±0.0
Olein 9 °C	0.2±0.0	1.0±0.0	28.7±0.1	2.9±0.0	0.3±0.0	**33.1±0.1**	0.2±0.0	51.5±0.1	**51.7±0.1**	15.0±0.1	0.2±0.0	**15.2±0.1**	70.7±0.2
Olein 12 °C	0.2±0.0	1.0±0.0	29.2±0.0	3.0±0.0	0.3±0.0	**33.7±0.1**	0.2±0.0	51.0±0.1	**51.2±0.1**	14.8±0.0	0.2±0.0	**15.0±0.0**	70.0±0.2
Olein 15 °C	0.2±0.0	1.0±0.0	29.8±0.0	3.1±0.0	0.3±0.0	**34.4±0.0**	0.2±0.0	50.5±0.0	**50.7±0.0**	14.6±0.0	0.2±0.0	**14.8±0.0**	69.2±0.0
Olein 18 °C	0.2±0.0	1.0±0.0	30.5±0.1	3.2±0.0	0.3±0.0	**35.2±0.1**	0.2±0.0	50.0±0.1	**50.2±0.1**	14.4±0.0	0.2±0.0	**14.6±0.0**	68.5±0.1
Olein 21 °C	0.2±0.0	1.0±0.0	31.1±0.1	3.2±0.0	0.3±0.0	**35.8±0.1**	0.2±0.0	49.6±0.1	**49.8±0.1**	14.1±0.1	0.2±0.0	**14.3±0.1**	67.6±0.1
PL 9	0.2±0.0	0.9±0.0	28.9±0.1	0.3±0.0	0.3±0.0	**33.3±0.1**	0.3±0.0	51.2±0.0	**51.5±0.0**	15.1±0.0	0.2±0.0	**15.3±0.0**	70.7±0.0
PS 9	0.2±0.0	1.2±0.0	45.1±0.0	5.0±0.1	0.5±0.0	**52.0±0.1**	0.2±0.1	37.4±0.1	**37.6±0.2**	10.4±0.1	0.1±0.0	**10.5±0.1**	50.4±0.1

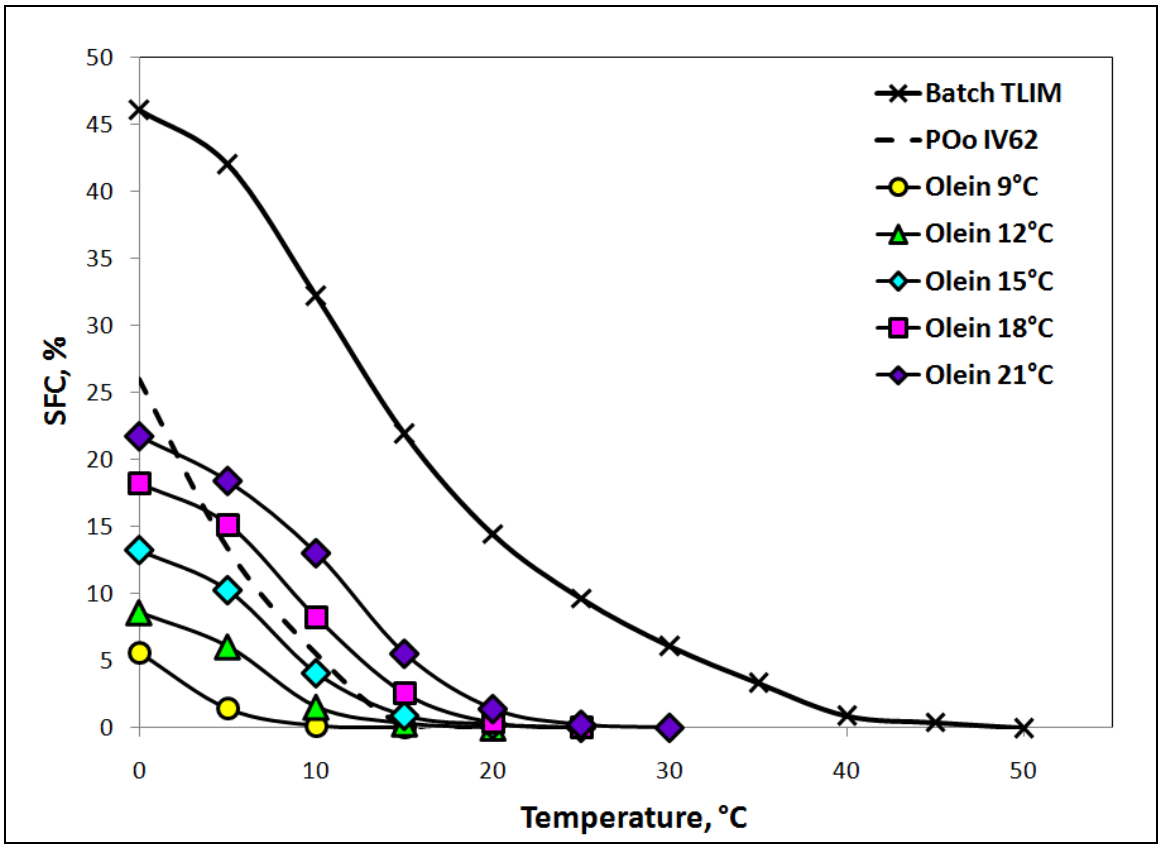
Both TAGs and fatty acid composition results showed no significant difference between the lab-scale and pilot-scale fractionation oleins. This indicated that lab-scale fractionation can be used to predict the olein quality to be produced in larger scale fractionation when the same cooling program was applied. Different filtration tools will only affect the stearin quality as well as the accuracy of the olein yield (Timms, 2005). This can be proven in the TAG and fatty acid composition results of the lab-scale stearin fractions that illustrated in Appendix K and L, respectively. The stearins compositions were not as expected. The olein yield of pilot-scale fractionation was higher due to lesser olein entrapment in the stearin fraction.

The SFC of the feed palm olein, the batch TLIM interesterified product, and the lab-scale oleins were measured as a function of temperature, as shown in Figure 3.10. SFC of the batch TLIM interesterified product was increased due to the formation of adequately high amount of trisaturated TAGs. Majority of the trisaturated TAGs was removed after lab-scale fractionation, thus resulted in reducing the SFC of the olein fractions overall (Table 3.5). When fractionation temperature was reduced, lower SFC was observed in the olein fractions due to the reduction of total disaturated TAGs as well as the increment of total diunsaturated TAGs (Table 3.5).

The figure also showed that the olein fractionated at 9, 12, and 15 °C have lower SFC at all tested temperatures, whilst olein 18 °C and 21 °C contained higher SFC at tested temperature above 5 °C. This was basically due to the presence of some trisaturated TAGs in olein 18 °C and 21 °C, ranging from 0.4 to 0.8%. Basically, the presence of high

unsaturated fatty acids with bend-structures will disturb the arrangement of TAGs during crystallization, and reduced the SFC of the oil (Lawler and Dimick, 2002). Therefore, higher content of total triunsaturated TAGs in all the olein fractions had retarded the crystallization of the diunsaturated and disaturated TAGs, which resulted in lower SFC at 0 °C.

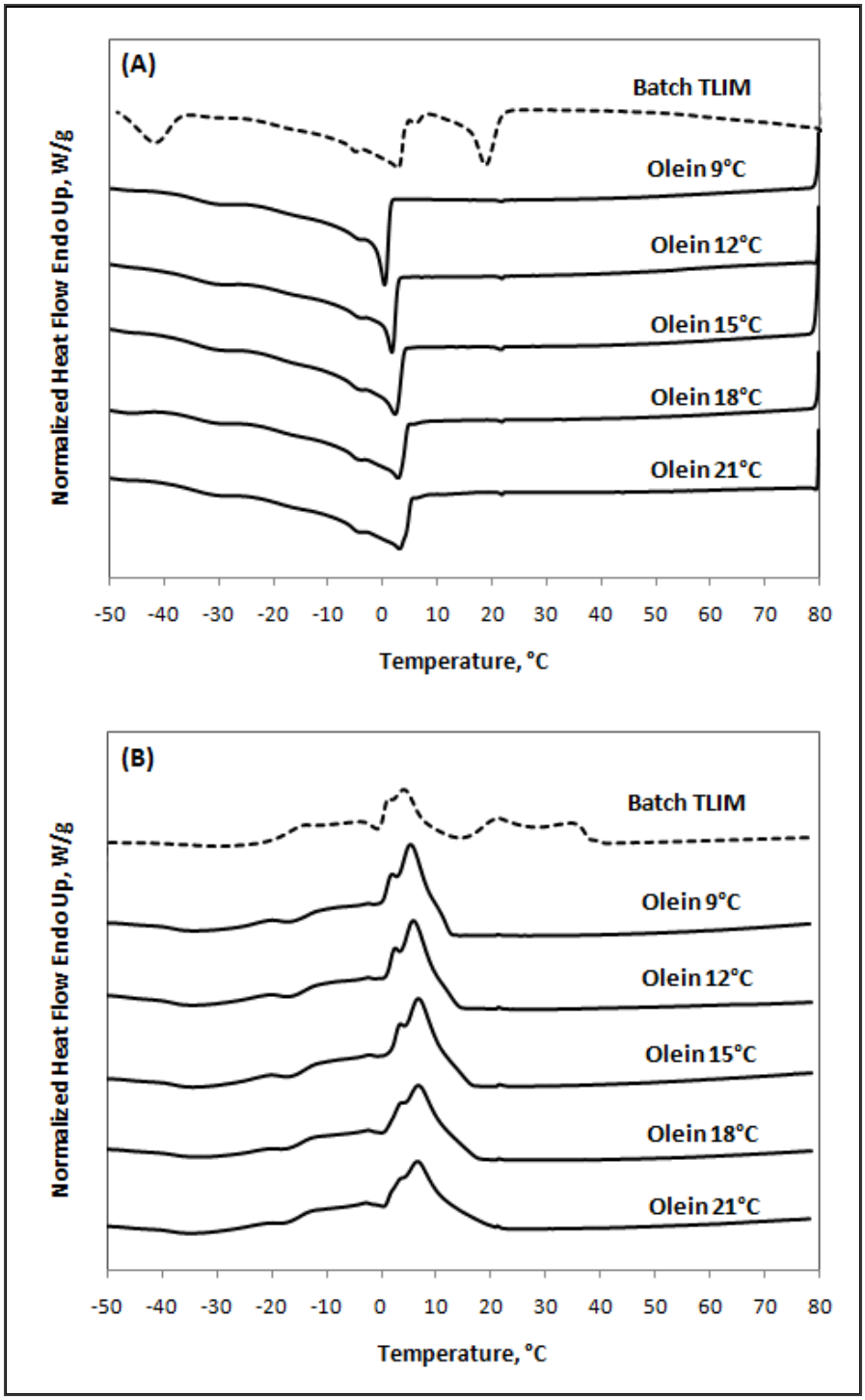
Figure 3.10
Comparison of the SFC of feed palm olein, the interesterified products of batch TLIM and the lab-scale olein fractions fractionated at 9, 12, 15, 18, and 21 °C



Similar to the DSC diagrams of oleins from batch RMIM interesterification, majority of the high melting components were removed in the lab-scale fractionation. The exothermic peaks in crystallization thermogram of the oleins (Figure 3.11, A) were shifted to higher temperature and became broader when higher fractionation temperature was applied. This

was basically due to the incomplete removal of trisaturated TAGs for high temperature fractionation, such as olein 18 °C and 21 °C.

Figure 3.11
Comparison of (A) crystallization, and (B) melting properties of feed palm olein, and the lab-scale olein fractions from fractionation of batch TLIM at 9, 12, 15, 18, and 21°C



The further removal of trisaturated and disaturated TAGs (mainly POP) at 12 ℃ had contributed to the appearance of sharper peak (olein 12 ℃) as compared to olein 18 ℃ and 21 ℃. Similar findings were observed in Figure 3.11 (B), whereby the olein 9 ℃ were fully melted at 12 ℃ and the melting temperature was gradually increased to about 20 ℃ for olein 21 ℃.

3.2.3 Fractionation of Packed-bed TL IM

Table 3.7 shows the TAG profiles of the lab-scale oleins, the pilot-scale olein and stearin fractions. In lab-scale fractionation of packed-bed TLIM product, the yield of olein fractions were lower as fractionation temperature was reduced from 21 °C to 9 °C. The olein yields were decreased gradually from 51.8% to 46.9 % for fractionation temperature of 21 °C to 12 °C. At 9 °C, the olein yield dropped significantly to 24.8%.

Under the crystallization condition (9 °C), β-crystals (triclinic parallel structure) were formed, retarded the filtration of the slurry (Lawler and Dimick, 2002), and thus reduced the olein yield drastically. Although the olein yields were significantly different, the TAG composition of olein 9 °C was generally similar to olein 12 °C, with only 1.3% higher in diunsaturated TAGs and 1.6% lower in disaturated TAGs. Therefore 9 °C was too low to be used for fractionation of the interesterified products.

Table 3.7
Yield and acylglycerols composition of the olein fractions from lab-scale fractionation of packed-bed TLIM

Yield/ Acylglycerols	POoIV62	Oln 9°C	Oln12°C	Oln15°C	Oln18°C	Oln21°C	PL 12	PS 12
Yield (%)	-	24.8±1.3	46.9±1.1	48.3±0.7	49.5±0.3	51.8±1.1	67.1±0.0	32.9±0.0
Total DAG	**6.2±0.0**	**8.1±0.3**	**8.1±0.1**	**8.1±0.1**	**7.8±0.0**	**7.8±0.0**	**8.0±0.0**	**8.5±0.1**
OLL	0.6±0.0	2.7±0.0	2.8±0.0	2.7±0.1	2.7±0.1	2.6±0.0	2.6±0.1	1.1±0.0
OLO	2.6±0.0	8.7±0.0	8.6±0.3	8.1±0.0	8.3±0.0	8.2±0.0	8.2±0.1	4.0±0.0
OOO	5.7±0.1	10.3±0.0	9.9±0.0	9.6±0.1	9.3±0.1	9.3±0.0	9.4±0.1	5.5±0.0
Total triunsaturated	**8.9±0.2**	**21.7±0.0**	**21.3±0.3**	**20.4±0.2**	**20.3±0.0**	**20.1±0.0**	**20.1±0.3**	**10.6±0.1**
PLL	3.3±0.1	3.2±0.0	3.3±0.1	3.1±0.0	3.1±0.3	3.1±0.0	3.4±0.1	1.5±0.0
PLO	15.0±0.0	16.3±0.0	15.7±0.1	15.4±0.2	14.8±0.0	14.6±0.0	15.5±0.1	7.4±0.1
POO	31.9±0.2	26.1±0.0	25.4±0.1	25.0±0.1	24.1±0.1	24.2±0.0	24.9±0.0	12.3±0.1
SOO	3.5±0.1	2.7±0.0	2.6±0.0	2.6±0.0	2.6±0.1	2.5±0.0	2.7±0.1	1.1±0.1
Total diunsaturated	**53.7±0.2**	**48.3±0.0**	**47.0±0.2**	**46.1±0.2**	**44.6±0.2**	**44.4±0.1**	**46.4±0.1**	**22.2±0.3**
MLP	0.4±0.1	0.6±0.0	0.7±0.1	0.7±0.0	0.8±0.0	0.7±0.0	1.0±0.1	0.7±0.1
PLP	9.5±0.0	5.5±0.1	5.6±0.2	5.9±0.2	6.2±0.2	6.1±0.0	6.3±0.1	7.2±0.0
POP	17.4±0.0	12.7±0.3	13.7±0.0	15.4±0.2	16.0±0.0	16.6±0.1	14.5±0.2	25.2±0.3
POS	3.2±0.1	2.3±0.0	2.6±0.0	2.8±0.2	3.0±0.0	3.2±0.0	2.7±0.1	5.0±0.0
SOS	0.4±0.0	0.3±0.0	0.4±0.0	0.4±0.1	0.4±0.0	0.1±0.1	0.4±0.1	0.7±0.1
Total disaturated	**30.9±0.0**	**21.4±0.4**	**23.0±0.2**	**25.2±0.3**	**26.4±0.1**	**26.7±0.0**	**24.7±0.2**	**38.7±0.4**
PPP	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.3±0.1	0.2±0.0	0.1±0.0	15.1±0.1
PPS	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	4.4±0.1
Total trisaturated	**0.1±0.0**	**0.1±0.0**	**0.1±0.1**	**0.2±0.0**	**0.3±0.1**	**0.2±0.0**	**0.1±0.0**	**19.5±0.2**

Originally, the feed olein contained higher amounts of total diunsaturated TAGs (PLP, POP, POS, and SOS) and total diunsaturated (PLL, PLO, POO, and SOO) as compared to the olein fractions. After the interesterification, the fatty acids had been rearranged, resulted in increased of the total diunsaturated and disaturated TAGs. Therefore, the olein products

contained significantly lower amounts of these TAGs compared to the starting material. Additionally, total triunsaturated TAGs content of all the olein fractions were much higher than that of the feed olein, which comprises with only 8.9% of total triunsaturated TAGs. Total triunsaturated TAGs increased from 20.1% to 21.7% for fractionation temperature of 21 °C to 9 °C.

The improvement in unsaturation content in olein fractions was further confirmed by the fatty acid composition results. From the fatty acid composition results shown Table 3.8, the unsaturation content of the olein fractions had improved as compared to the starting material. Fractionation reduced the SFA content; while increased the MUFA and PUFA as fractionation temperature was reduced. Olein 9 °C contained the lowest amount of SFA which was only 32.9%, as compared to 33.2% in olein 12 °C. However, the yield of olein 9 °C was apparently much lower (22.1%) than olein 12 °C, and this again proved that 12 °C was the more suitable temperature required for the removal of the saturation content.

Another indicator usually used in describing the degree of unsaturation is IV. In food industry, IV is used to evaluate the liquidity of the oil. The higher the IV, the greater is the oil unsaturation. The olein obtained from the fractionation process had greater IV compared to the feed palm olein IV of 62. The IV of olein 21 °C was 68.8 which had improved by 6.8 from the original feed olein. The IV of the oleins were gradually increased to 69.6 (olein 18 °C), 69.8 (olein 15 °C), 71.1 (olein 12 °C), and 72.0 (olein 9 °C).

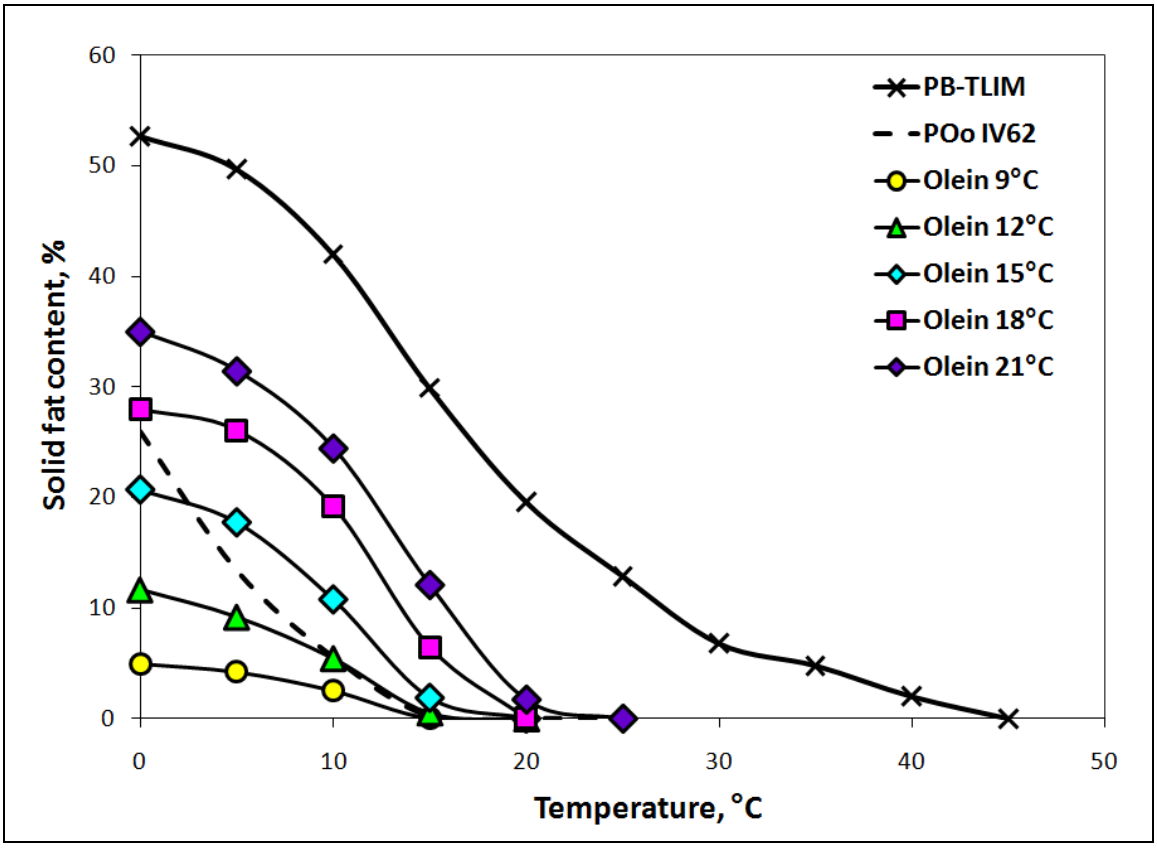
Table 3.8
Fatty acid composition and iodine value (IV) of the olein fractions from lab-scale fractionation of packed-bed TLIM

Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
POo IV 62	0.3±0.0	1.1±0.0	36.3±0.1	3.6±0.0	0.4±0.0	**41.7±0.1**	0.2±0.0	44.9±0.1	**45.1±0.1**	13.0±0.0	0.2±0.0	**13.2±0.0**	62.0±0.0
Olein 9 °C	0.2±0.0	1.0±0.0	27.9±0.1	2.9±0.0	0.3±0.0	**32.3±0.1**	0.2±0.0	51.7±0.1	**51.9±0.1**	15.5±0.1	0.3±0.0	**15.8±0.1**	72.0±0.2
Olein 12 °C	0.2±0.0	1.0±0.0	28.7±0.1	3.0±0.0	0.3±0.0	**33.2±0.1**	0.2±0.0	51.0±0.1	**51.2±0.1**	15.3±0.0	0.3±0.0	**15.6±0.0**	71.1±0.1
Olein 15 °C	0.2±0.0	1.0±0.0	29.7±0.1	3.1±0.0	0.3±0.3	**34.3±0.1**	0.2±0.0	50.3±0.2	**50.8±0.2**	14.9±0.1	0.3±0.0	**15.2±0.1**	69.8±0.1
Olein 18 °C	0.2±0.0	1.0±0.0	30.1±0.1	3.1±0.0	0.3±0.0	**34.7±0.1**	0.2±0.0	50.0±0.1	**50.2±0.1**	14.9±0.1	0.3±0.0	**15.2±0.1**	69.6±0.2
Olein 21 °C	0.2±0.0	1.0±0.0	30.5±0.0	3.2±0.0	0.4±0.1	**35.3±0.1**	0.2±0.0	49.6±0.1	**49.8±0.1**	14.7±0.1	0.3±0.0	**15.0±0.1**	68.8±0.2
PL 12	0.2±0.0	1.0±0.0	29.4±0.3	3.0±0.0	0.3±0.0	**33.9±0.2**	0.3±0.0	50.2±0.2	**50.4±0.2**	15.2±0.1	0.5±0.0	**15.7±0.1**	70.7±0.3
PS 12	0.3±0.0	1.3±0.0	52.6±0.1	5.6±0.0	0.5±0.0	**60.3±0.1**	0.1±0.0	30.9±0.1	**31.0±0.1**	8.4±0.0	0.3±0.0	**8.7±0.0**	41.9±0.2

In pilot-scale fractionation conducted at 12 °C, olein yield had increased 20.2% to 67.1% compared to the lab-scale fractionation (Table 3.7). This trial again indicated that membrane filter press was much more effective in reducing the olein entrapment, as compared to the regular vacuum filter. However, the application of high pressure during the filtration has squeezed out trace amount of very fine crystals, which increased the IV of the pilot-scale olein by 0.4, compared to the lab-scale olein.

The SFC of the feed olein, and the packed-bed TLIM interesterified palm olein, and the lab-scale oleins were shown in Figure 3.12.

Figure 3.12
Comparison of the SFC of feed palm olein, the interesterified products of packed-bed TLIM and the lab-scale olein fractions fractionated at 9, 12, 15, 18, and 21 °C



The results showed that the interesterified product and the olein fractions were similar in SFC trend which were totally different from the feed olein. The SFC of the olein fractions was much lower than the interesterified product as saturated components had been removed during fractionation process.

Olein fractionated at the highest temperature (21 °C) achieved the highest percentage of SFC at each tested temperature from 0 °C to 25 °C, followed by olein 18 °C, olein 15 °C, olein 12 °C, and olein 9 °C. This was because the olein fractionated at higher temperature contained higher amounts of saturated fat (Table 3.7). During fractionation, the oil will be cooled down and begins to crystallize. When crystallization begin, saturated TAGs will slowly concentrate in the solid phase (stearin), leaving behind a more unsaturated TAGs in the liquid phase (olein) (Kellens et al., 2007). At lower fractionation temperature, more saturated TAGs will concentrate in the solid phase, thus producing olein fractions with higher unsaturation content.

Besides, it was found that only the SFC of olein 12 °C and olein 9 °C were generally lower than the original palm olein IV 62. At temperature below 10 °C, the SFC of these olein fractions was lower than the feed olein, meaning that these olein fractions were more resistant against crystallization at temperature below 10 °C. As for olein at 15 °C, its SFC at 0 °C was lower than the feed olein. At temperature above 2.5 °C, olein 15 °C achieved higher SFC as compared to the feed oil.

Figure 3.13

Comparison of (A) crystallization, and (B) melting properties of the interesterified palm olein, and the lab-scale olein fractions from fractionation of packed-bed TLIM at 9, 12, 15, 18, and 21°C

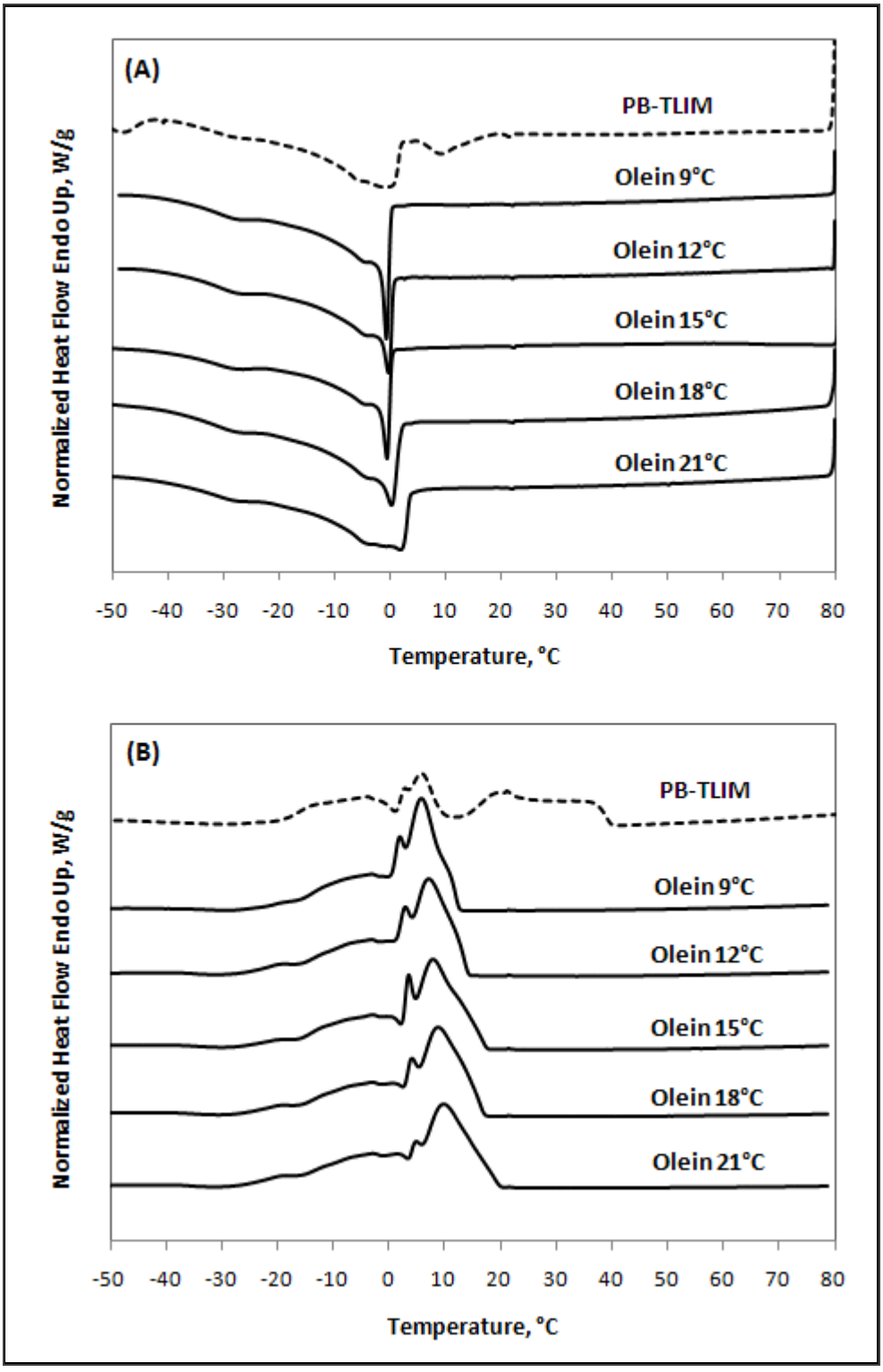


Figure 3.13 showed the thermal properties of the packed-bed interesterified palm olein, and its lab-scale olein fractions. Generally, the fractionation at 18 °C and 21 °C had completely removed the trisaturated TAGs, and resulted in the disappearance of the high melting peaks in both the crystallization and melting thermograms (Figure 3.13). Further reduction in the fractionation temperature had resulted in the removal of more disaturated TAGs that leads to the presence of sharper crystallization peak in the thermogram of olein fractionated at lower temperatures (Table 3.7). The DSC melting diagram in Figure 3.13 (B) showed that both olein 9 °C and olein 12 °C had comparable thermal properties, as both oleins were fully melted at about 16 °C.

Similar to other lab-scale stearin fractions, the stearin TAGs and fatty acid compositions of packed-bed TLIM were not as expected due to the inaccuracy in the filtration steps, data were illustrated in Appendix M and Appendix N.

3.3 Evaluation of the Products Quality

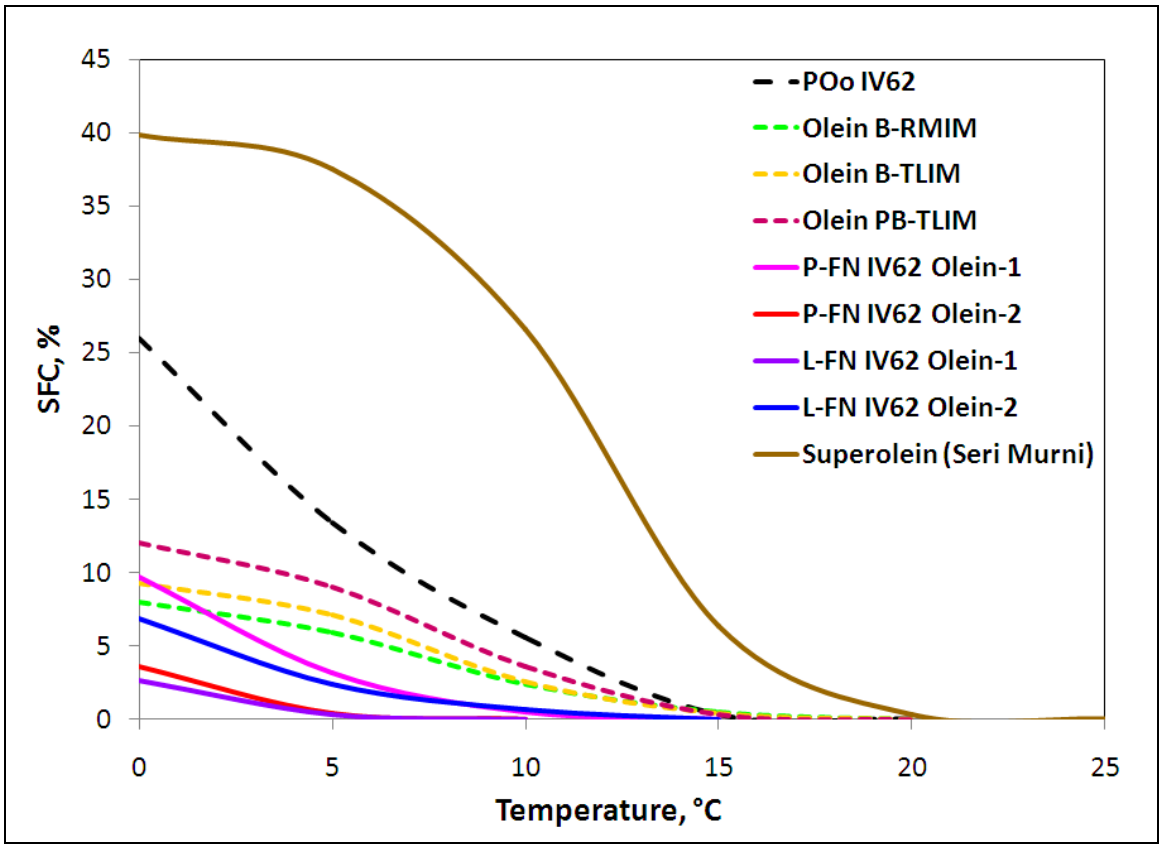
There are a few new products obtained from the modification processes, which can be divided into three categories; the low saturation liquid palm products, the subsequent solid products, and the intermediate palm olein from the enzymatic interesterifications. Other than the low saturation liquid palm products, the final solid fractions and the intermediate products from enzymatic interesterification are also valuable and have potential to be used as hard-based palm products with higher IV.

3.3.1 New Palm Liquid Products versus Other Palm Liquid Products

Figure 3.14 showed the comparison of SFC of the final olein obtained from batch RMIM, batch TLIM and packed-bed TLIM, with the feed olein, the commercial superolein from Seri Murni Sdn. Bhd., and the oleins obtained from fractionation of the feed palm olein. The SFC of the commercial superolein from Seri Murni Sdn. Bhd. was very much higher than the feed olein, indicating that the superolein from the market was lower in unsaturation level. Due to the removal of some saturated fats, all of the oleins derived from palm olein of IV 62, either from dry fractionation or the combination of enzymatic interesterification and dry fractionation contained adequately lower SFC than the feed palm olein of IV 62.

Figure 3.14

SFC of the final olein products in comparison with the feed palm olein (POo IV62), lab-scale (L-FN) and pilot-scale fractionation (P-FN) of the feed olein, and the superolein (Seri Murni)

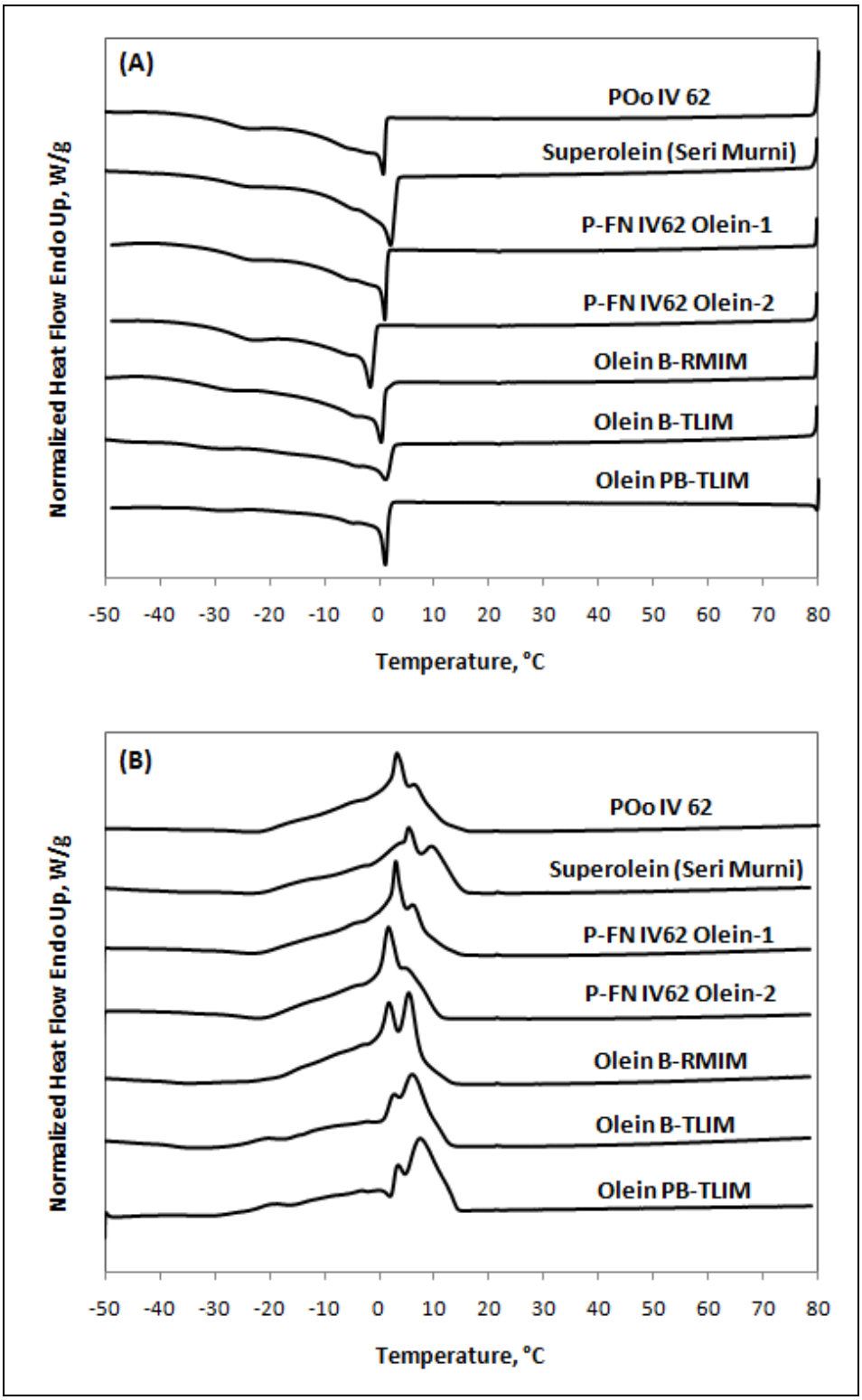


In comparison, the oleins from dry fractionation generally contained lower SFC than the oleins from enzymatic interesterification and dry fractionation. However, controlling of the crystallization during fractionation was very difficult due to absent of high melting TAGs such as trisaturated TAGs (Calliauw et al., 2007a). Due to this matter, crystallization of palm olein IV 62 had to be conducted at very low temperature. In lab-scale fractionation, palm olein IV 62 stay clear at 8 °C for more than an hour. Crystallization can only occur constantly at temperature below 5 °C; whereas in pilot-scale fractionation, crystallization of palm olein IV 62 had to be carried out at temperature as low as 2 °C.

The difficulty in controlling crystallization had resulted in the production of oleins with different yield and quality. Figure 3.14 indicated that the SFC of the oleins from pilot-scale fractionation of palm olein (P-FN IV62 Olein-1 and -2) was significantly different although they were produced under same fractionation conditions. In addition, the oleins from lab-scale fractionation (L-FN IV62 Olein-1 and -2) also showed significantly different SFC. Similar results were found in Figure 3.15, in which the crystallization and melting properties of P-FN IV62 Olein-1 and P-FN IV62 Olein -2 were very different.

This was basically because of the instability of the crystals in palm olein slurry which could easily damaged during filtration step. The less stable crystal forms were mainly associated with high intersolubility of the crystals in the liquid phase. Such miscibility or intersolubility of TAGs in solid form often presents problems in fractionation of palm olein (Calliauw et al., 2007b).

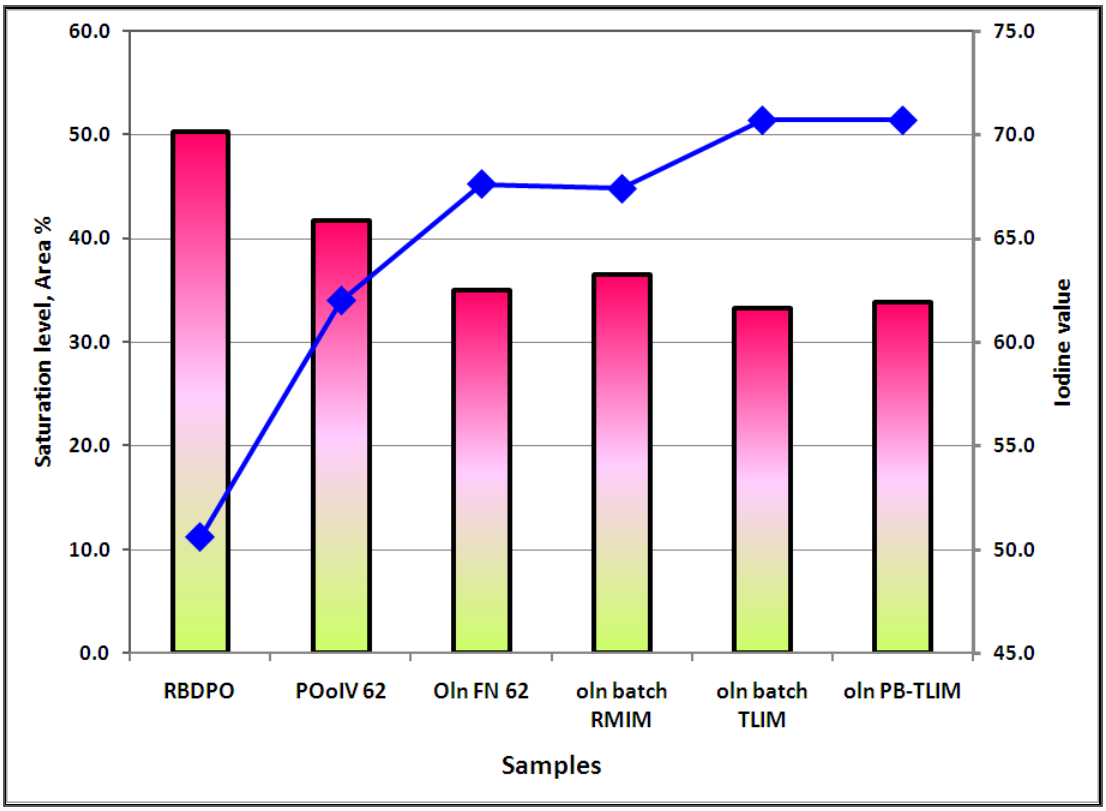
Figure 3.15
Comparison of (A) crystallization, and (B) melting properties of the three pilot-scale olein products from batch RMIM, batch TLIM, and packed-bed TLIM with feed palm olein (POo IV62), pilot-scale fractionation (P-FN) of the feed olein, and the commercial superolein



This problem was solved with interesterification of the palm olein. The interesterification reactions formed some trisaturated TAGs that could facilitate the crystals formation, and improved the crystallization process (Lawler and Dimick, 2002). Controlling of crystallization in the interesterified palm olein was much easier compared to that in the original feed olein. Trisaturated TAGs in the interesterified palm olein can act as seeds or nucleus for crystallization (Kellens et al., 2007).

Figure 3.16 showed the saturation level and the IV of some palm oil products including RBDPO, the feed olein (POo IV 62), olein obtained from pilot-scale fractionation of the feed olein (Oln FN 62), and the three major products obtained from batch RMIM, batch TLIM and packed-bed TLIM. Basically, the saturation content in palm oil products was calculated from C12:0, C14:0, C16:0, C18:0, and C20:0. Dry fractionation of RBDPO can easily reduce the saturation level to produce palm olein with IV of 56 and 62 (Kellens et al., 2007).

Figure 3.16
Comparison of the saturation level and IV of some palm oil products



From the study, dry fractionation of palm olein IV 62 reduced the saturation level from 41.7% to 35.0% with increasing of IV to 67.6, whereas enzymatic interesterification and dry fractionation produced the oleins with saturation content ranging from 36.5% to 33.3%, with IV of 67.4 to 70.7. Although the olein from batch RMIM had the lowest SFC, crystallization and melting temperatures, as shown in Figure 3.14 and Figure 3.15, the saturation level was higher than that in batch TLIM and packed-bed TLIM.

Here, it can be concluded that the oleins derived from batch and packed-bed TLIM contained the lowest SFA, which was 33.3% and 33.9%, respectively. Both processes are applicable for production of low saturation palm oil products in terms of the oil quality as

well as the economical aspects, as the cost of Lipozyme® TL IM is much lower as compared to the other enzymes.

3.3.2 New Palm Solid Products versus Commercial Palm Stearin

The physical properties of solid fractions from enzymatic interesterification and dry fractionation and palm stearin were evaluated, as shown in Figure 3.17 and Figure 3.18. The solid products were generally softer than the palm stearin. In comparison, the solid product from packed-bed TLIM had closer SFC as compared to the palm stearin.

Figure 3.17
Comparison of SFC of the solid products from batch RMIM, batch TLIM, and packed-bed TLIM (pilot-scale interesterfied products and the final stearin products) with commercial palm stearin

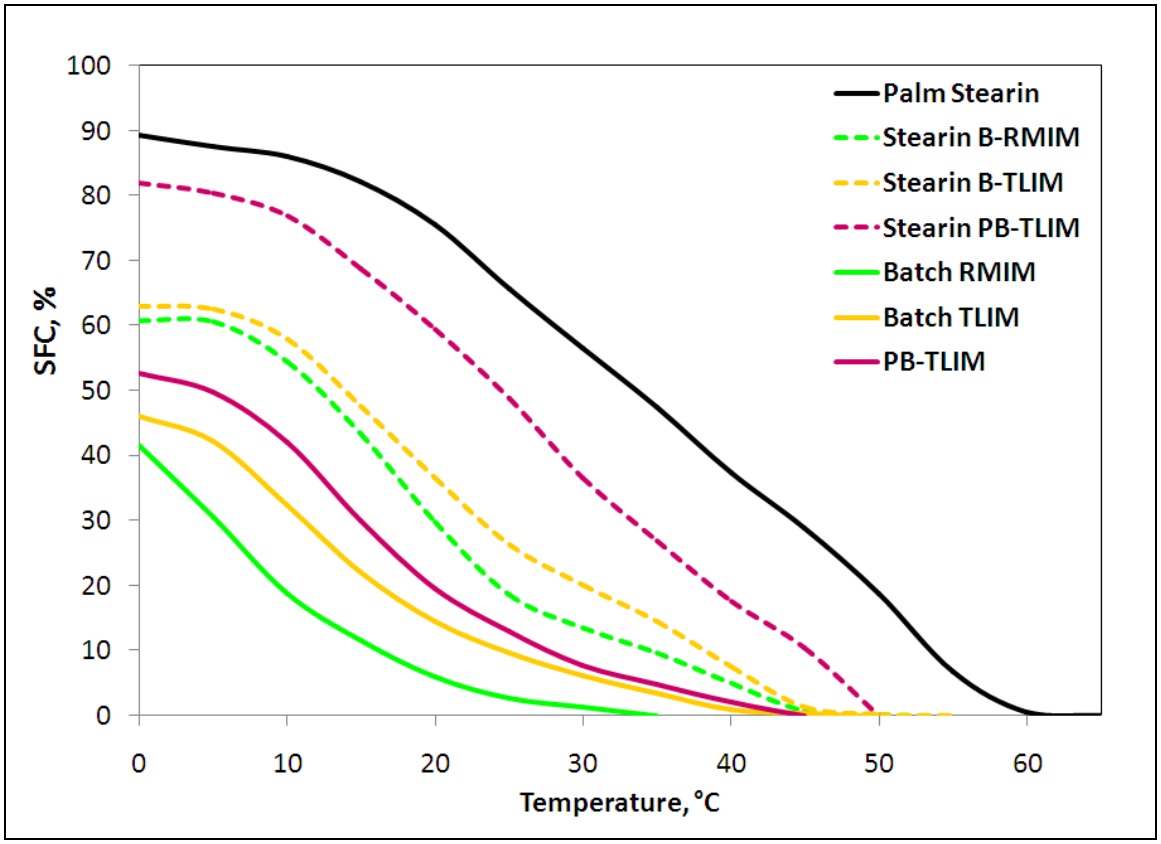
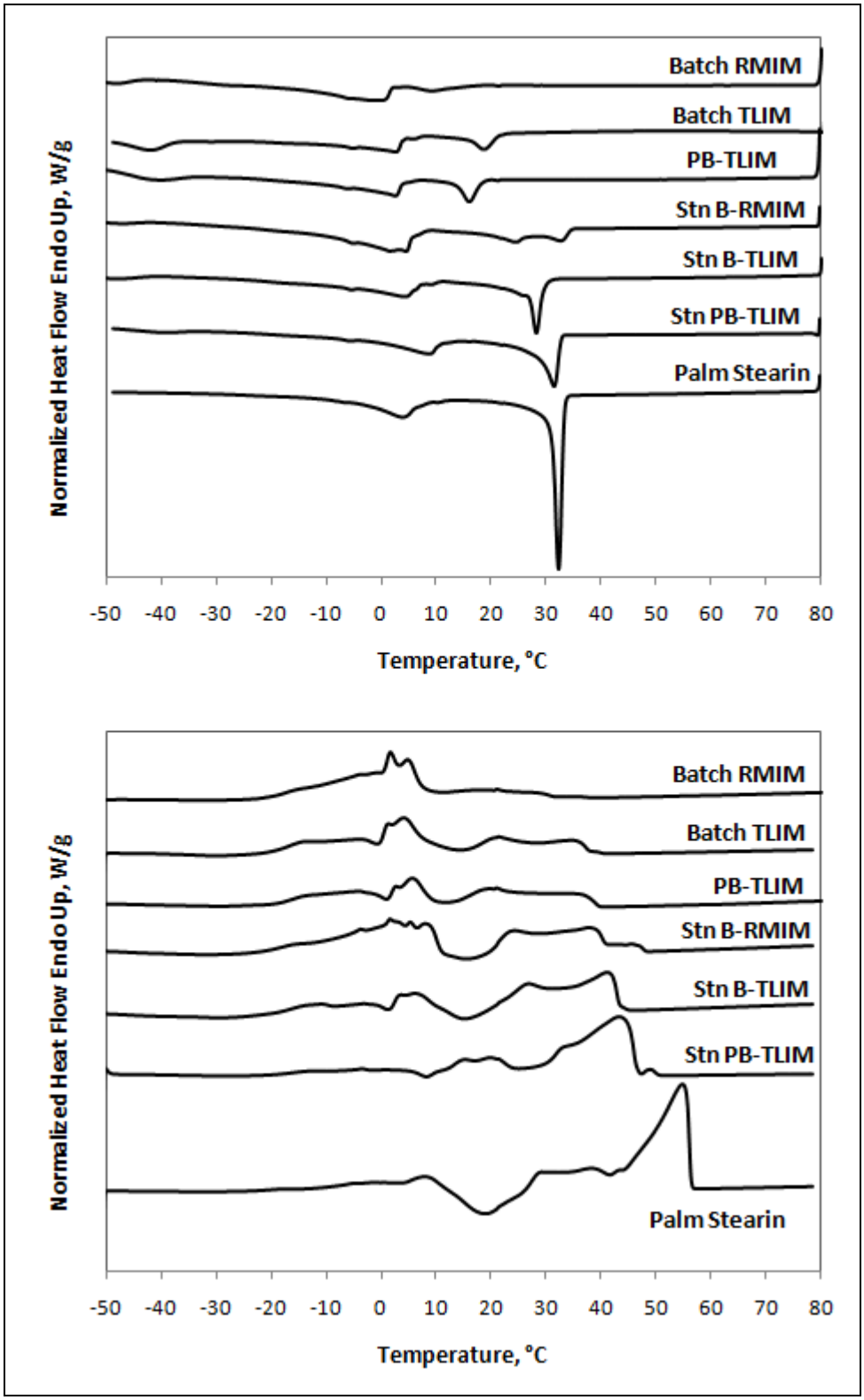


Figure 3.18
Comparison of (A) crystallization, and (B) melting properties of the products from batch RMIM, batch TLIM, and packed-bed TLIM, the relative stearin products and the commercial palm stearin



The solid products from batch TLIM and batch RMIM had much lower SFC and fully melt at lower temperature (45 °C). Due to the olein entrapment, the solid fractions were riched in triunsaturated TAGs, which ranging from 10.1% to 13.4%. These three solid products are of potential to be used as palm hard-based stock, in the formulation of margarine, vanaspati etc.

Besides, the SFC and thermal properties of the products from enzymatic interesterification were evaluated and shown in Figure 3.17 and Figure 3.18, respectively. In the enzymatic interesterification, the physicochemical properties of the palm olein of IV 62 had changed excluding the fatty acid composition and IV.

The products from enzymatic interesterification were generally harder in its physical appearance. The batch RMIM product was the softest among the interesterified products, followed by batch TLIM and packed-bed TLIM. The high SFC was basically due to the formation of some trisaturated TAGs during the interesterification reactions. These high melting components act as the backbone structure for crystal networking, thus increased the SFC.

CONCLUSION AND RECOMMENDATION

Enzymatic interesterification with combination of dry fractionation had successfully reduced the saturation content of palm olein IV 62 from 41.7% to 36.5%, 33.3%, and 33.9%, by using batch RMIM, batch TLIM and packed-bed TLIM, respectively. The IV was increased from 62.0 to 67.4 for batch RMIM olein product, and 70.7 for batch TLIM and packed-bed TLIM olein products.

In comparison, the oleins derived from dry fractionation of batch TLIM and packed-bed TLIM products contained generally lower SFA than the olein obtained from dry fractionation of the same feed oil (35.0%). The IV of the enzymatic modified oleins was 70.7, which was 3.1 greater than the olein from single-step dry fractionation. Batch RMIM olein product had similar fatty acid composition, IV, and SFC trend as the olein from dry fractionation, the IV achieved is 67.4, compared to 67.6 in the olein from dry fractionation. Although dry fractionation of the feed olein can reduce the saturation level to 35.0%, crystallization of palm olein was difficult due to the absent of high melting TAGs such as trisaturated TAGs.

Several aspects can be looked into to further reduce the saturation content of palm products. For example, developing the cooling program such as prolonging the crystallization time at higher fractionation temperature to have more stable/rigid crystals; interesterification of other palm products such as palm oil or palm olein with lower IV, to produce greater amount of trisaturated TAGs for ease of fractionation.

Besides, directed interesterification is also another way of reducing the saturation content. Directed interesterification allows crystallization during interesterification, in which crystallization takes place simultaneously with interesterification. The saturated components that produced during the reaction can be eliminated from taking part in the reaction continuously. However, the application of this concept is difficult due to the slow crystallization behavior of palm products. Long time interesterification may also cause formation of high amount of by products such as partial glycerides.

In conclusion, the combination of enzymatic interesterification and dry fractionation is a better way of reducing the saturation content in palm olein, as the level of saturation can be reduced up to 33.3%. This olein has fulfilled the requirement for healthy oil according to WHO dietary guidelines (WHO, 2003). Some saturation is necessary for better oil stability especially during cooking at high temperature.

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APPENDICES

Appendix A
The application sheet of Lipozyme® TL IM

Enzymatic interesterification with Lipozyme® TL IM

For margarines and shortenings low trans fats and the correct melting properties are required. Enzymatic interesterification is an efficient and environmentally friendly way to produce these without the use of chemicals.

Benefits

High product quality

- No chemicals used in processing
- No by-product formation, no trans fat formation, and low diglycerides formation
- No color change in the fat blend

Simple and easy process

- Fewer unit operations than in alternative processes
- No need for washing or postbleaching
- Improved industrial hygiene/safety
- No wastewater

Cost efficient

- Capital investment is low as only simple reactors are required
- Total variable costs are competitive with alternative processes

Environment-friendly

- Reduced production of greenhouse gases
- Reduced energy consumption

Appendix A, continued

Product

The product for interesterification is Lipozyme TL IM. It is produced by immobilization of a microbial lipase from *Thermomyces lanuginosus* on a granulated silica carrier.
The lipase is a 1,3-specific triacylglycerol lipase (EC 3.1.1.3) and is produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism.
Lipozyme TL IM is food grade and kosher/halal approved.
Further information on the abovementioned product is available from the Novozymes Customer Center.

Performance

Lipozyme TL IM rearranges the fatty acids preferentially, but not uniquely, in the 1- and 3-positions of the triglycerides. As a result of this rearrangement the melting properties of the fat blend are changed as illustrated in Figure 1.

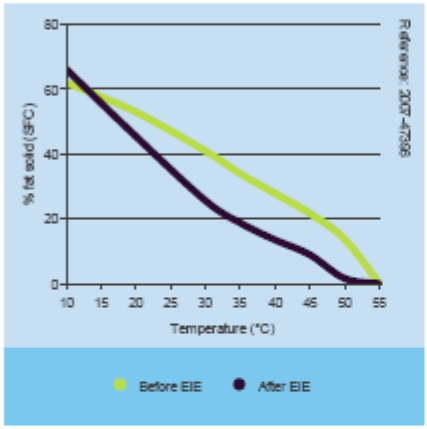

Fig. 1. SFC of a 70% palm stearin:30% soyabean oil before and after enzymatic interesterification.

Usage

Lipozyme TL IM is an immobilized lipase. As it is immobilized, it can be reused many times, and Lipozyme TL IM is not present in the interesterified fat blend.
For pilot-scale and industrial-scale interesterification we recommend a continuous process with Lipozyme TL IM in a fixed-bed reactor.
For initial interesterification trials in the laboratory we suggest setting up a batch process.

Application

Continuous process

Lipozyme TL IM is packed as a fixed bed, and the fat is interesterified continuously while passing through the catalyst bed. The flow rate controls the residence time of the fat in the reactor, which in turn controls the level of interesterification.
A number of reactors can be connected in series for the most optimal configuration (Figure 2). As the oil blend is pumped through the reactor series the enzyme in the first reactor loses activity, and in a single reactor system, flow rate would need to be reduced to maintain the conversion.

Appendix A, continued

In this configuration with four to six reactors, flow is maintained at a near-constant level by allowing all the activity in the first reactor to become exhausted.

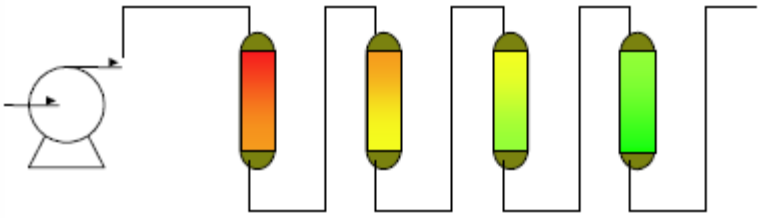

Fig. 2. Process layout for enzymatic interesterification.

At this point, it is disconnected from the flow while the spent enzyme is removed and fresh enzyme filled into the column. It is then re-connected to the flow becoming the last reactor in the chain. The reactor that was previously number 2 in the series now receives the unconverted oil blend, and enzymatic rearrangement continues until this reactor is exhausted. In a steady-state system, the first reactor normally contains < 20% of the initial activity and serves as a protective column for the remaining ones which carry out the main conversion.

Oil quality

The economy of the process is maximized by having the highest possible productivity (kg blend converted/kg enzyme). Residual particulate matter (soaps and phosphatides), oxidation products (peroxide and anisidine values), and residual mineral acids (from bleaching and/or degumming) will have a negative effect on enzyme activity and working life. The desired maximum levels of these factors and other parameters are shown in Table 1.

Moisture + impurities	0.1%
FFA	0.1%
Soaps	1 ppm max.
P	3 ppm max.
Fe	0.1 ppm max.
Ni	0.2 ppm max.
Cu	0.01 ppm max.
Peroxide value	1 meqO_2/kg max.
Anisidine value	5 max.
Citric acid	25 ppm max.
Temperature	70 °C
Water extract of oil	pH > 6.0, < 9.0

Table 1. Oil quality requirements for enzymatic interesterification.

Appendix A, continued

Postpurification
There is no need for washing or bleaching of the fat after enzymatic interesterification. A minimal deodorization is recommended in order to remove any free fatty acids that are generated during interesterification. Spent enzyme can be disposed of in a similar manner to that used for spent bleaching earth.

A detailed description of how to carry out interesterification in a fixed-bed reactor at pilot-scale and industrial-scale can be found in the document *Handbook of Enzymatic Interesterification*. The handbook is available from the Novozymes Customer Center.

Batch process
In the laboratory it is easier to set up a batch reactor than a fixed-bed reactor because a batch process can be carried out using standard laboratory hardware.
The principle of the batch process is to allow sufficient contact time between fat and enzyme to achieve the desired interesterification. Lipozyme TL IM and the fats are mixed in a container (e.g., a beaker). During the course of the reaction, the enzyme/fat mixture is continuously stirred in order to keep the enzyme particles in suspension and ensure good contact between the catalyst and the fat. When the reaction is complete and the mixing has been stopped, the enzyme particles sediment quickly, and the fat can be separated from the catalyst either by filtration or decanting.
A batch process is very useful for the production of small amounts of fat for physical and chemical characterization. The relationship between the reaction time and the resultant interesterification can be obtained by collecting small fat samples over time and carrying out relevant analysis of these samples.
We have prepared a detailed description of the procedure and equipment used at Novozymes for carrying out batch interesterification experiments. This description is available from the Novozymes Customer Center.

Dosage
Under the recommended operating conditions and with oil blends that meet the quality specifications, Lipozyme TL IM will deliver a productivity of at least 2,500 kg/kg. For batch reactions a dosage of 4% w/w enzyme is normally applied.

Activity and stability
Lipozyme TL IM has an optimal operating temperature of 70 °C. Operation at temperatures above this will increase the rate of conversion but will also increase the sensitivity of the enzyme to oxidation compounds. This will result in an increased conversion but a shorter working life. Temperatures above 80 °C will cause rapid and irreversible enzyme inactivation.

Appendix A, continued

Storage in application

It is recommended that Lipozyme TL IM be stored in unopened drums at a temperature below 25 °C. If the drums need to be partly emptied during reactor filling, they should be securely closed again in order to avoid an increase in the moisture level of the product.

Safety in use

Lipozyme TL IM is a robust product when being used for Interesterification. However, enzyme dust can be formed during filling and masks should be worn. Any spillage should be removed via a vacuum cleaner. Full handling details are available from the Customer Center.

Safety, handling, and storage

Safety, handling, and storage guidelines are provided with all products available from the Customer Center.

Appendix B
The application sheet of Lipozyme® RM IM

Lipozyme® RM IM

Valid From 2007-07-11

Product Characteristics:

Declared Enzyme	Lipase
Declared Activity	150 IUN/g
Colour	Brown Colour can vary from batch to batch. Colour intensity is not an indication of enzyme activity.
Physical form	Immobilized Granulate
Production organism	Aspergillus oryzae
Donor organism	Rhizomucor miehei
Production Method	Produced by submerged fermentation of a genetically modified micro organism. The enzyme protein, which in itself is not genetically modified, is separated and purified from the production organism.

Product Specification:

	Lower Limit	Upper Limit	Unit
Interestification Units IUN	150		/g
Loss on Drying	-	5	%
Total Viable Count	-	50000	/g
Coliform Bacteria	-	30	/g
Enteropathogenic E.Coli	Not Detected		/25 g
Salmonella	Not Detected		/25 g

The product complies with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC).

Packaging: See the standard packaging list for more information.

Appendix B, continued

Recommended Storage:

Best before	When stored as recommended, the product is best used within 3 months from date of delivery.
Storage at customer's warehouse	0-10°C (32°F-50°F)
Storage Conditions	In unbroken packaging - dry and protected from the sun. The product has been formulated for optimal stability. Extended storage or adverse conditions such as higher temperature or higher humidity may lead to a higher dosage requirement.

Safety and Handling Precautions

Enzymes are proteins. Inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes and mucous membranes upon prolonged contact. Powdered enzymes are readily inhaled and should be handled only with specific precautions to prevent inhalation of dust. All equipment and handling procedures must be designed to control airborne dust. Personal respiratory protection is recommended in all cases where full dust control is not secured . All spills, however minor, should be removed immediately. Use respiratory protection. Major spills should be carefully shovelled into plastic-lined containers. Minor spills and the remains of major spills should be removed by vacuum cleaning or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters. Wear suitable protective clothing, gloves and eye/face protection as prescribed on the warning label. Wash contaminated clothes. A Material Safety Data Sheet is supplied with all products. See the Safety Manual for further information regarding how to handle the product safely.

Appendix C

The normalized triacylglycerols compositions (Area %) of the 2 %w/w Lipozyme® TL IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**12.1**	**13.6**	**14.8**	**16.0**	**18.4**	**20.9**
OLL	0.9	1.1	1.3	1.6	1.7	2.2	2.7
OLO	2.8	4.0	4.7	5.2	5.8	6.9	8.1
OOO	6.1	7.1	7.6	8.1	8.6	9.3	10.1
Total diunsaturated	**56.4**	**54.6**	**53.1**	**52.1**	**50.0**	**47.0**	**44.3**
PLL	3.7	3.5	3.4	3.5	3.3	3.2	3.2
PLO	15.8	16.1	16.1	16.2	15.8	15.5	15.0
POO	33.0	31.6	30.3	29.4	27.8	25.5	23.7
SOO	3.9	3.4	3.3	3.1	3.1	2.8	2.4
Total disaturated	**33.6**	**32.5**	**32.0**	**31.5**	**31.4**	**30.7**	**29.4**
MLP	0.8	0.8	0.8	0.8	0.8	0.9	0.9
PLP	10.5	9.8	9.2	8.9	8.5	7.4	6.5
POP	18.3	18.4	18.3	18.2	18.2	18.4	18.0
POS	3.6	3.3	3.5	3.5	3.5	3.6	3.6
SOS	0.5	0.3	0.3	0.2	0.5	0.5	0.5
Total trisaturated	**0.4**	**0.9**	**1.4**	**1.7**	**2.7**	**4.0**	**5.5**
PPP	0.4	0.7	1.1	1.3	2.2	3.1	4.2
PPS	0.0	0.2	0.3	0.4	0.5	0.9	1.3

Appendix D

The normalized triacylglycerols compositions (Area %) of the 5 %w/w Lipozyme® TL IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**15.3**	**17.9**	**18.8**	**19.8**	**21.2**	**21.8**
OLL	0.9	1.7	2.1	2.1	2.3	2.7	2.8
OLO	2.8	5.4	6.7	7.2	7.6	8.2	8.8
OOO	6.1	8.3	9.2	9.6	9.9	10.3	10.2
Total diunsaturated	**56.4**	**51.0**	**48.0**	**47.0**	**46.0**	**44.4**	**43.2**
PLL	3.7	3.4	3.3	3.1	3.1	3.2	3.2
PLO	15.8	15.9	15.7	15.5	15.4	15.0	14.2
POO	33.0	28.6	26.3	25.7	24.9	23.8	23.4
SOO	3.9	3.1	2.8	2.8	2.6	2.5	2.4
Total disaturated	**33.6**	**31.8**	**30.9**	**30.4**	**30.0**	**29.2**	**29.5**
MLP	0.8	0.8	0.9	0.7	0.8	0.9	1.0
PLP	10.5	8.6	7.7	7.2	6.9	6.6	6.4
POP	18.3	18.4	18.3	18.4	18.2	17.8	17.8
POS	3.6	3.6	3.6	3.6	3.6	3.5	3.6
SOS	0.5	0.4	0.5	0.5	0.5	0.5	0.9
Total trisaturated	**0.4**	**2.0**	**3.2**	**3.8**	**4.4**	**5.3**	**5.6**
PPP	0.4	1.6	2.5	3.0	3.4	4.1	4.4
PPS	0.0	0.4	0.7	0.8	1.0	1.2	1.3

Appendix E

The normalized triacylglycerols compositions (Area %) of the 10 %w/w Lipozyme® TL IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**14.5**	**16.6**	**18.6**	**19.2**	**21.2**	**21.2**
OLL	0.9	1.4	1.7	2.2	2.2	2.8	2.8
OLO	2.8	5.0	6.1	6.9	7.3	8.3	8.2
OOO	6.1	8.1	8.8	9.5	9.7	10.2	10.2
Total diunsaturated	**56.4**	**51.8**	**50.0**	**48.0**	**47.0**	**43.8**	**43.9**
PLL	3.7	3.4	3.3	3.3	3.1	3.2	3.2
PLO	15.8	16.0	16.0	15.8	15.6	14.7	14.8
POO	33.0	29.6	27.8	26.3	25.7	23.4	23.6
SOO	3.9	2.9	3.0	2.7	2.7	2.6	2.4
Total disaturated	**33.6**	**32.1**	**30.9**	**30.2**	**29.9**	**29.3**	**29.3**
MLP	0.8	0.8	0.8	0.9	0.7	0.9	0.8
PLP	10.5	9.1	8.2	7.6	7.0	6.4	6.4
POP	18.3	18.4	18.3	18.1	18.2	18.0	18.0
POS	3.6	3.4	3.3	3.3	3.6	3.6	3.6
SOS	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total trisaturated	**0.4**	**1.6**	**2.6**	**3.3**	**4.0**	**5.7**	**5.7**
PPP	0.4	1.3	2.0	2.6	3.1	4.5	4.4
PPS	0.0	0.3	0.6	0.7	0.9	1.3	1.3

Appendix F

The normalized TAG compositions (Area %) of the 2 %w/w Lipozyme® RM IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**11.4**	**12.1**	**13.6**	**14.2**	**15.9**	**19.1**
OLL	0.9	1.0	1.2	1.4	1.4	1.8	2.3
OLO	2.8	3.6	3.9	4.7	4.9	5.7	7.3
OOO	6.1	6.8	7.1	7.6	8.0	8.5	9.6
Total diunsaturated	**56.4**	**54.7**	**53.9**	**52.8**	**52.0**	**49.1**	**47.4**
PLL	3.7	3.5	3.6	3.5	3.4	3.4	3.3
PLO	15.8	16.1	15.9	16.4	16.3	15.8	16.0
POO	33.0	31.7	30.9	29.9	29.3	26.9	25.4
SOO	3.9	3.5	3.5	3.1	3.1	3.1	2.7
Total disaturated	**33.6**	**33.4**	**33.4**	**32.7**	**32.7**	**33.1**	**30.9**
MLP	0.8	0.8	0.9	0.9	0.9	1.0	0.9
PLP	10.5	9.9	9.8	9.4	9.3	8.7	7.5
POP	18.3	18.5	18.7	18.7	18.8	19.2	18.4
POS	3.6	3.7	3.6	3.6	3.6	3.8	3.6
SOS	0.5	0.5	0.5	0.2	0.2	0.5	0.5
Total trisaturated	**0.4**	**0.6**	**0.7**	**1.0**	**1.1**	**1.9**	**2.8**
PPP	0.4	0.5	0.6	0.8	0.9	1.6	2.2
PPS	0.0	0.1	0.1	0.2	0.2	0.3	0.6

Appendix G

The normalized TAG compositions (Area %) of the 5 %w/w Lipozyme® RM IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**13.5**	**15.5**	**16.2**	**16.7**	**17.2**	**19.0**
OLL	0.9	1.3	1.6	1.7	1.7	1.9	2.3
OLO	2.8	4.7	5.6	5.9	6.2	6.3	7.2
OOO	6.1	7.6	8.4	8.6	8.9	9.0	9.5
Total diunsaturated	**56.4**	**52.7**	**50.3**	**49.4**	**48.6**	**48.7**	**47.0**
PLL	3.7	3.4	3.3	3.3	3.2	3.2	3.3
PLO	15.8	16.1	16.1	16.0	16.0	16.1	15.9
POO	33.0	29.9	27.9	27.2	26.5	26.6	25.1
SOO	3.9	3.3	3.1	3.0	2.9	2.8	2.8
Total disaturated	**33.6**	**32.8**	**32.7**	**32.8**	**32.9**	**32.3**	**31.0**
MLP	0.8	0.7	0.9	0.8	0.9	0.8	1.0
PLP	10.5	9.3	8.8	8.5	8.5	8.1	7.4
POP	18.3	18.8	19.0	19.3	19.4	19.2	18.4
POS	3.6	3.6	3.7	3.8	3.7	3.8	3.7
SOS	0.5	0.5	0.5	0.5	0.5	0.5	0.6
Total trisaturated	**0.4**	**1.0**	**1.5**	**1.7**	**1.9**	**1.9**	**3.2**
PPP	0.4	0.8	1.3	1.4	1.6	1.5	2.5
PPS	0.0	0.2	0.3	0.3	0.3	0.4	0.7

Appendix H

The normalized TAG compositions (Area %) of the 10 %w/w Lipozyme® RM IM lab-scale interesterification products after 1, 2, 3, 4, 8 and 24 hours of reaction

Triacylglycerols	POo IV 62	1	2	3	4	8	24
Total triunsaturated	**9.7**	**16.0**	**17.9**	**17.7**	**18.6**	**19.4**	**20.7**
OLL	0.9	1.7	2.1	1.9	2.1	2.3	2.5
OLO	2.8	5.8	6.7	6.6	7.0	7.4	8.0
OOO	6.1	8.5	9.2	9.2	9.5	9.8	10.2
Total diunsaturated	**56.4**	**49.9**	**48.2**	**48.3**	**47.7**	**47.6**	**45.9**
PLL	3.7	3.4	3.4	3.2	3.3	3.2	3.2
PLO	15.8	16.3	16.1	16.0	16.0	16.2	15.5
POO	33.0	27.4	26.0	26.2	25.7	25.5	24.6
SOO	3.9	2.9	2.8	2.9	2.8	2.7	2.6
Total disaturated	**33.6**	**32.7**	**32.0**	**32.0**	**31.4**	**30.2**	**29.2**
MLP	0.8	0.9	1.0	0.8	0.9	0.9	0.9
PLP	10.5	8.6	8.1	8.0	7.8	7.4	6.7
POP	18.3	19.0	18.8	19.0	18.6	18.1	17.8
POS	3.6	3.7	3.7	3.8	3.7	3.4	3.1
SOS	0.5	0.5	0.5	0.5	0.5	0.5	0.7
Total trisaturated	**0.4**	**1.5**	**2.0**	**2.1**	**2.4**	**2.9**	**4.4**
PPP	0.4	1.2	1.6	1.7	1.9	2.3	3.5
PPS	0.0	0.3	0.4	0.4	0.5	0.6	0.9

Appendix I

TAG composition of stearin fractions from lab-scale fractionatin of batch RMIM

Triacylglycerols	Stearin 8 °C	Stearin 10 °C	Stearin 12 °C	Stearin 15 °C	Stearin 18 °C
Total triunsaturated	**10.5**	**9.9**	**9.7**	**10.5**	**10.6**
OLL	0.9	0.8	0.9	0.9	0.9
OLO	3.8	3.6	3.5	3.7	3.7
OOO	5.9	5.6	5.3	5.9	6.1
Total diunsaturated	**30.9**	**30.2**	**28.0**	**29.9**	**30.3**
PLL	1.8	2.0	2.0	2.0	2.1
PLO	9.8	9.5	8.8	9.6	9.6
POO	17.4	16.9	15.7	16.7	16.7
SOO	2.0	1.9	1.6	1.7	2.0
Total disaturated	**37.5**	**40.6**	**40.4**	**35.9**	**33.1**
MLP	0.7	0.7	0.7	0.6	0.6
PLP	7.8	8.1	7.8	7.3	6.9
POP	23.9	26.0	26.1	23.0	20.9
POS	4.9	5.2	5.1	4.5	1.2
SOS	0.4	0.8	0.7	0.6	0.6
Total trisaturated	**7.6**	**7.2**	**8.6**	**10.0**	**12.2**
PPP	6.1	5.8	7.0	8.0	9.7
PPS	1.5	1.4	1.7	2.0	2.5

Appendix J

Fatty acid composition of stearin fractions from lab-scale fractionatin of batch RMIM

Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
Stearin 8 °C	0.2	1.2	45.4	4.9	0.5	**52.2**	0.2	37.5	**37.7**	10.0	0.1	**10.1**	49.9
Stearin 10 °C	0.2	1.2	46.2	5.0	0.5	**53.1**	0.2	36.9	**37.1**	9.7	0.1	**9.8**	48.8
Stearin 12 °C	0.2	1.3	48.3	5.0	0.5	**55.2**	0.2	35.3	**35.5**	9.1	0.1	**9.2**	46.4
Stearin 15 °C	0.2	1.2	47.9	5.0	0.5	**54.9**	0.2	35.4	**35.6**	9.4	0.1	**9.5**	47.0
Stearin 18 °C	0.2	1.2	49.3	5.1	0.5	**56.2**	0.2	34.2	**34.4**	9.2	0.1	**9.3**	45.6

Appendix K

TAG composition of stearin fractions from lab-scale fractionatin of batch TLIM

Triacylglycerols	Stearin 9 °C	Stearin 12 °C	Stearin 15 °C	Stearin 18 °C	Stearin 21 °C
Total triunsaturated	**12.6**	**12.8**	**13.2**	**14.1**	**14.1**
OLL	1.3	1.3	1.3	1.4	1.4
OLO	4.8	4.9	5.1	5.4	5.4
OOO	6.6	6.9	6.9	7.4	7.3
Total diunsaturated	**26.1**	**26.4**	**27.5**	**29.0**	**29.1**
PLL	1.9	2.0	2.0	2.1	2.2
PLO	8.5	8.6	9.0	9.5	9.6
POO	14.3	14.5	15.0	15.8	15.8
SOO	1.5	1.4	1.6	1.6	1.6
Total disaturated	**28.4**	**30.0**	**28.1**	**25.7**	**25.9**
MLP	0.5	0.6	0.6	0.5	0.6
PLP	5.3	5.7	5.3	5.1	5.1
POP	18.1	19.2	17.9	16.3	16.4
POS	3.9	4.0	3.8	3.4	3.5
SOS	0.7	0.6	0.6	0.5	0.4
Total trisaturated	**12.4**	**13.3**	**13.3**	**12.6**	**14.3**
PPP	9.6	10.3	10.3	9.8	10.9
PPS	2.9	3.0	3.0	2.9	3.4

Appendix L

Fatty acid composition of stearin fractions from lab-scale fractionatin of batch TLIM

Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
Stearin 9 °C	0.2	1.2	46.3	5.1	0.5	**53.3**	0.2	36.4	**36.6**	10.0	0.0	**10.0**	48.7
Stearin 12 °C	0.2	1.2	47.4	5.2	0.5	**54.5**	0.2	35.6	**35.8**	9.7	0.0	**9.7**	47.4
Stearin 15 °C	0.2	1.2	46.5	5.1	0.5	**53.4**	0.2	36.3	**36.5**	10.0	0.0	**10.0**	48.5
Stearin 18 °C	0.2	1.2	44.6	4.9	0.5	**51.4**	0.2	37.8	**38.0**	10.5	0.0	**10.5**	50.8
Stearin 21 °C	0.2	1.2	45.7	5.0	0.5	**52.6**	0.2	36.8	**37.0**	10.2	0.1	**10.3**	49.6

Appendix M

TAG composition of stearin fractions from lab-scale fractionatin of packed-bed TLIM

Triacylglycerols	Stearin 9 °C	Stearin 12 °C	Stearin 15 °C	Stearin 18 °C	Stearin 21 °C
Total triunsaturated	**14.1**	**14.8**	**15.7**	**15.5**	**15.5**
OLL	1.5	1.8	1.9	1.9	1.9
OLO	5.4	5.8	6.2	6.1	6.1
OOO	7.1	7.3	7.6	7.5	7.6
Total diunsaturated	**30.7**	**31.1**	**32.5**	**32.6**	**32.5**
PLL	2.0	1.6	1.5	2.3	2.1
PLO	9.8	10.3	11.0	10.9	10.7
POO	17.0	17.5	18.3	17.9	17.9
SOO	1.8	1.8	1.6	1.6	1.8
Total disaturated	**35.6**	**33.8**	**31.2**	**30.4**	**29.5**
MLP	0.7	0.6	0.6	0.6	0.6
PLP	6.8	6.6	6.3	6.1	5.8
POP	22.7	21.5	19.7	19.3	18.8
POS	4.8	4.5	4.1	3.9	3.9
SOS	0.6	0.6	0.5	0.5	0.4
Total trisaturated	**12.2**	**12.5**	**11.8**	**12.6**	**13.2**
PPP	9.5	9.7	9.1	9.8	10.2
PPS	2.7	2.8	2.7	2.8	3.0

Appendix N

Fatty acid composition of stearin fractions from lab-scale fractionatin of packed-bed TLIM

Sample	Fatty acid composition (Area %)												IV
	C12:0	C14:0	C16:0	C18:0	C20:0	SFA	C16:1	C18:1	MUFA	C18:2	C18:3	PUFA	
Stearin 9 °C	0.2	1.2	44.0	4.8	0.5	**50.7**	0.2	38.4	**38.6**	10.6	0.1	**10.7**	51.7
Stearin 12 °C	0.2	1.2	43.9	4.7	0.5	**50.5**	0.2	38.4	**38.6**	10.7	0.2	**10.9**	52.0
Stearin 15 °C	0.2	1.2	42.5	4.6	0.5	**49.0**	0.2	39.7	**39.9**	11.1	0.1	**11.2**	53.6
Stearin 18 °C	0.2	1.2	43.0	4.6	0.5	**49.5**	0.2	39.0	**39.2**	11.0	0.2	**11.2**	53.2
Stearin 21 °C	0.2	1.2	43.2	4.7	0.5	**49.8**	0.2	39.1	**39.3**	10.9	0.1	**11.0**	52.8

LIST OF PUBLICATIONS

The work presented in this thesis has been published / presented either fully or partially in the following: -

Journal

Saw M. H., Chuah C. H., and Siew W. L. (2009). Characterization of low saturation palm oil products after continuous enzymatic interesterification and dry fractionation. *J Food Science*. **74**. E177-E183.

Conference

Saw M. H., Siew W. L., and Chuah C. H. (2007). Regiospecificities of sn-1,3 specific lipases for enzymatic interesterification for RBD palm olein. Poster presented in Malaysian Science and Technology Congress 2007, 4-6th September 2007, Subang Jaya, Selangor.

Saw M. H., Siew W. L. and Chuah C. H. (2008). Enzymatic interesterification of RBD palm olein by lipase *Rhizomucor Miehei*. Poster presented in 2008 National Seminar on Palm Oil Milling, Refining Technology, Quality and Environment, 15-16th October 2008, Kota Kinabalu, Sabah.

Saw M. H., Siew W. L. and Chuah C. H. (2009). Physical properties of enzymatic interesterification of palm olein. Poster presented in MPOB International Palm Oil Congress 2009 (PIPOC 2009), 9-12th November 2009, Kuala Lumpur.