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Enzymatic Interesterification of Hard Stocks

A project report presented in partial fulfillment of the requirements of the Master of Food Technology at Massey University

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Abstract

The objective of this study was to use enzyme interesterification to produce two hard stocks which were based on hard stocks used in the manufacture of margarine and pastry fats. Both of these two hard stocks produced need to be fast crystallizers; one with a low melting point for spreadable margarine whiles the other with a higher melting point for pastry fats. Commercial hard stocks were provided by Bakels Edible oil (BEO) Ltd. The study was divided into three stages. In the first stage, three commercial lipase enzymes supplied by Novozymes, including Novozyme 435, Lipozyme RM IM and Lipozyme TL IM, were used to interesterify tallow stearin, palm stearin and fully hardened coconut oil mixed in different ratios. The most promising fat blend with the lipase enzyme was selected for optimisation trials in stage two of the study. In stage two, the amount of lipase enzyme was investigated along with the time required to process these fats in order to optimise the interesterification method as both enzymes and production time are cost factors associated with the successful application of this hard stock. The commercial lipase enzymes are the key to the interesterification process and are expensive hence in stage three of this study, the reusability of the enzymes was looked into in order to determine the maximum number of uses that can take place for one dose of enzyme during batch processing. The resulting interesterified fats at the end of each stage were tested for physical properties such as melting point, solid fat content, rate of crystallization, and change in specific heat during crystallization and chemical composition of triglyceride content. The best result for spreadable margarine was a blend of palm stearin and fully hardened coconut oil at 50%:50% and interesterified with 4% of Lipozyme TL IM at 65°C for 8 hours to achieve a melting point of 44°C. The best processing method for pastry margarine was blend of tallow stearin and fully hardened coconut oil at 70%:30% interesterified with 4% of Lipozyme RM IM at 65°C for 8 hours to achieve a melting point of 44°C. Both of these interesterified hardstocks were also fast crystallisers as determined using differential scanning colorimetry and nuclear magnetic resonance instrumentation. Each batch of enzyme was able to be reused up to seven times if washed with acetone and deionized water.

Acknowledgements

I would like to express my sincere gratitude and thanks to my supervisor Associate Professor Marie Wong for the continuous support, encouragement, patient guidance and her wisdom. Her expert technical knowledge has helped me to overcome many obstacles during this project.

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Contents

List of Abbreviations

Abbreviation	Full name
SFC	Solid fat content
DSC	Differential scanning calorimetry
HPLC	High pressure liquid chromatography
TAG	Triglyceride
ECN	Equivalent carbon number
TS	Tallow stearin
PS	Palm stearin
FHCO	Fully hardened coconut oil
TSFHCO	Tallow stearin and fully hardened coconut oil
PSFHCO	Palm stearin and fully hardened coconut oil
TSPS	Tallow stearin and palm stearin
Cp	Caprylic acid
C	Capric acid
L	Lauric acid
M	Mysteric acid
P	Palmitic acid
O	Oleic acid
S	Stearic acid

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Chapter 1 Introduction

The production of margarine and baking fats requires modification of the physical fat properties of the hard stocks used (Liu & Meng, 2009). Hard stock fats are fats used during the production of margarine in order to improve the physical characteristics of the margarine. Current methods used to manufacture hard stocks include hydrogenation, fractionation and chemical interesterification (O'Brien, 2009). Enzyme interesterification is a new method of hard stock production. In enzymatic interesterification, commercial lipases were reacted with the hard stock raw materials and the physical characteristics of the hard stocks are changed by rearranging the structure of the triglycerides present. Unlike other methods (hydrogenation, fractionation and chemical interesterification), the enzymatic rearrangement process does not lead to formation of trans-fatty acids nor does it require a large amount of heat as the reactions are carried out at temperatures between 50 to 70°C, and no harmful chemicals are discharged as waste hence less concerns regarding effluent treatment and more environmentally responsible (Bhaggan, 2010).

The objective of this study was to use enzyme interesterification to produce two hard stocks which are base fats used in the manufacture of margarine and pastry fats. Both of these two hard stocks produced need to be fast crystallizers; one with a low melting point for spreadable margarine whiles the other with a higher melting point for pastry fats.

Chapter 2 Literature review

2.1 Introduction

Interesterification is a chemical process used by food manufactures to tailor the physical and chemical properties of food lipids (Hamilton, 1999). Food lipids such as vegetable and animal fats and oil consist largely of triglycerides which are made up of an ester of glycerol and three molecules of fatty acids as indicated in Figure 1 (Padley & Gunstone, 1997). Interesterification takes place when fatty acid esters on one triglyceride are exchanged for another fatty acid ester on another triglyceride. This process does not affect the degree of saturation, the isomerization of a fatty acid double bond or change the composition of fatty acids but rearranges the fatty acids on the glycerol molecule as indicated in Figure 1. Interesterification can be defined as a redistribution of the fatty acid moieties present in triglyceride e.g. the fatty acids palmitic acid (P), oleic acid (O) and lauric acid (L) can be interesterified (IE) to the following combinations LPO, OLP and PLO (Dijkstra, 2011). Interesterified lipids are primarily used as coating fats, margarines, and baking fats or deep frying fats; the purpose of any rearrangement is to modify the melting point, slow rancidity and to create an oil with a low content of saturated fats (Rosseau, 2002). Interesterification can happen naturally but takes a very long time hence manipulation of this process is used for commercial refining (Bockisch, 1998). Interesterification is a term often used to describe many reactions which includes acidolysis, alcoholysis and transesterification (Senanayake, 2005a).

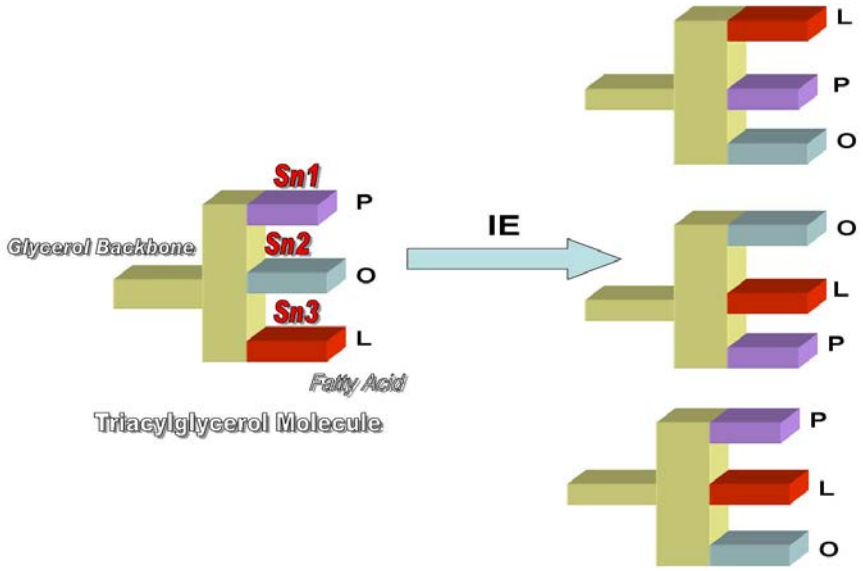

Figure 1. Structure of triglyceride and rearrangement of fatty acids (Karupaiah & Sundram, 2007) P = Palmitic acid, O = Oleic acid, Lauric acid,

Sn1 =Stereospecific number 1 denotes the enantiomer at carbon position 1 on the glycerol backbone (Senanayake, 2005a).

Sn 2 = Stereospecific number 1 denotes the enantiomer at carbon position 1 on the glycerol backbone (Senanayake, 2005a).

Sn 3 = Stereospecific number 1 denotes the enantiomer at carbon position 1 on the glycerol backbone (Senanayake, 2005a).

2.1.1 Acidolysis

Acidolysis involves the transfer of an acyl group between free fatty acids (FFA) and a triglyceride (TAG) molecule under acidic conditions. The acid added to this reaction may cause partial hydrolysis of a fatty acid from a triglyceride molecule (TAG). Hence forming a diacylglycerol (DAG) or monoacylglycerol (MAG) with free hydroxyl groups that are able to react with FFA to form new TAGs. This reaction can produce a mixture of reactants and products or can be driven to completion by physically removing one of the products (Kuo & Gardner, 2002) (Figure 2).

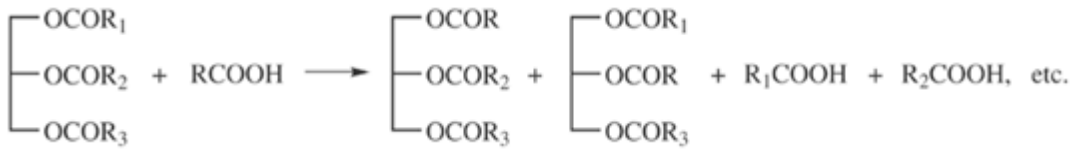

Figure 2. Acidolysis interesterification reaction from reactant to products (Senanayake, 2005a).

2.1.2 Alcoholysis

Alcoholysis occurs when a TAG reacts with alcohols in the presence of strong acids or bases producing a mixture of alkyl esters and glycerol. The primary application of alcoholysis is the production of DAGs and MAGs (Figure 3). However, DAGs and MAGs are undesirable by-products in food lipids hence alcoholysis is often avoided in the interesterification reactions (Senanayake, 2005a).

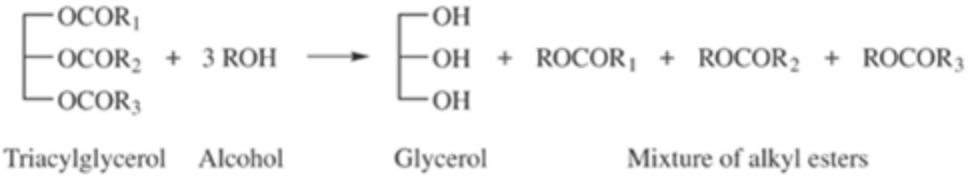

Figure 3. Alcoholysis interesterification reaction from reactant to products (Senanayake, 2005a).

2.1.3 Glycerolysis

Glycerolysis is an alcoholysis reaction in which glycerol acts as an alcohol in order to produce DAGs and MAGs (Figure 4). Excess amounts of glycerol are combined with TAGs with an alkali catalyst at elevated temperatures until random distribution of the TAGs is achieved. The reaction mixture after reaching equilibrium is cooled and deactivated by food grade acid and the catalyst separated by filtration while excess glycerol is removed by vacuum distillation. This reaction is capable of generating substantial yields of DAGs and MAGs (O'Brien, 2009).

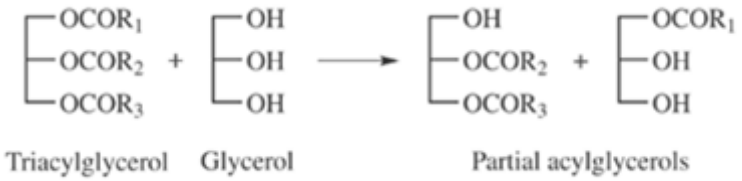

Figure 4. Glycerolysis interesterification reaction from reactant to products (Senanayake, 2005a).

2.1.4 Transesterification

Transesterification is the most common type of interesterification, when the fatty acids within individual TAGs and from other TAGs are mix and matched. This reaction involves the cleavage of ester bonds between fatty acid residues and the glycerol backbone followed by formation of a TAG by re-esterification of newly liberated fatty acids until equilibrium is reached, Equation 1 indicates the equal mixture of mono, di and tri-saturate TAG components once the reaction has reached equilibrium (Senanayake, 2005a).

$$SSS <=> (SUS <=> SSU) <=> (SUU <=> USU) <=> UUU$$

Equation 1: The equilibrium state of saturated and unsaturated fatty acids of transesterification (Senanayake, 2005a)

S = saturated fatty acids

U = unsaturated fatty acids

2.2. Interesterification in oil refining

The treatment of interesterification is carried out on refined oil before bleaching and deodorization. The oil needs to be properly dried to avoid catalyst deactivation prior to interesterification (Bockisch, 1998). The process flow diagram in Figure 5 shows the typical process of chemical interesterification (CIE) where the catalyst refers to the most commonly used, sodium methylate at 0.2 - 0.5% by weight of the oil (Minal, 2003).

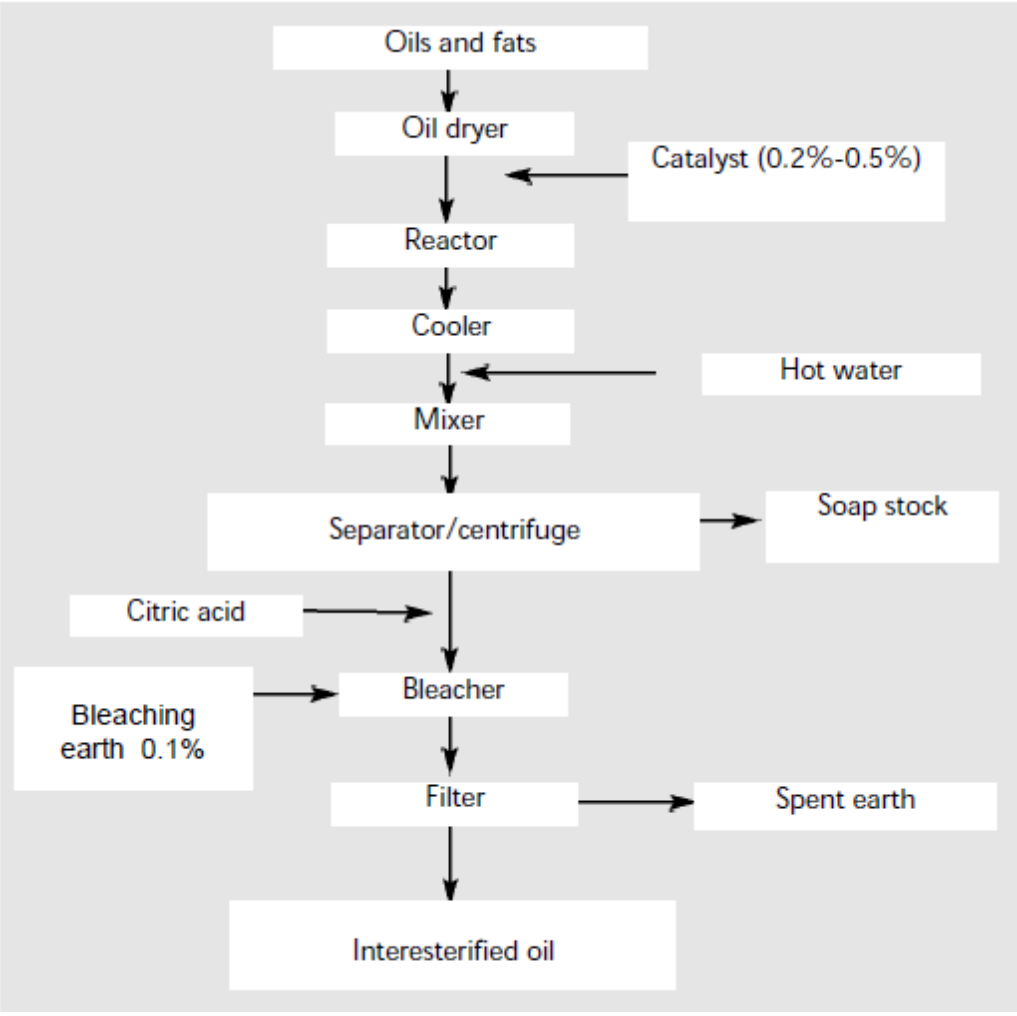

Figure 5: Flow chart of the chemical interesterification process (Minal, 2003).

2.2.1 Chemical Interesterification

Interesterification is also known as “randomization” due to the random shuffling of fatty acids which are detached from the glycerol back bone in the presence of the catalyst. Several catalysts can be used to assist the completion of this reaction yet little control can be exercised over the rate of the reaction. The percentage of product interesterified can only be determined by estimation or probability due to the randomized formation of newly interesterified fats (Padley & Gunstone, 1997).

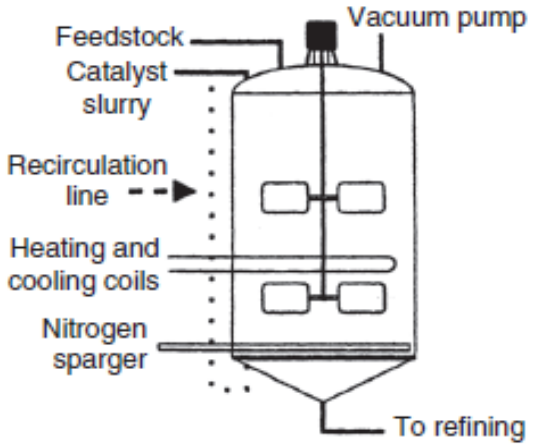

Figure 6. Batch reactor for chemical interesterification (Rosseau, 2002).

Figure 6 shows a batch reactor system used during chemical interesterification. In a batch chemical interesterification process, the incoming fat was first dried under vacuum and heated to 120 - 150°C to reduce the moisture level of the oil. The oil was then cooled to 70 - 100°C before the addition of catalyst under vacuum (Liu & Lampert, 1999). The most common catalyst used was the alkali powder sodium methoxide (sodium methylate). The amount of catalyst added is dependent on the oil quality after bleaching. Excess amount of catalyst may be required to neutralize the fat and decrease the amount of free fatty acids hence the formula used to calculate the required amount is (FFA × 0.19) + 0.06% (w/w). This calculation is generally employed as a rough estimate on the amount of catalyst required for chemical interesterification (O'Brien, 2009). The mixture is agitated for 30 to 60 minutes until the white slurry is transformed to a brown colour at which point a sample is taken for analysis in order to determine the reaction end point. The catalyst is neutralized towards the endpoint of the reaction and the neutralization agents used include phosphoric acid, citric acid or carbon dioxide. The deactivation by neutralization is to avoid the formation of sodium

hydroxide and methyl alcohol prior to washing in order to reduce product loss from soap formation (O'Brien, 2009).

2.2.2 Catalyst

Interesterification can happen naturally but takes a very long time hence manipulation of this process is used for commercial refining. The types of catalysts used can be grouped into acid, base and their corresponding metal and salts (Liu & Lampert, 1999). The name, dosage and operating conditions for some of the commonly used CIE catalysts are listed in Table 1.

Table 1. List of selected catalyst and operating conditions for CIE (Minal, 2003***).***

Type of catalyst	**Dosage (% oil weight)**	**Temperature**	**Time (min)**	**Example**
Metal Alkylate	0.1 - 1.0	25 - 270	5 - 120	Sodium methylate
Alkali metal amides	0.15 - 2.0	80 - 120	10 - 60	Sodium amide
Alkali metal hydroxides	0.2 - 2.0	170	30 - 120	Sodium hydrides
Alkali hydroxides	0.5 - 2.0	250	45 - 90	Sodium hydroxide, potassium hydroxide or sodium hydroxide & glycerol

Metal alkylates such as sodium methylate (sodium methoxide) are some of the most widely used chemicals to initiate catalytic reactions by CIE refineries due to their ability to operate at low temperatures, ease of dispersion in fat, relatively low cost and can operate without vacuum conditions. However, sodium methylate is very reactive hence care must be exercised during handling and sodium methylate has tendencies to form unwanted by-products as well as leading to fat losses during termination (Rosseau, 2002). The catalyst must be inactivated and removed at the end of the process. The loss ratio related to the use of sodium methylate is approximately 11 to 1 (w/w) with every kilogram of catalyst used, with further loss during bleaching and deodorizing (O'Brien, 2009).

2.2.3 Reaction mechanisms

There are several mechanisms explaining the repositioning of fatty acids between triglyceride hydroxyl sites. Claisen condensation describes the mechanisms for the formation of an enolate ester and carbonyl ion from a triacylglycerol molecule with the catalyst sodium methylate (Senanayake, 2005a). Figure 4 describes the mechanisms of enolate formation with catalyst during Claisen condensation.

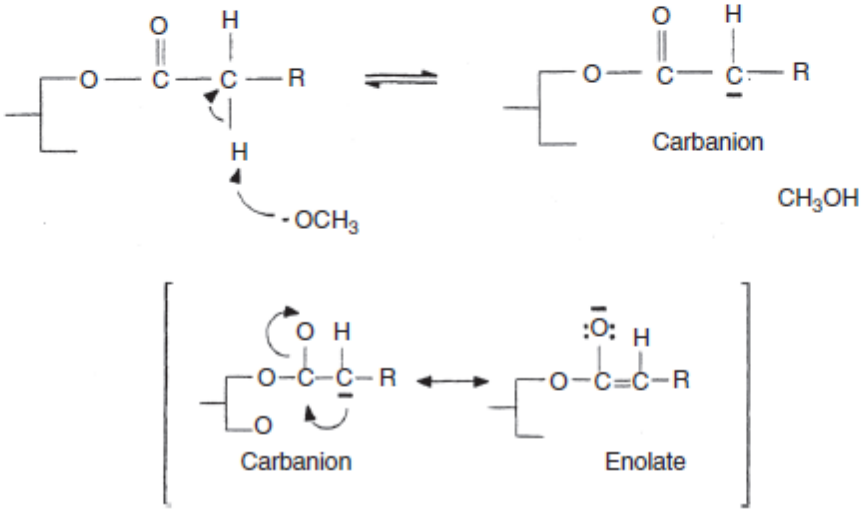

Figure 7. Formation of carbanion and enolate during Claisen condensation (Rosseau, 2002)

The carbanion is formed when the positively charged carbonyl carbon is attacked by the sodium methylate catalyst causing the formation of enolate ester (Rosseau, 2002). This carbanion is a strong nucleophile which will attack the carbonyl groups forming β–keto ester intermediates and glycerylate as shown in Figure 7. While the newly formed glycerylate further attacks esters intra-molecularly and inter-molecularly until equilibrium is reached (McMurry, 2011).

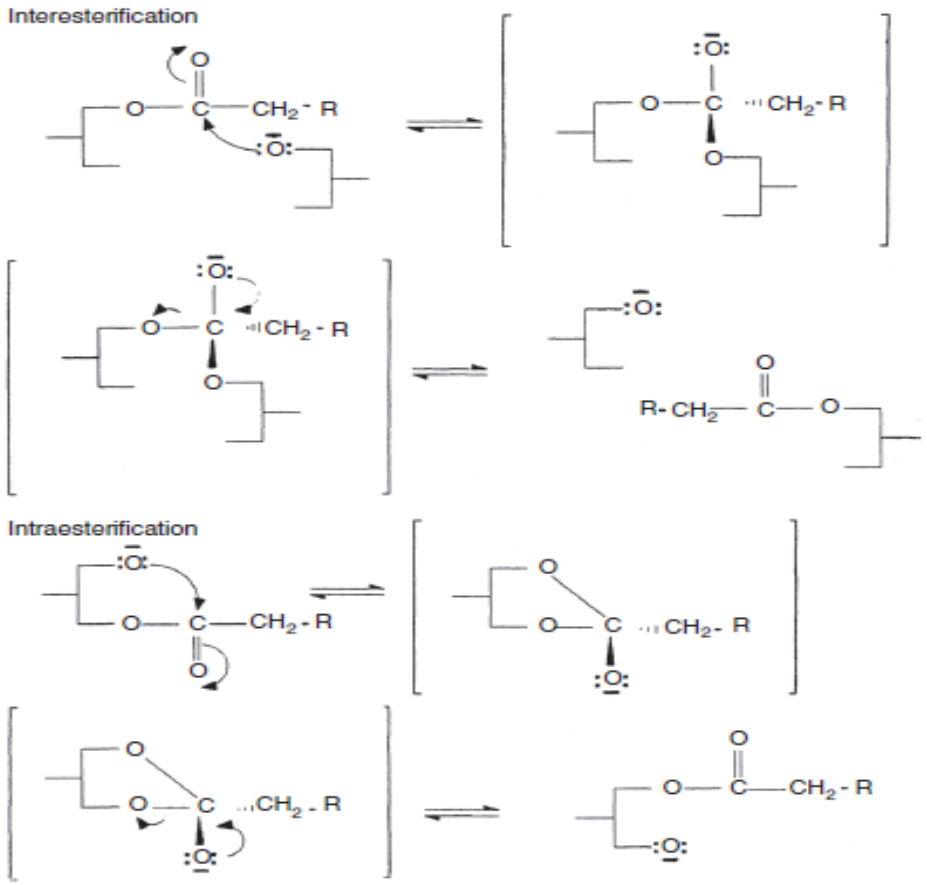

Figure 8. Reaction of glycerylate with triacylglycerol intra-molecularly and inter-molecularly (Rosseau, 2002).

The enolate ion formation mechanism is associated with the use of the catalyst sodium methylate and suggests that the catalyst reacts with the acidic hydrogen from the carbon (alpha) during intraesterification to produce an enolate ester as indicated in Figure 8. This reaction produces a carbanion which is a strong nucleophile that attacks the carbonyl groups forming a (beta) keto ester intermediate and a glycerylate. The glycerylate further attacks other carbonyl carbons and exchanges esters intra & inter-molecularly until equilibrium is reached.

2.2.4 Random interesterification

The random interesterification method is the most commonly used approach to interesterify oils and fats. The fatty acyl groups freely move from one position to another in a single TAG molecule or from one TAG to another during random chemical interesterification (O'Brien, 2009). This reaction continues and does not terminate until equilibrium is reached at which point the arrangement of fatty acids in the triglyceride is completely random (Rosseau, 2002). Alteration of physical characteristics such as melting points and melting point profile occurs as the starting fats and oil are changed from ordered arrangement to random arrangement (Senanayake, 2005a).

Random interesterification consists of three major rearrangement steps which includes: pre-treatment of oil, reaction with the catalyst and deactivation of the catalyst (O'Brien, 2009). It usually involves mixing at least two oils that have different fatty acid (FA) composition. The classical interesterification (IE) is characterized by a randomization in the distribution of acyl moieties in the TAG molecules after applying a chemical catalyst (El-Shattory & Aly, 1998). CIE causes a statistical randomization of fatty acid distribution that leads to a modification of the TAG composition and consequently, a modification to their physical properties (Minal, 2003). Interesterification reactions performed at temperatures above the melting point of the highest melting component in a mixture results in complete randomization of fatty acids and the percentage yield among all triacylglycerol is determined by the laws of probability during re-esterification (Rosseau, 2002).

2.2.5 Direct chemical interesterification

Direct interesterification can be a valuable extension to random interesterification as this reaction is directed to produce a particular type of TAG hence it is referred to as direct interesterification (Bockisch, 1998). The randomness in the formation of new TAGs is reduced by lowering the temperature of the oil below the melting point of the highest melting point fatty acid and allows it to crystallize. The crystallized high melting point fats are removed and this selective process can be repeated to produce fats with less random arrangement (Senanayake, 2005a). During direct chemical interesterification, the tri-saturates are crystalized and removed from the liquid phase simultaneously. The crystallized tri-saturates can no longer be part of the reaction equilibrium because they are not in the same phase (Gunstone, 2003). The fatty acid composition of the remaining liquid phase keeps changing as the reaction proceeds with the formation of more tri-saturated TAGs than would have otherwise occurred as the liquid phase continues to re-establish the distributed equilibrium (Rosseau, 2002). Crystallization continues until all the triacylglycerol capable of crystallizing has been eliminated from the reaction phase (Gunstone, 2003). In contrast to random interesterification, longer reaction times are required due to the low temperature hence low temperature catalysts such as sodium and potassium alloys are required. The reaction is normally conducted in a continuous process as removal of crystalized fatty acids is difficult to control in batch reactors (Senanayake, 2005a).

2.2.6 Enzymatic interesterification

Enzymatic interesterification requires the use of the enzyme lipase which is obtained from micro-organisms such as bacteria, yeasts and fungi (Padley & Gunstone, 1997). Lipases produced are released into their growth environment to digest lipid materials extra-cellularly (Willis, 2002). The lipase produced is used to catalyse the exchange of fatty acids attached to the glycerol backbone of a fat molecule. Enzymatic interesterification in which fatty acids are exchanged between two fats or within a fat has been used as an alternative to hydrogenation due to their specificity (Cowan, 2011a). The study conducted by Ahmadi, Wright and Maragoni (2008) suggested that both CIE and EIE was able to manipulate the physical charactersterics of fats. Husum (2003) claimed that the EIE method was much preferred than the CIE method due to the specificity of the enzymes (Husum, 2003). The 1, 3-specific lipases allows fatty acids in the sn-1 and sn-3 position to be shifted around while the sn-2 position is left unchanged. This means unsaturated fatty acid that are usually present in the sn-2 position in vegetable oils can be left unaltered hence preserving nutritional value and this specificity cannot be matched by chemical interesterification (Husum, 2003). Holm & Cowan (2008) pointed out that the CIE method was complicated and requires many stages of processing and uses higher temperatures as mentioned in Section 2.2.1 of CIE processing hence more energy was required as indicated in Figure 9. The report also compared waste disposal of the CIE and EIE methods and suggested the EIE method was more efficient as the spent enzyme contains high protein and can be used as animal feed but the CIE spent waste was chemically treated before disposal (Holm & Cowan, 2008).

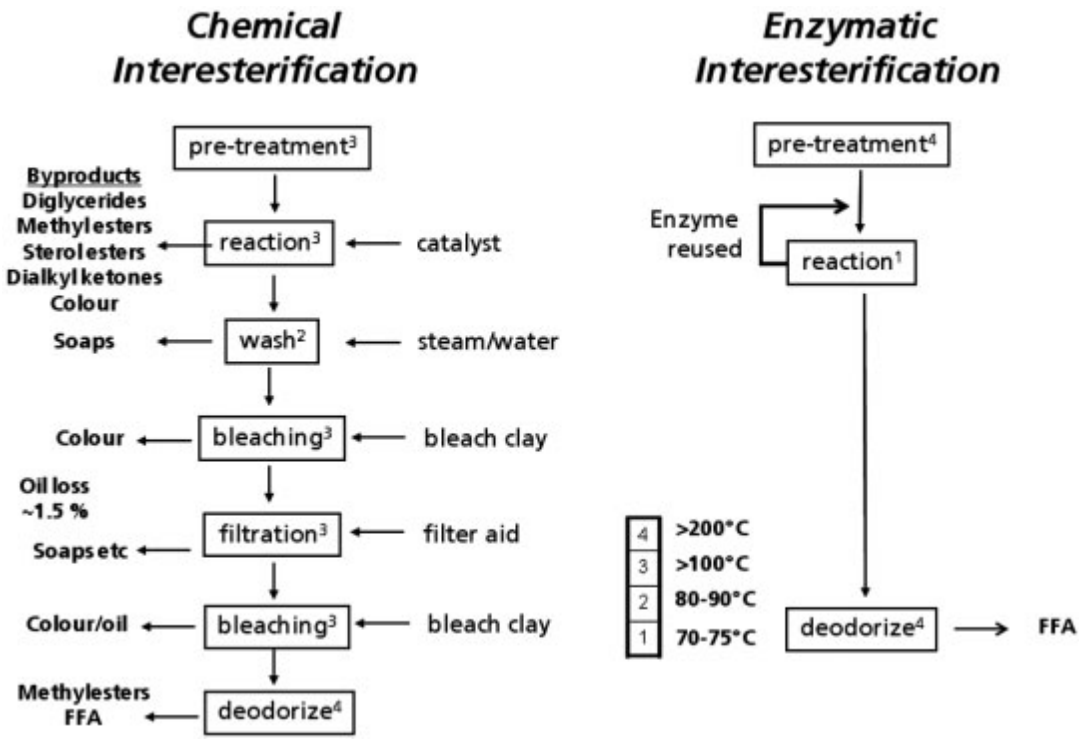

Figure 9. Contrast of processing method between CIE and EIE (Holm & Cowan, 2008).

Enzymatic interesterification can be carried out in a batch or continuous process as the latter is generally used in industrial scaled production (Zhang, 2007). In a continuous process the fat blends are pumped down a column where the lipase enzymes are immobilized. The enzymes catalyse the exchange of fatty acids in order to produce a product of desired melting properties as shown in Figure 10. The reaction vessel is insulated with water jackets and the substrates are introduced from the top of the vessel and passed down the column and continuously removed from the bottom of the reactor through split screens installed in the vessel to prevent the enzymes been carried off by the substrate. The screen is shaped as an inverted triangle with typical mesh sizes of 150 - 450nm and is capable of removing all lipase enzymes and as no residuals were formed during the reaction less processing was required in contrast to the CIE method (Cowan, 2011 b). Figure 11 is a photograph of the continuous enzymatic interesterification plant.

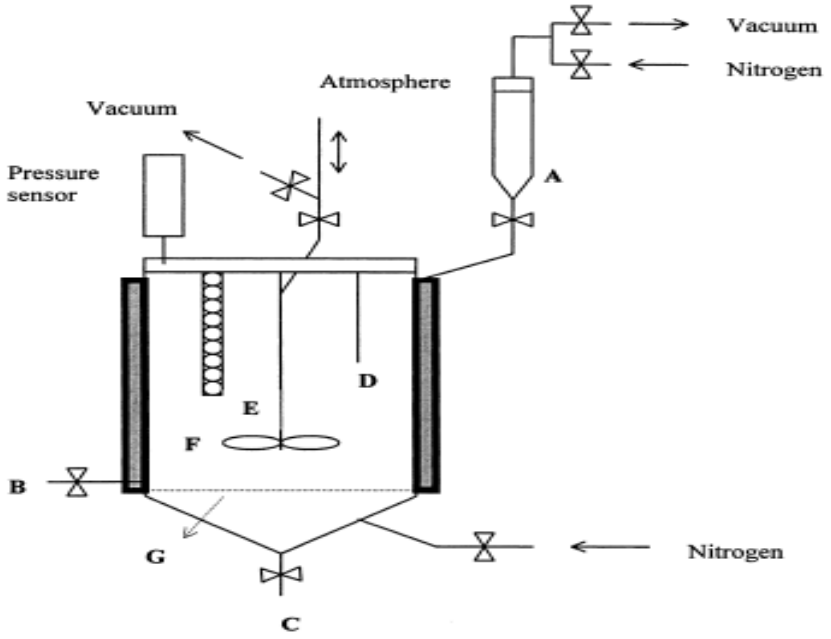

Figure 10. Schematic diagram of the 1kg scale batch reactor (Høy, 2000).

A: funnel; B: sampling valve; C: product valve; D: temperature sensor; E: electric heater; F: Impeller; G: filter

Figure 11. Photo on continuous interesterification reactor, reactor columns are insulated by water jackets (Gibon, 2011).

Table 2. The comparison between chemical and enzymatic process (Gupta, 2008)

Basis	**Chemical process (CIE)**	**Enzymatic process (EIE)**
Type of catalyst	Sodium methoxide	Immobilized lipase enzyme
Initial cost of catalyst	Low	High
Number of uses for the catalyst	Once	Multiple
Initial capital cost for the installation	higher	Lower
Operating cost	Lower	Higher
Net cost (Capital + Operating)	Same as enzymatic process	Same as chemical process
Side reactions	Produces monoglyceride, diglyceride, soap and methanol	No side reactions
Process loss	Due to several side reactions Oil absorption by the bleaching clay in post-bleaching	Low
Environmental impact	Less friendly	Environmentally friendly
Feed oil	Refined and bleached	Refined, bleached and deodorized
Feed oil quality required	Very high	Very high
Reaction temperature	80-100°C	70°C
Final product attributes Physical (solid content) Functionality	The physical properties and the functionality are identical to the product from the enzymatic process	The physical properties and the functionality are identical to the product from the chemical process
Stability of the transesterified product: oxidative flavour	Both oxidative and flavour stability tend to be lower than the original oil blend (in the deodorized state). This is partly because the oil blend is bleached before and after the interesterification process (referred to as post-bleaching), causing higher loss of tocopherols.	Both oxidative and flavour stability are closer to those of the original oil blend (in the deodorized state). The oil blend is bleached only once before the interesterification reaction.
Retained tocopherols (ppm)	30 – 39% of the original feed	64 – 79% of the original feed

The commercial parameters of CIE and EIE methods was contrasted and presented in Table 2 and as the results suggests, the net operating cost for CIE and EIE methods were the same yet EIE method was more environmentally friendly and requires less energy. The EIE method also has higher tocopherol content in the end product.

2.3 Lipases

Lipases are a class of enzyme that are water soluble and can act on water insoluble substrates and are stable in both polar and non-polar environments (Willis, 2002). The reaction system of enzymatic interesterification over two or more fats consists of the lipase catalyst working with a very small amount of water dispersed in a continuous organic phase (Gunstone, 2003). The enzyme lipase can act within the bulk body of the interesterified fats or at the interface yet their performances are much better at the interface and this phenomenon is also known as interfacial activation (Willis, 2002). The three commercial lipases used for this project includes Novozyme 435, Lipozyme RM IM and Lipozyme TL IM. The manufacture of the three lipase enzyme Novozyme claims all three enzymes are position Sn1, 3-specific.

2.4 Factors affecting lipase activities during enzymatic interesterification

Enzymatic activity and stability during interesterification is predominantly affected by operational temperature, pH, water content, reaction time, reusability, concentration of enzyme, purity of the enzyme and presence of other surface active agents (Cowan, 2011a).

2.4.1 Temperature

An increase in temperature will lead to an increase in the rate of interesterification, yet extreme temperatures can reduce the rate of reaction and may lead to irreversible denaturation of the enzyme (Kuo & Gardner, 2002). The temperature for reaction must be kept high enough to keep the substrate in a liquid state and low enough for the organic solvents to remain in the system. Immobilization has been found to improve the stability of the lipase enzyme under very high temperature and contributes towards reduction on the half-life of the lipase hence increasing the enzyme's reusability (Ozturk, 2001). The catalytic efficiency will increase with temperature over a finite range. Immobilization fixes the enzyme in one conformation which lowers the enzyme's susceptibility to denaturation by heat, with optimal activity range between 30 - 62 °C. (Weber, Weitkamp, & Mukherjee, 2003) suggested that an increase in reaction temperature usually leads to an acceleration of reactions catalyzed by enzymes. Temperatures ranging from 50 - 90 °C were applied to keep the lard in a liquid state. The optimal operational temperature for Lipozyme RM IM was at 40 °C and 50 °C for Novozyme 435 and Lipozyme TL IM(Kowalski, Tarnowska, & Gruczynska, 2005).

$$log\Delta R = \frac{\Delta T}{10 \times logQ_{10}}$$

Equation 2: Change in reaction rate with increase of temperature (Zhang, Smith, & Adler-Nissen, 2004).

The quotient Q10 is often used in biology which is defined as the increase of reaction rate due to a 10 degree change in temperature. Q_{10} falls within the range of 2 - 3 for most chemical and enzymatic reactions and the relationship between the change in reacition rate ΛR, the change in temperature T and reaction rate Q_{10} is described in Equation 2.

The effect of temperature on immobilized lipozymes was investigated by (Zhang, 2007) who conducted trials between 50 - 75 °C. Little changes in the degrees of interesterification were observed within this temperature range but slight increases in FFA content occurred at higher temperature hence it was recommended that low reaction temperatures should be used (Zhang, 2007).

2.4.2 Water

The presence of water in the system is important for thermal stability, catalytic activity, protein stability, active site stability and structural integrity (Senanayake, 2005a).

A certain amount of water is required for the lipase catalyzed reaction to occur in a lipid substrate, but the content of water has varying effects on various lipases. Water is required in the reaction medium to maintain a layer of water around the enzyme molecule in order to maintain the activity of its three dimensional conformational state (Willis, 2002). The content of water should be kept as low as possible in order to avoid hydrolysis of the lipid substrate yet complete removal of water can cause lipase deactivation. The required water content for lipases can range from 0.04 % to 11% (w/w). The optimal water content for a specific enzyme such as *Candida Antarctica* is generally around 1 - 2 %(w/w), as too much water may also lead to an increase in lipase activity and increase in by-product production (Høy, 2000). The interesterification reaction was not affected with the addition of water under default conditions yet the percentage yield of TAG was affected due to the increase of DAGs and FFAs. The increase in water content causes a decrease in the formation of TAGs with an increase in the formation of byproducts like DAGs hence water content should be minimized (Zhang, 2007).

2.4.3 pH

The commercial lipases only remain catalytically active at certain pH depending on their origin and the ionization state of residues in their active sites. The tertiary structure of the lipases is sensitive to pH changes and can be easily disrupted. Lipases can be active over a wide range of pH from 4 to 10 yet the catalytic activity is only optimum at pH 7 - 9 where typical lipases from *Aspergillus Niger* are known to be acidic. The lipase reaction is generally conducted under neutral pH hence not monitored closely due to the versatility of the enzyme (Willis, 2002). However, denaturation of large quantities of enzymes can be observed visually as aggregated substances in the substrate. If activity of an enzyme is plotted against the pH, a bell shaped curve usually results with either sharp or broad pH optimum (Kuo & Gardner, 2002).

2.4.4 Surface active agents

Lipase activity during interesterification can be improved with the presence of surface active agents such as lecithin or sugar esters. However, the presences of surface active agents in the form of emulsifiers will cause a dramatic reduction in the rate of interesterification as they can prevent contact between lipase and the substrate. Phospholipase can cause inhibition of the active site preventing reaction of the TAG with the lipase active site. Phospholipase should be reduced to less than 500 ppm in order to prolong the half-life of the immobilized enzyme (Senanayake, 2005a).

2.5 Reactors

2.5.1 Methods for large scaled production of structured lipids:

Enzyme reactors differ from chemical reactors by being able to operate at lower temperature and lower pressures. An enzyme reactor is the container which a reaction is catalyzed by free or immobilized enzymes. The types of reactors commonly used include packed bed reactors, continuous stirred tank reactors, stirred tank batch reactors and membrane bioreactors (O'Brien, 2009).

2.5.2 Continuous stirred tank reactors

A continuous stirred tank reactor is a reactor in which the rate of substrate input is at the same rate as the product output. The consistency of liquid phase composition is ensured by proper agitation provided by the impeller. This is an ideal system for large scaled production where cost and time of production are considered as limiting factors (Kuo & Gardner, 2002). A diagram for continuous enzymatic interesterification reactor is shown in Figure 12.

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Equation 3: Michaelis Menten equation (Bisswanger, 2008)

The reaction rate of a single enzyme reacting on a single reactant can be described by the Michaelis Menten equation (Equation 3) where:

V_1 is the reaction rate

S is the concentration of substrate

Km is the Michaelis-Menten constant

V_{max} is the maximum reaction rate

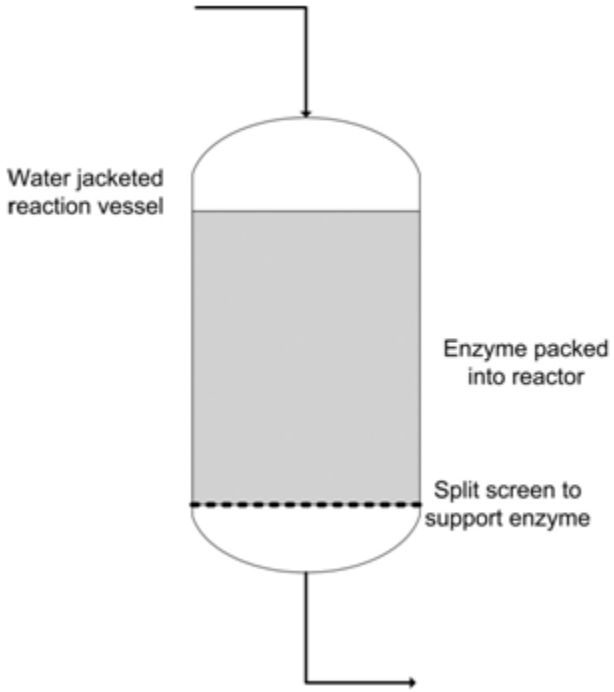

Figure 12. Indicates the design of a continuous stirred tank reactor (Cowan, 2011 b).

2.5.3 Stirred tank batch reactor

Stirred tank batch reactors at bench scale for lipase catalytic reactions are ideal for technical feasibility of the process as they are simple to operate and versatile (Gupta, 2008). In stirred batch reactors, the composition of the reactant varies during the reaction but stays consistent throughout the reactor system. Since there is no input or output from the system, the substrate to product ratio reduces over time which leads to reduction in the reaction rate (Senanayake, 2005a). Operational parameters such as pH, temperature and substrate to product ratio also remain constant during the course of the reaction. Adjustments such as increasing mixing speed, reducing substrate viscosity and efficient baffling in the reactor will result in better mixing though high shear may cause damage to the biocatalysts (Zhang, 2007). The high ratio between substrate and enzyme in batch reactors requires long reaction times, which encourages the formation of DAGs hence decreasing the purity of the substrate (Kuo & Gardner, 2002).

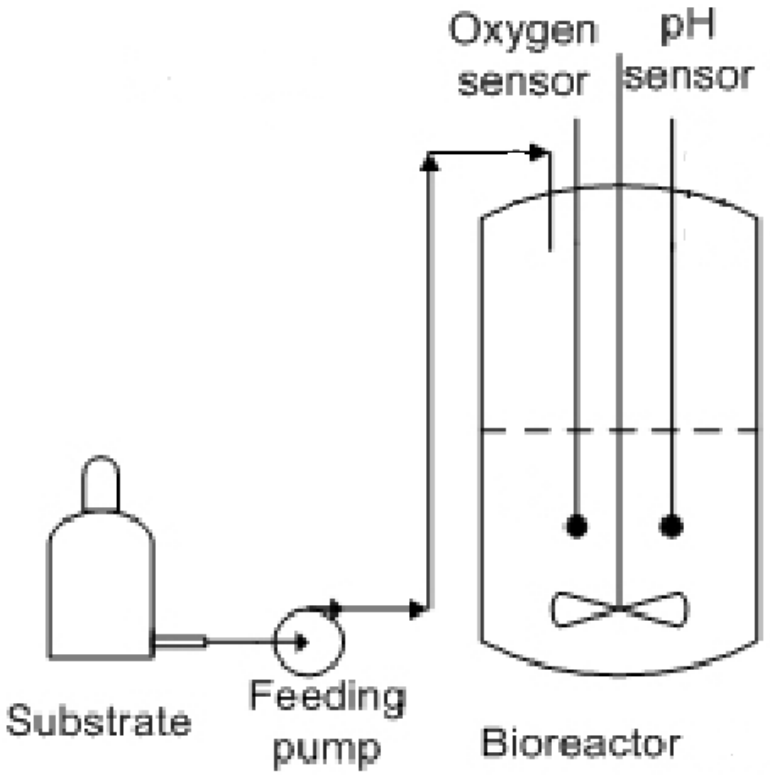

Figure 13. Diagram of batch stirred tank reactor (Salleh, 2013)

2.5.4 Packed bed reactors:

Packed bed reactors are commonly found in industrial scale applications due to their low production cost, ease of operation and ease of construction (Raju, 2011). The substrate feed can be fed into the reactor either from the top or the bottom of the reactor. The substrates are pumped in and out of the reactor at controlled rates (Zhang, 2007). The substrate moves along the column such that there is negligible mixing in the direction of flow, but it is well mixed in the radial direction. The molecules move as a fluid plug without mixing with the previous fluid elements in the reactor as indicated in Figure14 (O'Brien, 2009).

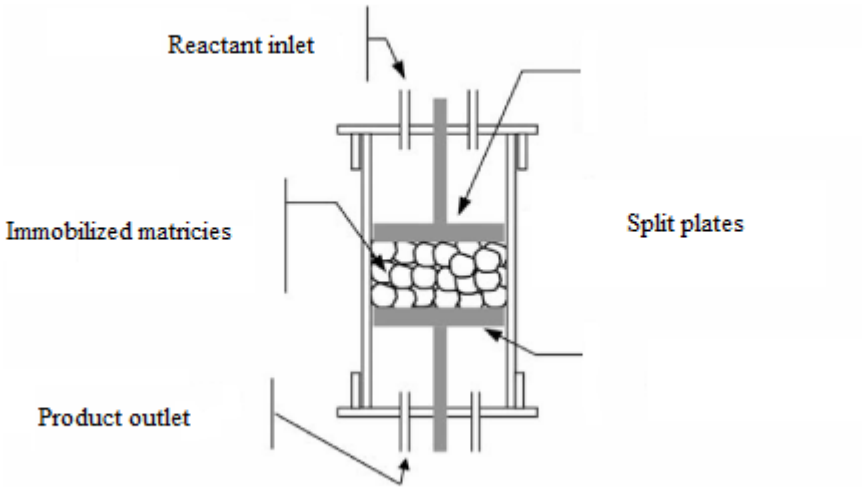

Figure 14. Diagram for packed bed ractor (Prieto, 2008)

2.6 Hardstock Substrates

2.6.1 Tallow stearin

Tallow stearin is the hard fraction of beef tallow after fractionation (Hamilton, 1999). Tallow stearin is used in the production of margarine product due to its economic advantages and vast availability in NZ (O'Connor & Eyres, 2007). The meat industry in New Zealand considers tallow as a by-product and exports approximately 190,000 tonnes annually (MIA, 2011). In 2011, New Zealand exported tallow generated a profit of $153 million (NZ) but these fats are mostly sold as raw materials for soap manufacturing carried out overseas hence interesterifying tallow stearin into edible oils will generate more profit domestically and also increase job opportunities as the processing can also take place domestically (MIA, 2011). Another reason for considering tallow stearin as a substrate was due to its fatty acid composition as beef tallow consists of high levels of long chained fatty acid as indicated in Table 4 which contributes to the physical requirement of hard stock fats. The physical parameters of typical beef tallow were included in Table 3 (Firestone, 1999).

Table 3. Physical properties of beef tallow (Firestone, 1999).

Characteristics	Typical	Range
Refractive index, 40 °C	-	1.448 - 1.460
Iodine value	45	40 - 49
Mettler dropping point, °C	46.5	45 - 48
Solidification point, °C	-	-

Table 4. Saturated and unsaturated TAGs of beef tallow (Senanayake, 2005a).

Characteristics	Typical (%)
SSS tri-saturated	21.5
SUS di-saturated	49.0
SUU mono-saturated	32.5
UUU tri-saturated	1.0
Crystal habit	B’

Table 5. Fatty acid composition of beef tallow (Firestone, 1999)

Characteristics	Typical (%)	Range (%)
C12:0 Lauric acid	0.2	<0.2
C14:0 Myristic acid	4.0	1.4 - 7.8
C14:1 Myristoleic acid	0.5	0.5 - 1.5
C15:0 Pentadecanoic acid	1.0	0.5 -1.0
C16:0 Palmitic acid	24.3	17.0 - 37.0
C16:1 Palmitoleic acid	2.5	0.7 - 8.8
C16:2 Hexadecadienoic acid	-	<1.0
C17:0 Margaric acid	2.1	0.5 - 2.0
C17:1 Margaroleic acid	1.3	<1.0
C18:0 Stearic acid	21.4	6.0 - 40.0
C18:1 Oleic acid	33.6	26.0 - 50
C18:1 Vaccenic acid (*trans*)	4.9	3.4 - 6.2
C18:2 Linoleic acid	1.6	0.5 - 5.0
C18:2 (*trans*)	1.1	0.6 - 1.7
C18:2 Rumenic acid (*Conjugated trans*)	0.9	0.6 - 1.7
C18:3 Linolenic acid	0.6	<2.5
C20:0 Arachidic acid	0.2	<0.5
C20:1 Gadoleic acid	0.1	<0.5
C20:4 Eicosatetraenoic acid	-	<0.5

Tallow contains large amounts of saturated fatty acids and has high melting points as indicated in Table 5. When the fat is fractionated, the solid stearin fraction contains an even higher percentage of saturated fatty acid and the melting point temperature will also be higher (Senanayake, 2005a).

2.6.2 Coconut oil

Coconut oil is known to contain high content of lauric acid which differs from other fatty acids due to their narrow melting range and the characteristic of sharp melting close to body temperature from brittle solids (O'Brien, 2009).

The odour and taste of coconut oil is caused by the presents of lactone as the taste of the oil was described to have a cool, clean and non-greasy sensation in mouth after melting (Amri, 2011; O'Brien, 2009). The physical parameters of coconut oil are listed in Table 6.

Majority of fatty acids in coconut oil are saturated as indicated in Table 7 and 8, this leads to good oxidative stability characteristic of the oil. The rate of hydrolysis for coconut oil can be improved by the presence of enzyme lipase (O'Brien, 2009). Coconut oil is considered for this project due to its high content of 12 carbon fatty acid content, high saturation and the distinctive sharp melting profile of lauric acid which may result in rapid melting characteristic of the modified fats (Bockisch, 1998).

Table 6. Physical properties of coconut oil (Firestone, 1999).

Characteristics	Typical	Range
Refractive index, 40 °C	-	1.448-1.449
Iodine value	10	7.5-10.5
Titer, °C	-	20-24
Mettler dropping point, °C	26.5	25-28
Solidification point, °C	-	14-22

Table 7. Saturated and unsaturated TAG of coconut oil (Senanayake, 2005a).

Characteristics	Typical (%)
SSS tri-saturated	84.0
SUS di-saturated	12.0
SUU mono-saturated	4.0
UUU tri-saturated	0
Crystal habit	B’

Table 8. Fatty acid composition of coconut oil (Firestone, 1999).

Characteristics	Typical (%)	Range (%)
C6:0 Caproic acid	0.5	0.4-0.6
C8:0 Caprylic acid	7.8	6.9-9.4
C10:0 Capric acid	6.7	6.2-7.8
C12:0 Lauric acid	47.5	45.9-50.3
C14:0 Myristic acid	18.1	16.8-19.2
C16:0 Palmitic acid	8.8	7.7-9.7
C18:0 Stearic acid	2.6	2.3-3.2
C18:1 Oleic acid	6.2	5.4-7.4
C18:2 Linoleic acid	1.6	1.3-2.1
C20:0 Arachidic acid	0.1	<0.2
C20:1 Gadoleic acid	trace	<0.2

Like tallow stearin, coconut oil also crystallizes in the B' form which was preferred in edible oils and fats due to its plasticity and crystallization rate (O'Brien, 2009).

2.6.3 Palm Stearin

Palm oil is one of the most widely fractionated vegetable oils in the world today and is commonly used in frying fats, margarines and specialty fats (Hamilton, 1999). The more solid stearin fraction contains a high content of saturated fatty acids as indicated in Table 10 which leads to higher melting point temperature of the fats as shown in Table 9. The crystallization properties of palm stearin are dependent on the composition of the fat. Palm stearin was selected as one of the substrate raw materials due to its long association with margarine production and the high composition of long chain fatty acids that can produce fats with different composition once interesterified with short chained fatty acid fats (Senanayake, 2005a; Khatoon, Khan, & Jeyarani, 2012).

Table 9. Physical properties of palm stearin (Lin, 2011).

Characteristics	Typical	Range
Iodine value	21.6 – 49.4	46.7
SMP (°C)	44.5-56.2	47.7

Table 10. Fatty acid composition for palm stearin (Lin, 2011).

Characteristics	Typical (%)	Range (%)
C12:0 Lauric acid	0.1	0.1 – 0.6
C14:0 Myristic acid	1.1	1.1 – 1.9
C16:0 Palmitic acid	49.3	47.2 – 73.8
C18:0 Stearic acid	4.9	4.4 – 5.6
C18:1 Oleic acid	34.8	15.6 – 37.0
C18:2 Linoleic acid	9.0	3.2 – 9.8
C18:3 Linolenicacid	0.2	0.1 – 0.6
C20:0 Arachidic acidacid	0.4	0.1 – 0.6

2.7 Analysis

The analysis conducted on the hard stocks were mostly focused on the physical parameters such as melting point, solid fat content, rate of crystallization and heat profile during transition. Chemical composition of the hard stocks was also conducted in order to monitor the changes of the interesterified hard stocks and to help explain the physical differences that took place with interesterification reactions.

2.7.1 Thermal analysis

Differential scanning calorimetry (DSC) is the most common technique used to measure the thermal profile of fats (Rehs, 2010). DSC is a technique used to monitor the difference in heat flow as a function of temperature for compounds showing thermal transition (Azis, Mohamud, & Roselina, 2011). Baseline standardization is normally conducted prior to sample testing in order to test the stability of the base line and if curvature was detected during the standardization, the degree of curvature can still be subtracted from the sample during the data analysis process. Heat is released by fat crystals upon solidifying and absorbed during melting (O'Brien, 2009). When a transition occurs with the solid fat sample, thermal energy was absorbed or released and the change of this transition energy was released and registered as peaks by the DSC instrument. The area below the peak is in direct correlation with the change of heat (Tan & Man, 2000). Pomeranz & Meloan (2000) stated that the DSC investigation of oil and fats can be used to demonstrate different melting and crystallization behaviour caused by the change of chemical composition. According to O’Brien (2004), DSC is the best way to determine the final point of interesterification of lard

and of lauric fats, since the crystallization curves are significantly representative of the final triacylglycerol composition desired (Basso, Gioielli, Gonçalves, Grimaldi, & Ribeiro, 2009). The triglyceride and DSC analysis are commonly used in conjunction in order to explain the physical change of fats by the change of chemical composition and the crystallization curve of an oil or fat can be subdivided into different exothermic regions reflecting different types of triacylglycerol (Meng, 2011).

2.7.2 Triglyceride (TAG) analysis

Triacylglycerol analysis was conducted in order to help identifying the compositional changes to the hard stocks during interesterification (Pomeranz & Meloan, 2000). The identification of TAG composition is commonly carried out with gas chromatography (GC) or high pressure liquid chromatography (HPLC) (Rehs, 2010). Both methods were based on the column separation technique which separates the sample into components and was then registered by detector and displayed as chromatographs (Cserháti, 1999). Each component in the sample was shown as a peak on the chromatograph and the order of the peaks was determined by the length of time each individual component took while travelling through the column (RT) (Pomeranz & Meloan, 2000).

Gas chromatography (GC)

The analysis of triacylglycerol using gas chromatography requires special conditions like non-polar mobile phase and high temperature operation at 300 to 360°C in order to keep the TAGs in the gaseous phase (Basso et al., 2009). The standard method for TAG analysis using GC was described in Ce 5-86 from AOCS official method where the sample was first heated to the gaseous state and transferred into the column by helium carrier gas(Firestone, 2009). The TAGs with the same carbon number were heated and separated with the set temperature program while travelling through the column. The most commonly used column for this analysis was the C18 capillary column (30 m × 0.25 mm i.d., 0.10 μm film thickness) with the interior fused with silica (Angers & Arul, 1999). The separated component of the TAG sample was then detected using a FID detector and the results were displayed as a percentage relative to the total triglyceride in the sample (Neff, Byrdwell, & List, 2001).

High Pressure Liquid Chromatography (HPLC)

HPLC analysis is the most common method of TAG analysis for interesterified fats (Noor Lida, Sundram, & Idris, 2006). In HPLC analysis, the sample was injected into the instrument in a liquid state and the solid fat sample dissolved in organic solvents (Rehs, 2010). Unlike GC analysis, the sample does not need to be turned into a gas hence can be directly injected into the instrument without heating at high temperature hence the column used for HPLC analysis was different. The most common column used for HPLC analysis was the reverse-phase C18 column (250 mm x 4.6 mm) (Ahmadi, Wright, & Marangoni, 2008; Castilho, Costa, Rodrigues, Branco, & Costa, 2004; Meng, 2011; Noor Lida et al., 2006)

The standard method for TAG analysis using HPLC was the Ce 5b-89 from AOCS official method and the retention time of the TAG component was related to the equivalent carbon number (ECN) and was used to determine the order of elution for the composition, the higher the carbon number the longer the retention time (De Clercq, Danthine, & Nguyen, 2012). The eluted components were registered by a refractive index detector and each component was presented as a peak (Lin, 2011). The area under the peak correlates to the amount of composition in the sample and as a percentage of the total amount of TAGs detected. The results of the HPLC analysis were also displayed in the form of chromatograph and each component was registered as a peak on the chromatograph and the size of the peak correlates to the amount of each composition as a percentage of the total amount of components detected (Cserháti, 1999).

2.7.3. Nuclear Magnetic Resonance (NMR)

The performance of an oil or fat product at a given temperature is largely determined by the solid to liquid ratio of the fat at that temperature and the solid to liquid ratio changes with the change of temperature hence this ratio cannot be displayed as a single value during the fat transition of crystallization or melting (O'Brien, 2009). Hence the solid to liquid ratio cannot be displayed by one single temperature, several temperatures are normally selected and the temperatures and reason for selection are listed in Table 11.

Table 11. The temperature selected for SFC analysis and reason for selection (O'Brien, 2009).

Temperature for analysis (°C)	Reason for analysis at the selected temperature
0	indicates the solid to liquid ratio when the fat or oil product is frozen
10	indicates the spread ability of the fat or oil at refrigeration temperature
20	indicates the spread ability of the oil or fat at room temperature
30	indicates the solid to liquid ratio of the fats during consumption
40	indicates the solid to liquid ratio of the fats in a near or completely melted state

The determination of solid to liquid ratio over a temperature range is commonly used by the nuclear magnetic resonance techniques (NMR). The NMR technique operates on the principle that distinguishes the hydrogen atoms in liquid and solid fats (Pomeranz & Meloan, 2000). A radio frequency pulse was fired from the transmitter of the pulsed NMR that excites the hydrogen atoms in the solid fat sample by transmitting a short burst of energy when the transmission of the energy stops, the excited hydrogen returns to the ground state through a relaxation process when energy was released but the relaxation time of different states of the sample differs (Rehs, 2010). The decay time for hydrogen atoms in a fat sample at solid state was much slower than the hydrogen atoms in fat samples in the liquid state and with the pulsed NMR technique, the measurement of total number of hydrogen atoms took place prior to a second measurement 70 µsec later that measures the hydrogen atoms in the liquid environment of the fats (O'Brien, 2009). The contrast was able to provide information on the percentage of liquid hydrogen atoms to the percentage of solid fat hydrogen atoms hence giving information on the ratio of solid to liquid or the content of solids in the fat sample (O'Brien, 2009).

2.8 Literature review conclusion

Interesterification reaction is the re-arrangement of fatty acids within and with other triacylglycerol molecule. Interesterification reaction includes acidolysis, alcoholysis, glycerolysis and transesterification. Transesterification reaction is the reaction most commonly applied interesterification reaction hence all transesterification reactions will be referred to as the interesterification reaction in this study. Interesterification is a modification process and whether by chemical or enzymatic method, the quality of incoming oil and fat prior to interesterification is very important hence it is generally performed after degumming

and bleaching.

Interesterification could occur naturally but it happens over a long period of time and a metal alkylate (sodium methoxide) catalyst is commonly used with heat to speed up the reaction and this method is also known as chemical interesterification. Chemical interesterification can be random or direct. The random method involves random arrangement of fatty acids on the triacylglycerol molecule after the hydrolysis reaction and produces low yields of desired triacylglycerol molecules. Direct chemical interesterification is more specific and is used for the synthesis of tri-saturated triacylglycerol molecules as this method involves the crystallization and removal of tri-saturated triacylglycerol molecules as they form during interesterification. Loss of oil occurs in both methods due to side chain reactions and the necessity of post-bleaching.

Post bleaching is not required for enzymatic interesterification reaction as the immobilized enzymes do not cause any side reactions and produces no waste during the interesterification reaction. The commercial lipase enzymes are commonly immobilized on silica granules and can be removed from reaction by simple filtration in batch reaction of having the oil or liquid fat passing through it in continuous reaction. The enzymes are position sn1, 3 specific and not random that aids in the prediction of the final product and also increases yield of desired products. For the enzymes to operate at optimum rate, the water content, pH and temperature of the reaction must be monitored and avoid the presence of surfactants during processing.

Palm stearin was selected as a raw material for the interesterification reaction because palm has always been traditionally used for the manufacture of fat products. Fully hardened coconut oil was used due to the distinct sharp melting and lower melting point of the lauric acid fats while tallow stearin was selected for its low cost and vast availability in New Zealand.

Analyses were conducted to monitor the change of the solid fats prior and after interesterification though most of the analyses measures the physical parameters, the triacylglycerol analysis with HPLC helps us to understand the change of fatty acids within the triacylglycerol molecule or the compositional change in the fat in order to explain the physical changes. Other analysis conducted includes melting point and solid fat content as well as thermal analysis when the release of heat was monitored during the transition of crystallization and the rate of crystallization analysis to help identify how fast the interesterified fats were crystallizing.

Chapter 3 Materials and Methods

3.1 Equipment	
Equipment	**Supplier**
Hot plate	Cemarec 3 Hotplate, Thermolyne, USA
Stainless steel ball bearing (3.178 mm)	Bakels edible oil, Mount Manganui, NZ
Digital Thermometer	51II Thermometer, Fluke, USA
Magnetic stirrer	48.5 mm X 9 mm, SciLabware, Staffordshire, UK
Filter paper (Grade 4, Qualitative)	Whatman, Buckinghamshire, UK
Ultrasonic bath	RK 510, Bandelin, Heinrichistr, Berlin
3.2 Materials	
Raw Materials	**Supplier**
Tallow stearin	Bakels Edible Oil, Mount Manganui, NZ
Palm stearin	Bakels Edible Oil, Mount Manganui, NZ
Fully hardened coconut oil	Bakels Edible Oil, Mount Manganui, NZ
Interesterified Palm and Fully hardened coconut oil fat (IPL hard stock)	Bakels Edible Oil, Mount Manganui, NZ
Interesterified Palm stearin fat (IE 45)	Bakels Edible Oil, Mount Manganui, NZ
Interesterified Palm stearin fat (Olinera)	Bakels Edible Oil, Mount Manganui, NZ
Enzyme	**Supplier**
Lipozyme RM IM	Novonzymes, Krogshoejvej, Denmark. Nutra Functional Food Ingredients, Auckland (NZ agent), NZ
Lipozyme TL IM	Novonzymes, Krogshoejvej, Denmark. Nutra Functional Food Ingredients, Auckland (NZ agent), NZ

Novozyme 435	Novonzymes, Krogshoejvej, Denmark. Nutra Functional Food Ingredients, Auckland (NZ agent), NZ
Reagents	**Supplier**
Acetone (Analytical grade) Acetonitrile (Analytical grade) Chloroform (Analytical grade)	Thermo Fisher Scientific, New Jersey, US Thermo Fisher Scientific, New Jersey, US Thermo Fisher Scientific, New Jersey, US
Standards	**Supplier**
Triglyceride mixture standard (Analytical grade) Trilauric acid standard (Analytical grade)	Sigma Aldrich, St Louis, USA Sigma Aldrich, St Louis, USA

3.3 Analytical methods

3.3.1 Barnicoat softening point

Determination of fat melting point is one of the analyses carried out to define the physical property of solid fats. The Barnicoat softening point method can be used to determine melting point, which is accurate, inexpensive and easy to perform (Deman, Deman, & Blackman, 1983). A small stainless steel ball bearing is placed on top of solidified fats in a small test tube; the test tube with fat is then heated to soften the fat. When the ball bearing drops half way down the test tube due to the softening of the fat, the temperature of the fat is recorded as the fat's melting point.

Method:

One millilitre of melted fat sample was transferred to a 10 ml test tube and then labelled. The test tube of fat was then placed into a freezer at -20 ± 1°C for 30 minutes. The tubes of fat were then stored in a 4 ± 1°C refrigerator overnight. The test tubes were retrieved from the refrigerator the next day and suspended in a 20 ± 1°C water bath for 15 minutes. A stainless steel ball bearing ($1/8^{th}$ inch) was placed on the surface of the solid fat. The test tube was then placed in a beaker of water at 20°C which was placed onto a hot plate (Cemarec 3 Hotplate, Thermolyne, USA) with a magnetic stirrer. The digital thermometer (51II Thermometer, Fluke, USA) was placed in the beaker of water to monitor the temperature and a flea for the magnetic stirrer was also placed in the water. The water was heated up by the hot plate at a rate of 0.5°C /min. The test tubes were observed to determine the temperature at which the ball bearing fell half the height of the fat in the test tube. This temperature was recorded as the melting point.

3.1.2 Thermal analysis

Differential scanning calorimetry was conducted to analyze the amount of heat absorbed and released by the fat sample during crystallization and melting (Basso et al., 2009). The instrument operates by comparing the difference between the heat absorbed or released by the sample and the heat absorbed or released from an empty sealed pan which acts as reference (Tan & Man, 2000). The reference was checked daily with a blank ran. Standardization of the

instrument was also carried out, the temperature range applied for the melting and crystallization was between -60 to 80°C (Basso et al., 2009).

Figure 15. Differential scanning calorimeter (DSC) (DSC Q100, TA Instrument, New Castle, Delaware, USA).

Equipment

Differential scanning calorimetric analysis was conducted with a Q100 (DSC Q100, TA Instrument, New Castle, Delaware, USA) with a RCS90 Refrigerated cooling system (TA Instruments, New Castle) that operates between -90 to 550°C and allows rapid heating and cooling. Nitrogen gas was used at a flow rate of 20ml/min. The hermitically sealed aluminium sample pans and lids with maximum operating temperature at 600°C (TA Instruments, New Castle) were also supplied by TA Instruments. The data was analyzed using the TA universal analysis software, version 4.2E program (explorer, 2005). Duplicated trials were analyzed with the same method in order to provide contrast. Figure 15 shows a photograph of the assembly of the DSC Q100 instrument with RCS90 refrigeration unit.

Method

Standardization

Standardization of the instrument was conducted with an indium standard (melting point 156.6°C) (TA Instruments, New Castle). Indium gives a sharp single peak instead of the complicated profile for triglycerides and the melting temperature of indium metal also covers the range of many fats (De Clercq et al., 2012). The aluminium hermetically sealed pan was handled only with forceps to avoid contamination of fats from direct contact.

Blank

Blank analysis was conducted daily at the beginning of the sample analysis as calibration. The blank analysis was subtracted from the sample curve to minimize differences between the reference hermetic pan and sample pan as well as avoiding possible baseline instability that might be caused by a change of room temperature or inefficient cooling or heating by the refrigeration unit. The empty hermitic pan was cooled to -60°C and heated to 80°C at a rate of 5°C/min which covers all of the operating temperature range of the fat samples (Firestone, 2009).

Sample preparation

The balance was calibrated with zero balance then an empty aluminium lid and pan were placed on the balance and the weight recorded. Approximately 7.00 ± 0.20mg of the solid fat sample was dispensed into the pan using a spatula and forceps, and transferred to center of the empty aluminium pan. The lid was placed on the sample pan and seal by pressing down firmly on the lever of the sealing mechanism. The fat sample pan was then weighed and the weight subtracted from the empty pan and recorded.

System Preparation procedure

The system was prepared by first opening the valves on the nitrogen gas cylinder and the valve connected to the instrument. The Q100 DSC instrument, the RSC90 refrigeration system and computer were turned on to allow both instruments to warm up for approximately 15 minutes. The TA instrumental explorer analysis program was selected and the beep indicated the connection of instruments and the computer software.

Sample Loading

The sample was loaded by removing the lid of the instrument by pressing the “Open” button on the instrument panel under the control menu followed by transferring the sample pan with forceps and placing it on top of the stool in the sample well. The “Close” button was pressed on the control menu to close the lid. Figure 16 shows the sample in the DSC instrument including the position of sample and reference well and the lid of the instrument

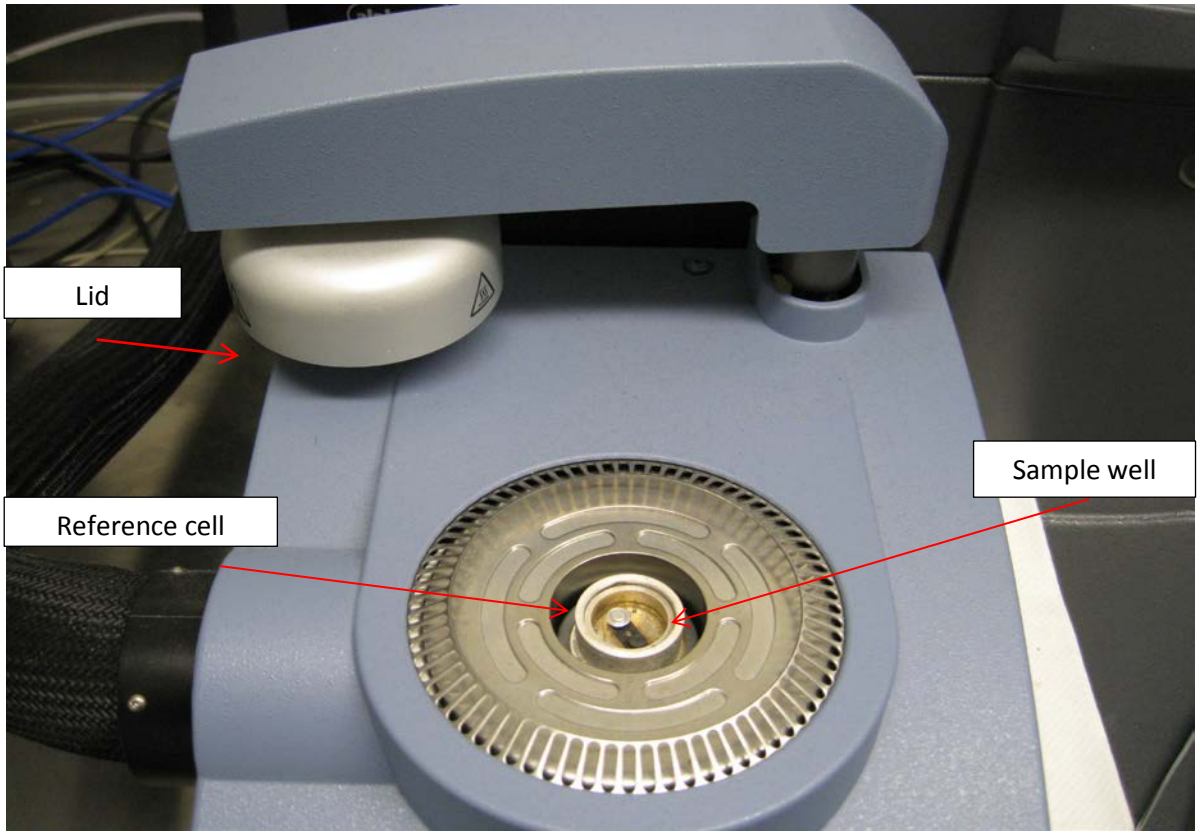

Figure16. Sample well of for DSC (DSC Q100, TA Instruments, New Castle, Delaware, USA)

Crystallization of fats using differential scanning calorimeter

The temperature of the instrument was first raised and kept at 80°C by setting the onset temperature on the control menu. The fat sample sealed in a hermetically sealed pan was then loaded into the instrument and placed in the sample well as shown in Figure 16. Once the solid fat sample was loaded, it was kept at 80°C for 10 minutes to allow the solid fat sample to completely melt prior to cooling and crystallisation. The fat sample was then cooled to -60°C at the rate of 10°C/minute and then kept at -60°C for 30 minutes allowing the fat sample to be completely crystallized. The heat released by the fats during crystallization was monitored and recorded in the form of a DSC crystallization thermograph.

Melting of fats using differential scanning calorimeter

The temperature of the instrument was lowered to -60°C by changing the onset temperature on the control menu. The fat sample in the sealed pan was loaded into the DSC the melting programme was initiated, where the solid fat sample was kept at -60°C for 30 minutes allowing it to crystallize then followed by melting with application of heat. The crystallized fat sample was heated at 5°C/minute until it reached 80°C. The heat absorbed by the fat sample was also monitored and recorded in a DSC melting thermograph.

Data analysis for thermal analysis

The crystallization and melting results analysed by DSC were displayed in the form of cooling (crystallization) and melting curves. The peaks observed during the crystallization and melting indicates the amount of heat released during cooling and heating. The base line curve obtained from the blank trial at the beginning of each day of analysis was subtracted from the cooling and heating curve, as calibration, in order to minimize any differences in heat flow between the reference pan and sample.

3.1.3 High Pressure Liquid Chromatography (HPLC) for trilglycerides

The composition of triglycerides in fat samples used as a monitoring analysis was recently adopted by many industries as a measure for the quality of oil and fats based on the specific composition (Aparicio & Aparicio-Ruiz, 2000). Interesterification caused by commercial lipase enzymes triggers the re-allocation of fatty acids within the triglycerides hence this analysis was conducted to provide some understanding of the changes to the overall triglyceride composition and helped to explain the physical changes of the interesterified fats (Firestone, 1999). Figure 17 shows the HPLC instrument modules used for this analysis.

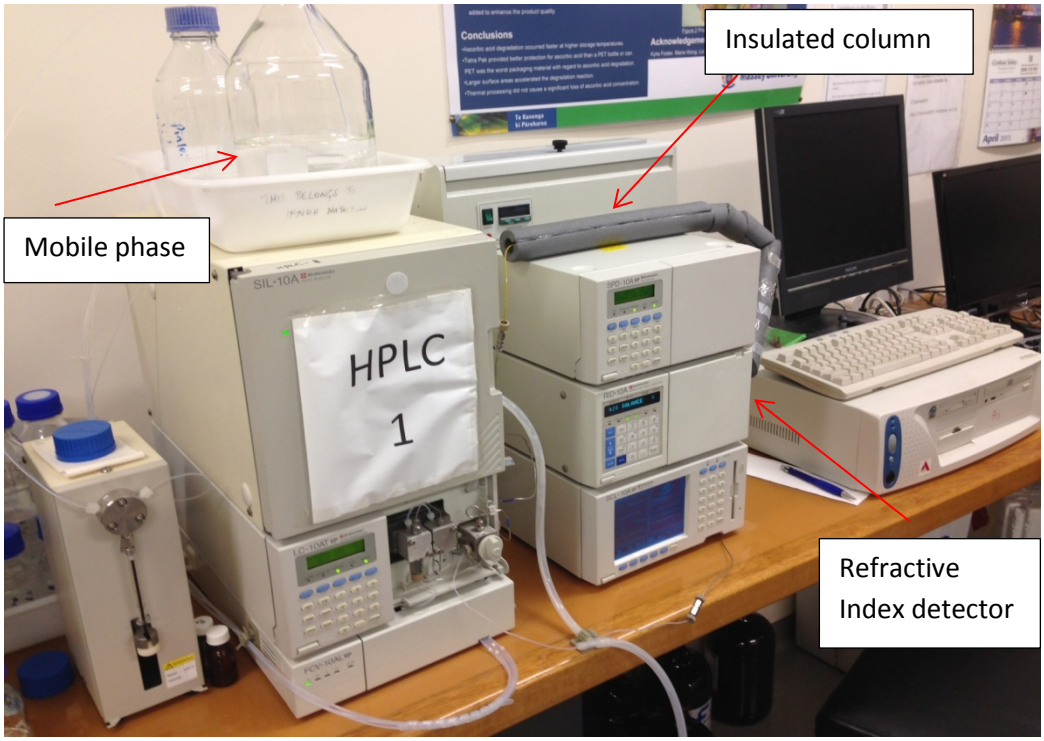

Figure 17. High pressure liquid chromatography (HPLC) (LC-10AD, Shimadzu Corp., Tokyo, Japan)

HPLC Equipment and settings

The triglyceride composition analysis was conducted using a Shimadzu HPLC (LC-10AD, Shimadzu Corp., Tokyo, Japan) instrument equipped with a reverse phase Synergi 4u Hydro-RP80 C18 column from Phenomenex (250 × 4.6mm, 4 μm) for separation of triglycerides. The separation was based on different molecular weights and polarity. The separated triglyceride molecules were detected by a refractive index detector (Shimadzu RID-10A) and translated by the software interface (Shimadzu LC solutions).

The isocratic mobile phase used to carry the sample solution through the system was a mixture of acetone and acetonitrile (Analytical grade, Thermo Fisher Scientific, New Jersey, US) at 50:50 (v/v) at the flow rate of 1.5 ml/min. The injection volume was set at 10 μl and the fat sample carried by mobile phase was dissolved in a mixture of chloroform and acetone (Analytical grade, Thermo Fisher Scientific, New Jersey, US) at 50:50 (v/v). The run time was set at 180 minutes which allowed all components separated to elute from the column. The column temperature was set at room temperature of 21 $\pm$ 1°C whereas the detector temperature was kept at 40°C and sensitivity was set at 1.0×10^{-3} (RIU).

Mobile phase preparation

The mobile phase used for this analysis was a mix of acetone (analytical grade, Thermo Fisher Scientific) and acetonitrile (analytical grade, Thermo Fisher Scientific) made up in a ratio of 1:1 v/v.

The mobile phase solution once mixed was filtered with a 0.22 μm pore size filter paper (Millipore, white GSWP) under vacuum and the solution was then transferred to a glass Schott bottle after filtration.

The filtered solution was degassed under vacuum with constant vibration in an ultrasonic water bath (RK 510, Bandelin, Heinrichistr, Berlin) for a period of 10 minutes. The solution was then transferred and connected to the HPLC instrument and ready for analysis.

Sample preparation

Approximately 0.5 g of the melted fat was weighed into at 10 ml volumetric flask and dissolved in the chloroform and acetone solution at 50:50 (v/v) (Analytical grade, Thermo Fisher Scientific, New Jersey, US), and made up to the 10 ml volume. The sample solution was then filtered through a 0.45micron filter (Millipore, white GSWP) using a 10ml syringe, then transferred to a 2 ml HPLC disposable glass sample vial (12 × 32mm, Grace Davison Discovery Scientific). The sample vial was then labeled and transferred to the sample tray of the instrument.

Blank injection

The blank trial solution consisted of only the solubilization reagent of chloroform and acetone at 50:50 (v/v), (Analytical grade, Thermo Fisher Scientific, New Jersey, US) without any fat sample in order to help identify the additional chloroform reagent peak and any residual compounds from previous injections. Blank injections had run times of 15 minutes and there should have been no peaks present apart from the solvent peaks.

Batch mode injection

Analysis of interesterified samples was carried out in batches with a blank placed before and after each sample for the identification of any residual components from previous injections. Duplicate injection of a mixed triacylglycerol triglyceride mixture standard (TAG), (Analytical grade) Sigma Aldrich, St Louis, USA took place during each batch analysis, one just after the first blank injection with the second after the last fat sample. The retention times of standards in the two injections of the reference standards was compared at the end of the analysis to monitor changes or “shifts” of the peaks that might occur along the baseline during the analysis. The two reference samples can also provide a time frame should the shifting of the baseline occur. Figure 18 shows the reference standards for TAG analysis

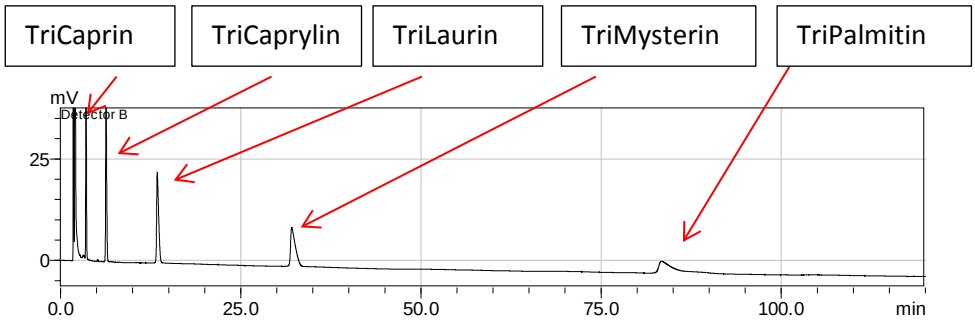

Figure 18. HPLC chromatogram showing the five TAG peaks included in reference mixed standard (Sigma Aldrich).

Triacylglycerol (TAG) identification

The identification of triglyceride peaks was determined after the peaks were integrated with the Shimadzu LC solution software. The integrated peaks were differentiated based on their retention time and were compared to the reference mixed standard from Sigma Aldrich (Lipid standard, triglyceride mixture). The peaks were further identified based on their order of elution and retention time from previous published studies (Tan & Man, 2000; Kallio, Yli-Jokipii, Kurvinen, Sjövall, & Tahvonen, 2001; Neff et al., 2001; Kowalska, Bekas, Kowalska, Lobacz, & Kowalski, 2007; Khatoon, Khan, & Jeyarani, 2012; Qin, Yang, Huang, & Wang, 2012). The percentage of each triglyceride present as a percentage of the total triglycerides was determined from the ratio of the peak areas. Peak areas below 0.5% of the total peak area were not integrated and used in the final total area calculated. Equation 4 shows the calculation for Equivalent Carbon Number which helps to determine the elution order of the TAGs exiting from the column (Firestone, 2009). The order of elution goes from low ECN to high ECN as higher ECN TAGs had longer retention times in the reverse phase column.

$$ECN = CN - 2N$$

Equation 4. Equivalent carbon number calculation (Firestone, 2009)

ECN = Equivalent Carbon Number

CN = Carbon Number

N = Degree of unsaturation

3.1.4 Solid fat content

The solid fat content is a measurement that indicates the ratio of the solid to liquid fat fractions at various temperatures (Marangoni & Rousseau, 1995). The low frequency magnetic pulse fired by an nuclear magnetic resonance (NMR) instrument causes excitation and relaxation as the nuclei of a hydrogen atom in the fat sample returns to its ground state (Firestone, 2009). The time taken by the solid and liquid nuclei to return to their ground states during the relaxation period differs hence can be detected and is expressed in percentage (Basso et al., 2009).

NMR Instrument

The solid fat content was obtained using a Bruker Minispec Pulse NMR (mq 20, Bruker, Germany) with a Bakels Edible Oils in-house test method 2.025 (Bakels, 2006)

Method

The solid fat sample was first melted thoroughly in a beaker on a hot plate. Five test tubes were labeled for each sample and 2.0 ml of the melted sample was pipetted into each of the five tubes. The five tubes were placed in a beaker then transferred to a microwave oven and heated for 1 minute. The tubes were then removed from the microwave oven and held in heated block at 60°C for 30 minutes then transferred in a 0°C water bath for exactly 4 hours. At the end of the 4 hour period, the five test tubes were than separately placed into the 0, 10, 20, 30 and 40$\pm$1°C water baths for exactly 35 minutes. The tubes were removed from the water baths at different temperature, water droplets were wiped from the tubes and they were inserted into the wells of the NMR instrument for reading. Readings of the five fat samples in the NMR were taken in the order of 0°C to 40°C and the readings at each temperature were recorded.

3.1.5 Rate of crystallization

An alternative method to follow the rate of crystallization was developed at Bakels Edible Oils. This method measures the time it takes for a fat to crystallize from the completely melted liquid state to stable solid state under consistent cooling in a 0°C water bath over set time periods. The stable solid state was measured using a NMR instrument when the readings were taken a certain time interval of cooling and the process repeated until consistent reading from the NMR instrument was obtained.

Instrument

The solid fat content was obtained using the Bruker Minispec Pulse NMR with Bakels Edible Oils in-house test method 2.025 (Bakels, 2006) as described in Section 3.1.4, with some modifications to determine the rate of crystallization.

Method

The solid fat sample was completely melted in a microwave oven. Approximately 2.0 ml of the melted fat sample was dispensed into a solid fat content (SFC) glass tube then placed in a heating block at 60°C for 10 minutes. The SFC glass tube was removed from the heating block at the end of the 10 minutes and was placed in the well of the NMR instrument for initial reading for the solid fat content of the melted fat prior to crystallization at 60°C. The SFC tube was then transferred from the NMR instrument to the 0°C water bath and held in the water bath for 2 minutes allowing the melted fats to cool and crystallize. At the end of the 2 minute cooling time, the SFC glass tube was placed into the well of the NMR instrument for another reading before transferred back to the 0°C water bath for a further 2 minutes. This process was repeated until a stable reading was obtained from the instrument. However, if the reading was not stable after the first 20 minutes, the cooling and reading process was continually repeated at 10 minute intervals instead of 2 minute intervals until a stable reading was obtained.

3.2 Interesterification Processing Trials

3.2.1 Fat blending and optimization trial

The following methodology was conducted for both fat blending trials and the optimization trials. The fat blending trials investigated the impact of different ratios of raw materials and the optimization trials determined the effect of different concentrations of enzymes on the rate of interesterification when the reaction was monitored over 8 hours with samples taken every two hours. The three commercial lipases used for this project included Novozyme 435, Lipozyme RM IM and Lipozyme TL IM. Novozyme claims all three enzymes are position Sn1, 3-specific. Table 10 lists the blends of hard stocks used for the fat blending trials, the three raw materials of tallow stearin, palm stearin and fully hardened coconut oil were mixed two at a time at the ratios of 80%: 20%, 70%: 30% and 50%: 50% where palm stearin or tallow stearin were always the higher percentage due to their higher melting points and solid fat contents. The fat blending trails were conducted in duplicate in order determine the reproducibility of the results.

Table 12. Ratios of fats used for fat blending and rate trial

Fat blend	Abbreviation	Ratio (v/v)
Tallow stearin : Fully hardened coconut oil	TS : FHCO	80% : 20%
Tallow stearin : Fully hardened coconut oil	TS : FHCO	70% : 30%
Tallow stearin : Fully hardened coconut oil	TS : FHCO	50% : 50%
Palm stearin : Fully hardened coconut oil	PS : FHCO	80% : 20%
Palm stearin : Fully hardened coconut oil	PS : FHCO	70% : 30%
Palm stearin : Fully hardened coconut oil	PS : FHCO	50% : 50%
Tallow stearin : Palm stearin	TS : PS	80% : 20%
Tallow stearin : Palm stearin	TS : PS	70% : 30%
Tallow stearin : Palm stearin	TS : PS	50% : 50%

The experimental design (Table 12) used for the planning of the experiments was completed with Minitab 16 software using the Taguchi method. The results from computer output of the design were included in Appendix A1 the translated model is listed in Table A1. The fat

blending trials were carried out in duplicate in order to ensure the repeatability of the processing method.

Table 13. Experimental design for fat blending trial

Code number	Enzyme type	Fat mix	Fat ratio
Novo 8.2 (TS:FHCO)	Novozyme 435	TS:FHCO	80/20
Novo 7.3 (TS:FHCO)	Novozyme 435	TS;FHCO	70/30
Novo 5.5 (TS:FHCO)	Novozyme 435	TS:FHCO	50/50
Novo 8.2 (PS:FHCO)	Novozyme 435	PS:FHCO	80/20
Novo 7.3 (PS:FHCO)	Novozyme 435	PS:FHCO	70/30
Novo 5.5 (PS:FHCO)	Novozyme 435	PS:FHCO	50/50
Novo 8.2 (TS:PS)	Novozyme 435	TS:PS	80/20
Novo 7.3 (TS:PS)	Novozyme 435	TS:PS	70/30
Novo 5.5 (TS:PS)	Novozyme 435	TS:PS	50/50
RM 8.2 (TS:FHCO)	Lipozyme RM IM	TS:FHCO	80/20
RM 7.3 (TS:FHCO)	Lipozyme RM IM	TS:FHCO	70/30
RM 5.5 (TS:FHCO)	Lipozyme RM IM	TS:FHCO	50/50
RM 8.2 (PS:FHCO)	Lipozyme RM IM	PS:FHCO	80/20
RM 7.3 (PS:FHCO)	Lipozyme RM IM	PS:FHCO	70/30
RM 5.5 (PS:FHCO)	Lipozyme RM IM	PS:FHCO	50/50
RM 8.2 (TS:PS)	Lipozyme RM IM	TS:PS	80/20
RM 7.3 (TS:PS)	Lipozyme RM IM	TS:PS	70/30
RM 5.5 (TS:PS)	Lipozyme RM IM	TS:PS	50/50
TL 8.2 (TS:FHCO)	Lipozyme TL IM	TS:FHCO	80/20
TL7.3 (TS:FHCO)	Lipozyme TL IM	TS:FHCO	70/30
TL5.5 (TS:FHCO)	Lipozyme TL IM	TS:FHCO	50/50
TL 8.2 (PS:FHCO)	Lipozyme TL IM	PS:FHCO	80/20
TL7.3 (PS:FHCO)	Lipozyme TL IM	PS:FHCO	70/30
TL5.5 (PS:FHCO)	Lipozyme TL IM	PS:FHCO	50/50
TL 8.2 (TS:PS)	Lipozyme TL IM	TS:PS	80/20
TL7.3 (TS:PS)	Lipozyme TL IM	TS:PS	70/30
TL5.5 (TS:PS)	Lipozyme TL IM	TS:PS	50/50

Table 13 shows the experimental plan for the trials on impact of reaction time and enzyme concentration. Four enzyme concentrations were investigated for each of the three commercial lipase enzymes. A sample of the reaction mixture was taken every 2 hours in order to monitor the change in physical properties and composition of the fats caused by the enzymes due to interesterification. This stage of trial was a continuation from the fat blending trial and three fat blends were reselected to undergo further trials with different

concentrations of enzymes at different reaction times. The trials were not conducted in duplicate due to the large amount of analysis associated with each sample. Samples were analysed in duplicate.

Table 14. Type and concentration of enzymes used for the impact of concentration and time trials

Abbreviations	Lipase enzyme name	Percentage used (w/w)
Novo	Novozyme 435	0
Novo	Novozyme 435	2
Novo	Novozyme 435	4
Novo	Novozyme 435	8
RM	Lipozyme RM IM	0
RM	Lipozyme RM IM	2
RM	Lipozyme RM IM	4
RM	Lipozyme RM IM	8
TL	Lipozyme TL IM	0
TL	Lipozyme TL IM	2
TL	Lipozyme TL IM	4
TL	Lipozyme TL IM	8

Methodology

The solid fat raw materials were melted in a beaker on a hot plate and weighed separately into two beakers according to the ratios indicated in Table 14. The melted fats in the two beakers were then combined with the total weight of the fat blend at 50 $\pm$ 0.5g. A water bath at 65 $\pm$ 1°C was set up on top of two hot plates and a digital thermometer was inserted into the water bath. Once the temperature was stable, the beaker containing the fat blend was transferred to the water bath and a magnetic stirrer was placed in the fat blend beaker. Commercial lipase enzyme (4% w/w) was gently stirred into the fat blend. The fat with enzyme was maintained at 65 $\pm$ 1°C for 8 hours. The granular enzyme was then filtered from the melted fat with grade 4 Whatman filter paper; the filtration took place in an oven at 60°C to prevent solidification of the fast crystallizing hard stocks. The filtered enzymes was

discarded during the fat blending trials and the fat samples was transferred to clear plastic containers, labelled and stored under 4°C conditions for analysis.

3.2.2 Reusability of enzyme test

The commercial lipase enzymes are expensive and a relatively large amount is required for interesterification at 4 to 8% (w/w) hence reusability of the enzyme became a concern as it may affect the profitability of the enzymatic interesterification process.

The reusability trials were conducted after the fat blending trials and trials on determination of reaction time and enzyme concentration. The interesterification operational conditions were the same as the fat blending trials but fresh commercial enzymes was used during the reusability trials. During the reusability trials, the enzymes were collected after each interesterification trial by filtration. Once the commercial enzymes were collected after each interesterification trial, they were put through washing with different organic solvents including acetone, chloroform, ethanol and iso-octane to see which solvent can deliver the most number of reuses without the enzymes losing their activities (Kim. 2008; Ognjanovic et al. 2009; Souza. 2011; De Martini Soares et al., 2013). The washed enzymes was then rinsed with deionised water and dried under ambient temperature overnight. The dried enzymes were then collected and stored in clear plastic containers for further reusability trials. The interesterified fat samples were taken after each wash for analysis that includes triglyceride content (TAG) using HPLC and melting point analysis in order to determine the maximum number of washing cycles for the commercial lipase enzymes during batch production. The fat sample after each interesterification trial was collected and stored under refrigeration temperature at 4°C.

Methodology

The raw materials of tallow stearin and fully hardened coconut fats were both melted and combined in a beaker at a ratio of 70%:30% (w/w) with the total weight of 50 ± 0.5g. The beaker was placed in a water bath at 65 ± 1°C, and stirred gently with a magnetic stirrer. The commercial enzymes Lipozyme RM IM or Lipozyme TL IM were tested at a concentration of 4% w/w. The enzymes were gently stirred into the tallow stearin and fully

hardened coconut oil blend and the temperature was maintained at 65 ± 1°C for a period of 8 hours. The enzyme was separated from fat at the end of the 8 hours by filtering through filtering paper at 60 ± 1°C in an oven. Once the fats were separated from the enzyme they were stored left to crystallize under room temperature than stored at 4°C until analysed. The enzyme was then washed three times with 30 ml of organic solvent, which included ethanol, acetone, chloroform and iso-octane under room temperature. The enzyme was then separated from the organic solvent by decanting and filtering with grade 4 qualitative filtering paper (Whatman, Buckinghamshire, UK) then rinsed three consecutive times with deionized water followed by further filtering with grade 4 qualitative filtering paper (Whatman, Buckinghamshire, UK) and was dried under room temperature (22 ± 1°C) for overnight. The dried enzymes were collected on the following day and stored in clear, labelled container until next interesterification reusability trial. The interesterification process was repeated with a fresh sample of tallow stearin and fully hardened coconut (TS:FHCO) and the washed enzyme, this was repeated until the fat blends melting point and triglyceride content remained unchanged.

Chapter 4 Fat Blending Trials Results and Discussion

The objective of this study was to produce hard stock fat products suitable to be used as pastry fat or spreadable margarine by the process of enzymatic interesterification. The ideal properties for hard stock used to make margarine spreads are rapid crystallizers with relatively low solid fat content and melting point around 44°C (Eyres, 2013). The ideal properties for hard stock fats used for pastry fats are high in solid fat content, rapid crystallizers with melting point close to 44°C like the benchmark commercial hard stock IPL. The fat blending trials carried out evaluated the fat blends according to these key physical properties and allowed the identification of fat blends that were closest to the properties of the benchmark hard stock.

4.1 Preliminary trials on current commercial hard stocks

Preliminary trials and analysis were conducted prior to interesterification trials in order to gain a better understanding of the raw materials for hard stocks which included tallow stearin, palm stearin and fully hardened coconut oil. Some of the current hard stocks were also analysed in the preliminary trial to provide contrast for interesterification trials.

4.1.1 Melting point for preliminary trials

The melting point analysis was conducted on the raw materials including tallow stearin (TS), palm stearin (PS) and fully hardened coconut oil (FHCO) as well as commercial hard stocks of IE 45, Olinera and IPL. These analyses provided a better understanding of the raw materials and showed the targeted melting point required from interesterification trials. The tallow stearin (TS), fully hardened coconut oil (FHCO) and palm stearin were used as raw materials for interesterification. Figure 19 indicates that both TS and PS had melting points higher than 50°C while FHCO had much lower melting points at 36°C. The interesterified hard stock IPL was marked as the benchmark hard stock for this project with a melting point at 44°C. The commercial hard stocks of IE 45 and Olinera were both interesterified hard stocks but had different melting points with IE 45 at 49.8°C and Olinera at 40.8°C.

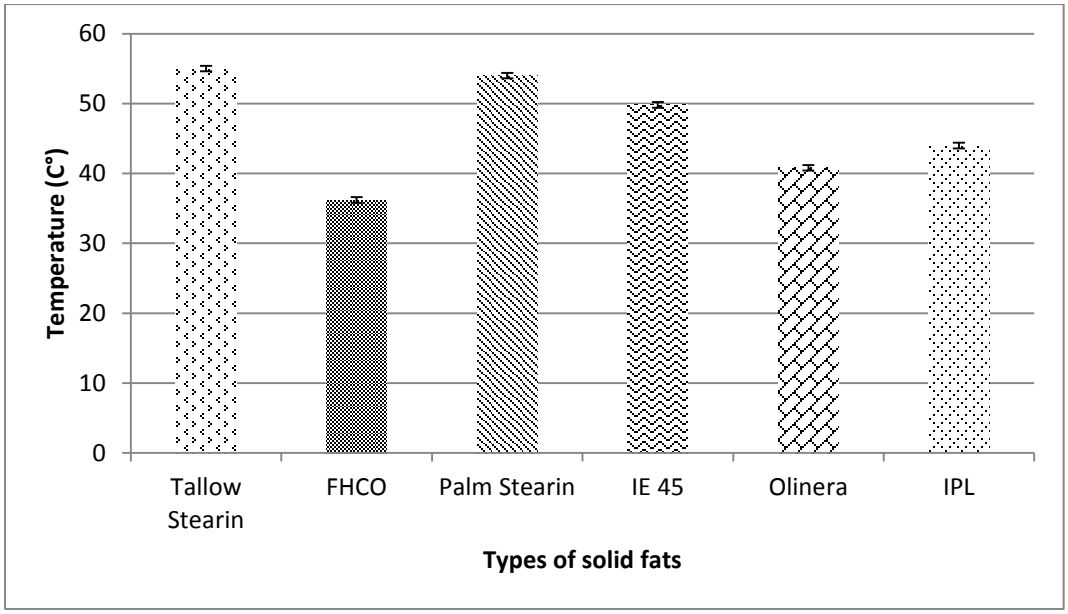

Figure 19. Melting points for solid fats analysed in the preliminary trials that includes both raw material (tallow stearin, fully hardened coconut oil and palm stearin) and commercial hard stocks (IE 45, Olinera and IPL). Data presented as mean ± standard error, each solid fat was analysed in quadruplicates (n=4).

4.1.2 Triglycerides (TAGS)

The HPLC analysis on TAGs for raw material and commercial hard stocks gives compositional information. The TAG name was displayed as a three letter code name with each letter representing a fatty acid on the TAG molecule. The composition of TAGs is reported as the percentage of total TAGs present in the fat sample. Each peak shown on the chromatograph represents the presence of a TAG molecule and the retention time shows the resident time of the TAG molecule in the column during separation (Figure 20). The TAG composition for palm stearin, tallow stearin and fully hardened coconut oil is shown in Table 15.

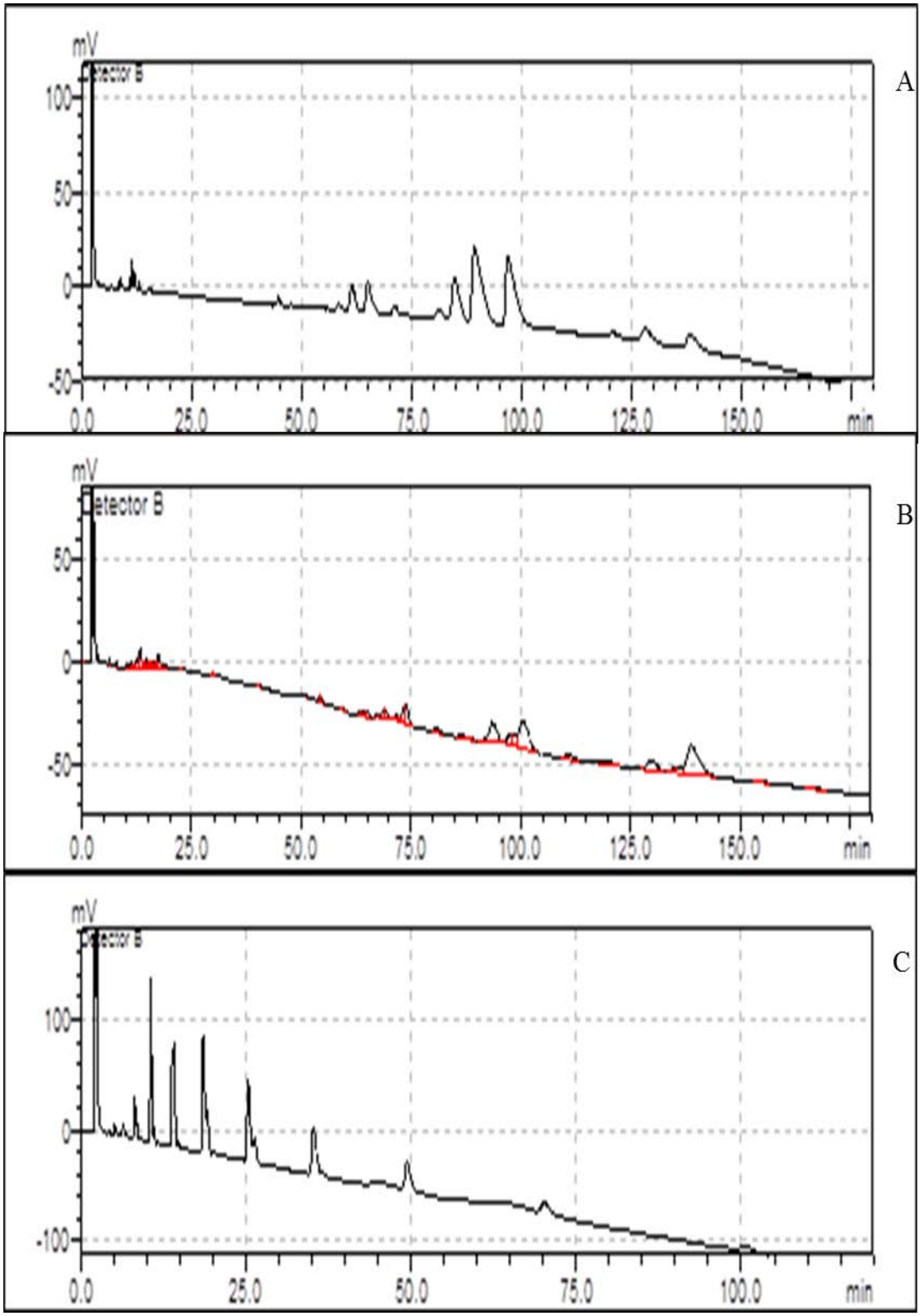

Figure 20. Examples of HPLC chromatographs of triglycerides ***(TAG) for the raw materials used in this project. A. chromatograph for palm stearin (PS), B. chromatograph for tallow stearin (TS), C. chromatograph for fully hardened coconut oil (FHCO)***

Table 15. The TAG percentage composition corresponding to the chromatographs showed Figure 20 on the three raw materials including PS, TS and FHCO. (The fatty acid abbreviations were listed in page ix in the index section of the report).

		Percentage of TAG (individual TAG/total TAGs present)		
ECN	**Name**	**PS**	**TS**	**FHCO**
24	CpCpCp		1.2	3.8
28	CpCL		1.6	4.1
28	CCL		1.1	12.9
30	CLL		2.1	19.1
32	LLL		6.1	21.4
34	LLM			18.7
36	LLO			5.4
36	LMM			7.6
42	LMO			3.1
44	LPP			1.4
44	MMP	1.8		
44	PLO	2.2	1.4	
44	PPL	4.8		
46	MPP	4.2		
46	PLS		10.1	
48	OOO	1.6	2.9	
48	POO	12.5	9.7	
48	POP	36.2	3.2	1.0
48	PPP	25.8	3.3	1.4
50	SOO	1.8		
	RT110.9		1.8	
50	POS	2.8	18.5	
50	PPS	1.6	11.6	
52	SOS	1.5	9.6	
52	PSS	2.6	15.3	

The chromatographs in Figure 20 indicate the TAG composition of the three raw materials used for the project, each TAG was shown as a peak on the chromatograph, the order of the peaks shown on the chromatographs was determined by the molecular weight of the TAG or the ECN number indicated in Table 15. The three large peaks observed in the palm stearin chromatographs (Figure 20 A) between 70 to 100 minutes represent the high molecular weight TAGs of POO, POP and PPP commonly found in palm products. The composition of these three TAGs was listed in Table 13 at 12.5, 36.2 and 25.8%. The chromatographs for tallow stearin indicates that it contains both low and high molecular weight TAGs. Table 15 shows the major TAGs in tallow stearin are POS, PSS and POO. The high percentage of high molecular weight TAGs result in the higher melting points in PS and TS as indicated in Figure 19. The TAG composition for FHCO in Figure 20 shows that the fat was largely made up of low molecular weight TAGs eluting before 50 minutes (retention time). The percentage composition of TAGs in FHCO (Table 15) indicates that the TAGs were largely made up of

CCL, CLL, LLL and LLM. The presence of these low molecular weight TAGs in FHCO results in its lower melting point compared to PS and TS.

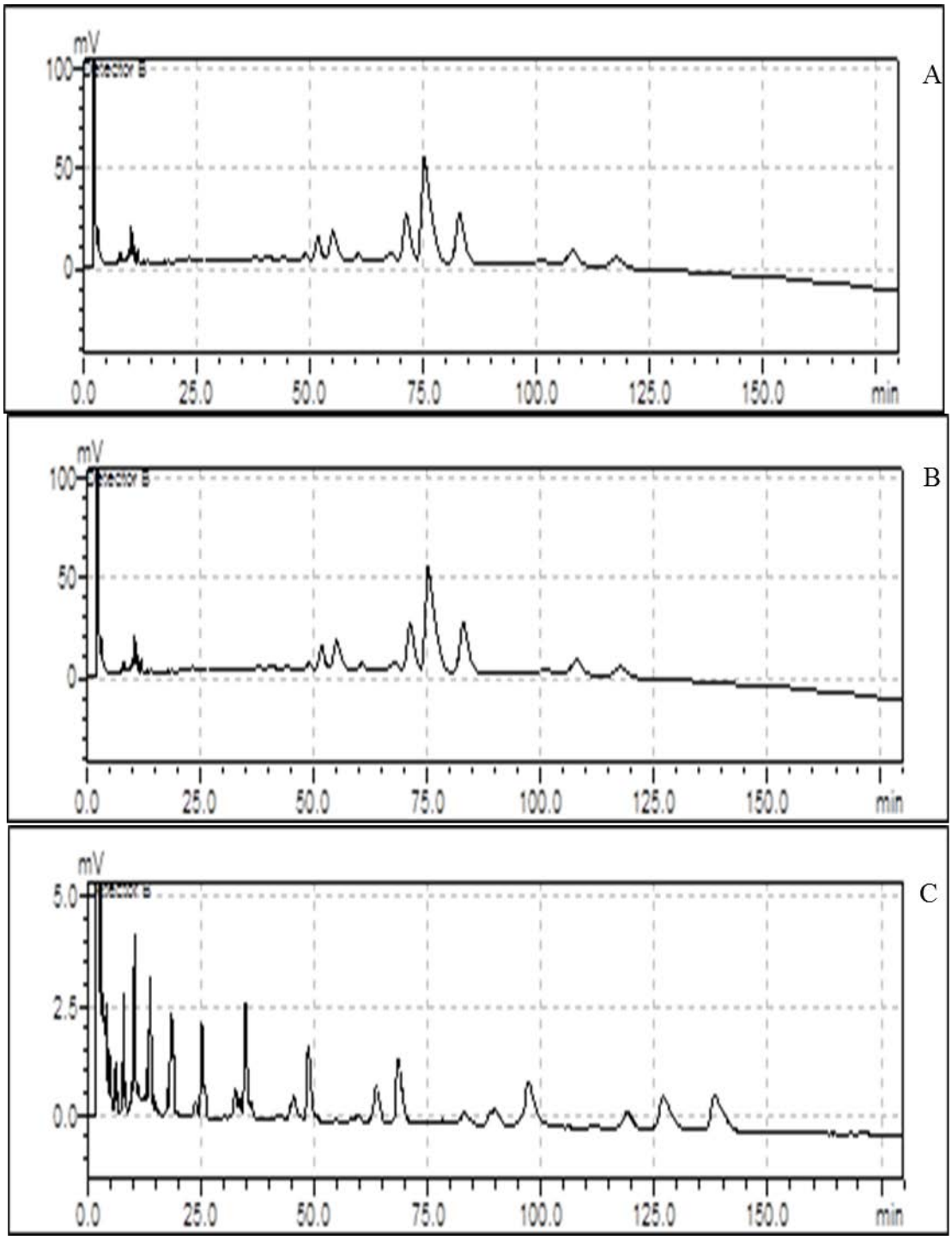

Figure 21. HPLC chromatographs of triglycerides ***(TAG) for the commercial hard stocks A. chromatograph for Olinera, B. chromatograph for IE 45, C. chromatograph for IPL***

Table 16. The TAG percentage composition corresponding to the chromatographs indicated in Figure 21 of the three commercial hard stocks including IE 45, Olinera and IPL. (The fatty acid abbreviations were listed in page ix in the index section of the report.)

ECN	Name	IE 45	Olinera	IPL
24	CpCpCp	2.1		2.6
	RT5.9	1.2		3.5
30	CCC	1.1		2.7
32	CCL	1.4	2.4	5.7
	RT11.8	1.3	2.7	1.9
	RT12.9			1.6
	RT12.3	1.2	1.7	5.8
36	LLL			7.7
38	LLM			3.6
	RT23.7			1.1
40	LMM			5.1
40	RT25.8			1.2
40	LLO			3.5
42	LMO			2.3
42	RT48.9	3.1	1.8	
42	LMP			7.5
44	PLO	5.2	6.1	5.3
44	PPL	4.1	6.2	6.2
46	PLS			4.9
48	POO	14.1	12.5	4.3
48	POP	37.1	35.3	4.6
48	PPP	17.2	19.1	4.1
	RT107.7		3.2	
	RT117.3	3.2	1.7	
50	POS	4.1	2.8	
50	PPS			6.5
52	PSS	3.3	3.1	7.2

The analyses of the three commercial hard stocks were conducted to provide benchmarks for the interesterification trials. From Figure 21, we can see that both IE 45 and Olinera have the same three high molecular weight peaks between 70 to 100 minutes, as seen in palm stearin (Figure 20). The TAG percentage composition from Table 16 shows that the two hard stocks have similar TAG composition with Olinera having fewer TAGs than IE 45. The IPL hard stock contains both high and low molecular weight TAGs. The IPL hard stocks were set as the benchmark of the project. TAG composition from Figure 21 indicates IPL contains traces of coconut oil, typically CLL, LLL and LMM, as well as a based palm product, typically with POO, POP and PPP.

4.1.3 Crystallisation profiles from DSC

The DSC thermal scan shows the change of heat during crystallization of the tallow stearin, palm stearin and fully hardened coconut oil. The crystallization profile for palm stearin (A) shows a first crystallization peak occurred at temperatures between 30 to 20°C, releasing 10 J/g°C (Figure 22). The second crystallization peak occurred between 5 to -15 °C with a much smaller heat release of 5 J/g°C. The cooling curves in Figure 21 show that tallow stearin (B) released a large amount of heat (16 J/g°C) between 40 to 20°C and gradually releases the remaining heat between temperatures of 20 to 0°C. The temperature range for crystallization for tallow stearin occurred between 40 to 0°C. The fully hardened coconut oil (C) crystallization curve shows that the release of heat did not occur until 20 °C and the heat released was consistent compared to tallow stearin and palm stearin at 8 J/g°C between the temperature ranges of 20 to -20°C.

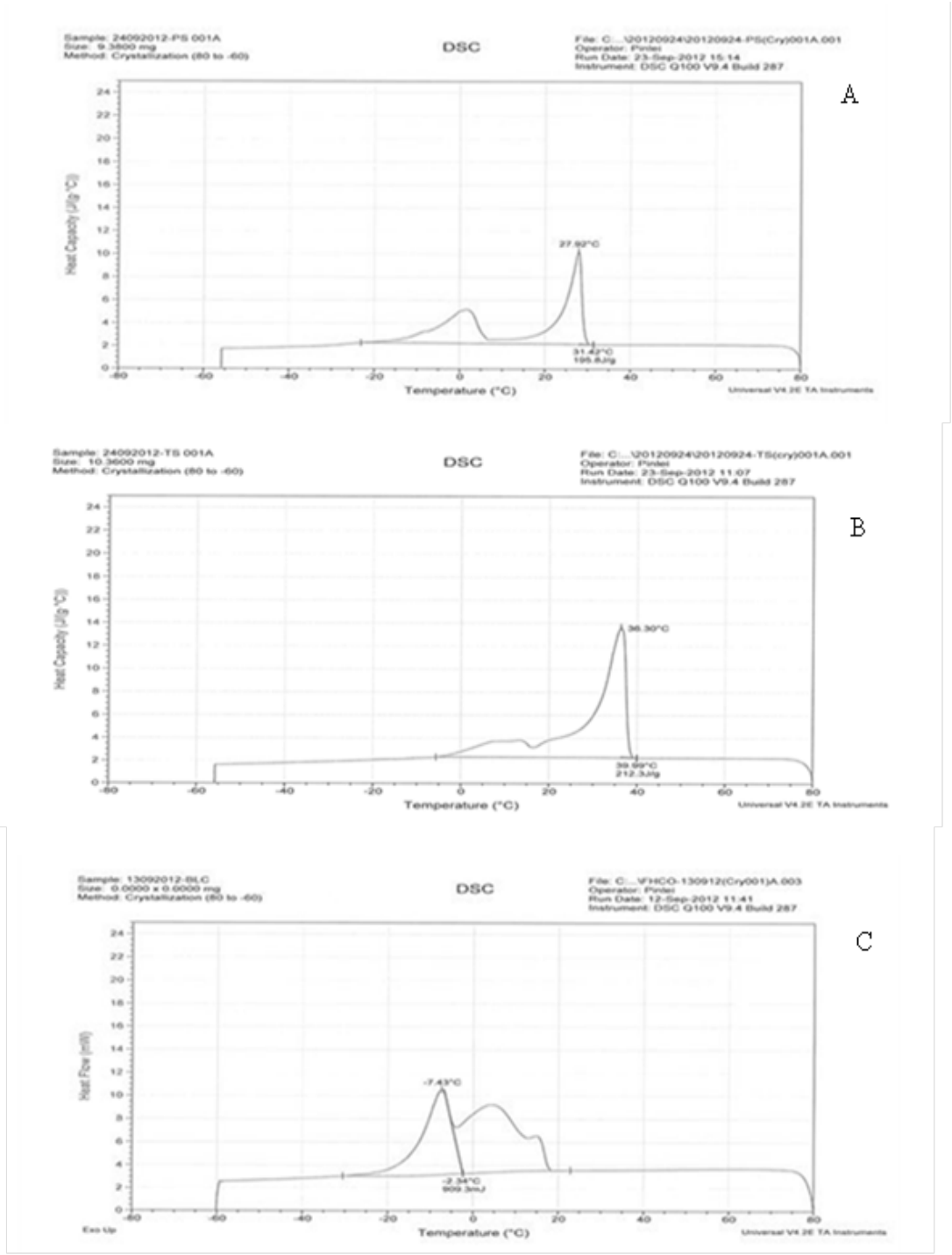

Figure 22. DSC cooling (crystallization) curve for non-blended, non-interesterified A. Tallow stearin, B. palm stearin and C. fully hardened coconut oil.

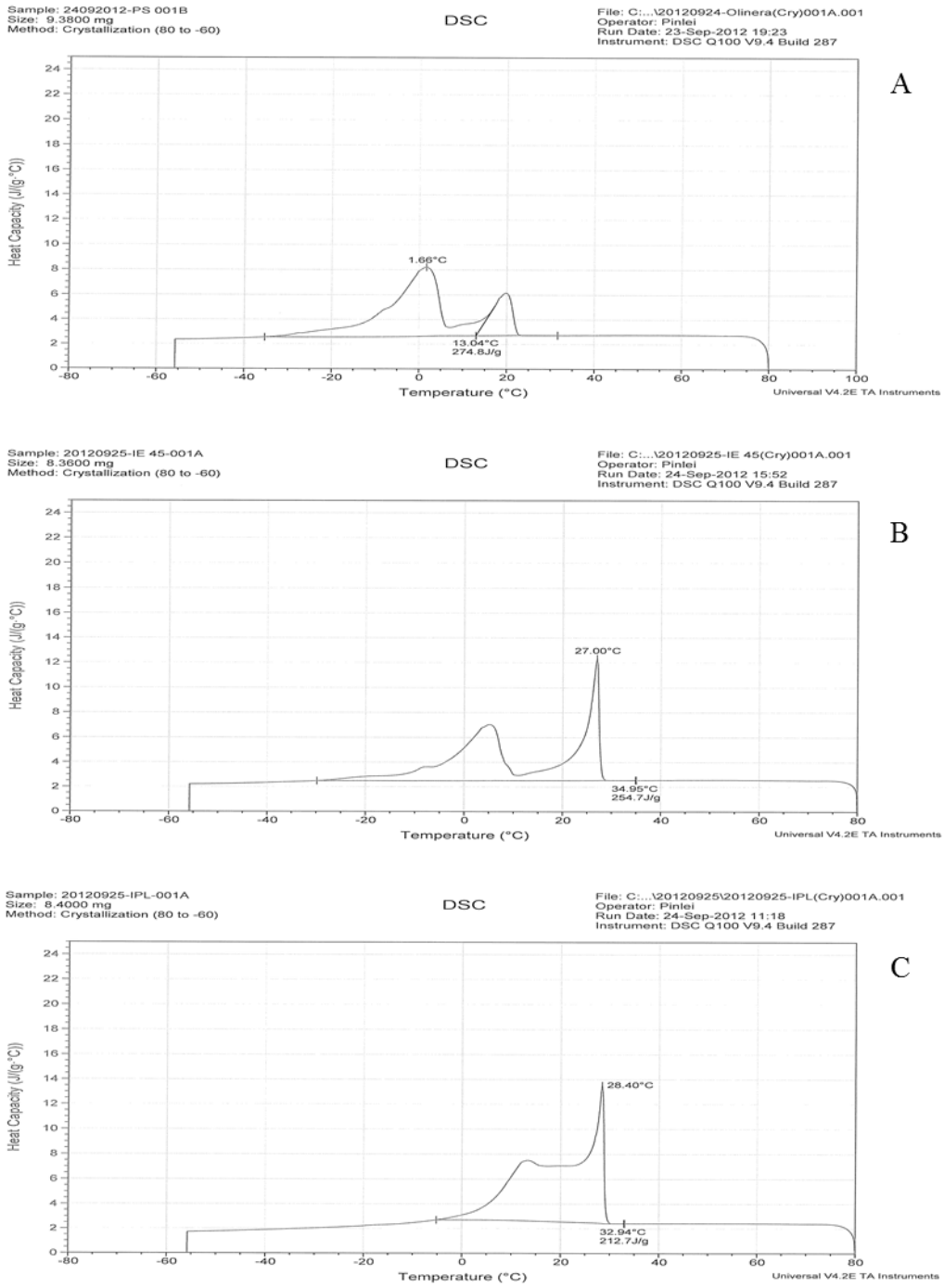

Figure 23. DSC cooling (crystallization) curve for commercially interesterified fats. A. Olinera, B. IE 45 and C. IPL.

The heat released by the interesterified Olinera hard stock occurred between 20 to -20°C (Figure 23). Two peaks were registered for heat release during crystallization for Olinera and both were small peaks at 8 J/g°C and 6 J/g°C. Although the TAG composition for Olinera and IE 45 were similar, two peaks were also detected for the IE 45 hard stock but the first peak at 30 to 10°C was larger than the first peak of the Olinera hard stock at 13 J/ g°C. This observation can be confirmed by the differences in melting points between the two hard stocks. The large first peak 30 to 10°C was the reason for IE 45's melting point at 49.8 compared to 40.8°C for Olinera. The cooling curve for IPL hard stock shows heat release occurred during 30 to 0°C. A large peak occurred at temperatures during 30 to 25 °C with heat release at 14 J/g°C for the IPL hard stock. This was followed by a flat shoulder peak that showed a constant heat release at temperatures between 25 to 0 °C. This result shows that IPL was able to crystallize within a shorter temperature range than the other two hard stocks under the same rate of cooling and confirms that it is a more rapid crystallizer than IE 45 and Olinera.

4.1.4 Solid Fat Content

The solid fat content measures the percentage ratio of solid fat to liquid fat at different temperatures. The low solid fat content indicates a lower ratio of solid to liquid fat present hence providing information amount of solid fat melted at a specific temperature. The hard stock benchmark IPL was compared to the raw materials in this stage of experiment in order to provide the ratios of fat blend required for interesterification trials.

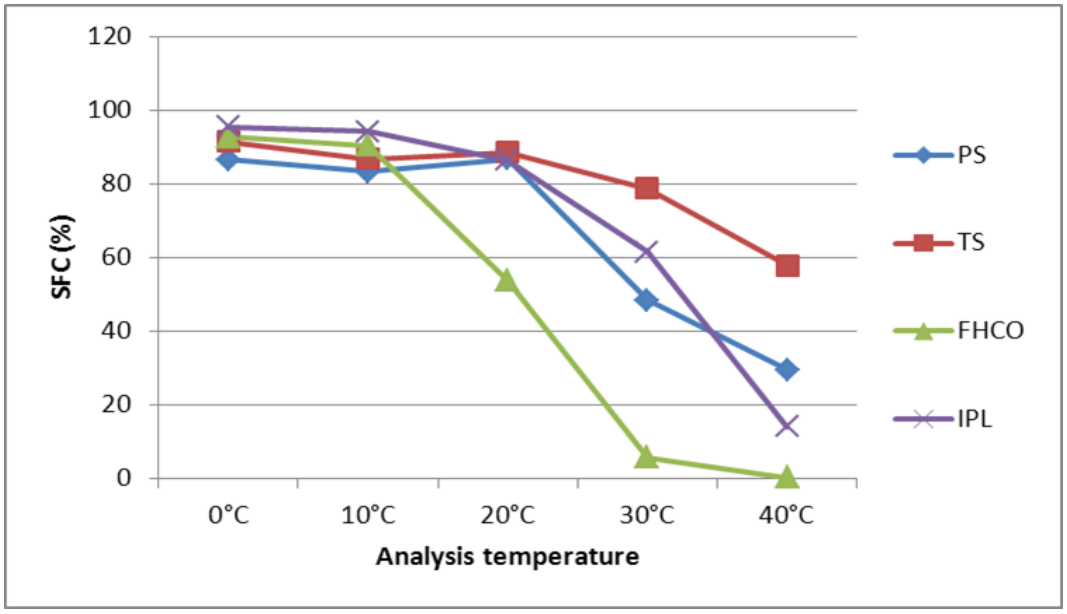

Figure 24. The comparison of raw materials with the benchmark hard stock IPL

The contrast between raw materials in terms of solid fat content to the IPL benchmark is shown in Figure 24. The IPL hard stock has a high solid to liquid ratio of 95.5% at 0°C and the solid fat content decreased gradually as the temperature increased to 30°C. A large decrease in the solid fat content was observed between 30 to 40°C and this change is preferred for pastry and spreadable margarine made hard stocks as the desired melting point for these is between 30 to 40°C. In contrast to IPL, tallow stearin and palm stearin had high solid fat content at 0°C but as the temperature was increased no large change was observed between the 30 to 40°C temperature ranges. At 40°C, the solid fat content for both tallow stearin and palm stearin still remained quite high, this observation confirmed the high melting point (Figure 19) of these raw materials of above 50°C hence the two raw materials were not completely melted by 40°C. The fully hardened coconut oil contains a large amount of lauric acid (Table 16 TAG). In Table 15 it can be seen that there are large changes in solid fat content between 10 to 20°C and again between 20 to 30°C. This observation confirmed the results from Figure 19 where FHCO melted at much lower temperature than TS and PS. This physical characteristic does not meet the benchmark requirement as spread and pastry margarine need to be relatively high in solid fat content at room temperature (22°C) whereas FHCO was already mostly melted between 20 to 30°C. The solid fat content from Figure 24 illustrated to us that the raw materials alone were not able to achieve the desired physical characteristics for the desired hard stock hence manipulation of the fats such as blending and interesterification was required.

4.1.5 Rate of crystallization

The rate of crystallization analysis was carried out in order to gain a better understanding of these raw materials. IPL was included in this analysis to provide contrast and understanding of the ideal crystallization rate for the interesterified hard stocks. The fat samples were first completely melted than allowed to crystallize in a 0°C water bath. The hard stocks with the shorter times were deemed to be faster crystallisers as all the fat was crystallised in a much shorter time.

Table 17. Time required for the sample to be fully crystallised at 0℃, for raw materials and the benchmark IPL

Solid fats	Time for sample to be fully crystallised (min)
TS	90
PS	110
FHCO	100
IPL	16

The crystallization results from Table 17 suggest that the crystallization time for tallow stearin at 90 minutes which is faster than fully hardened coconut oil. Palm stearin was the last to crystallize out of the three raw materials at 110 minutes. Yet none of the raw materials measures upto the bench mark IPL hard stock with crystallization time of 16 minutes during cooling at 0°C.

4.2 Melting point of interesterified fat blends.

4.2.1. Tallow stearin and fully hardened coconut oil (TS:FHCO)

The impact of increasing the tallow stearin content in the blend on the hard stock's melting point is shown in Figure 25 for all three commercial lipase enzymes tested. Raw data is presented in Appendix 1.

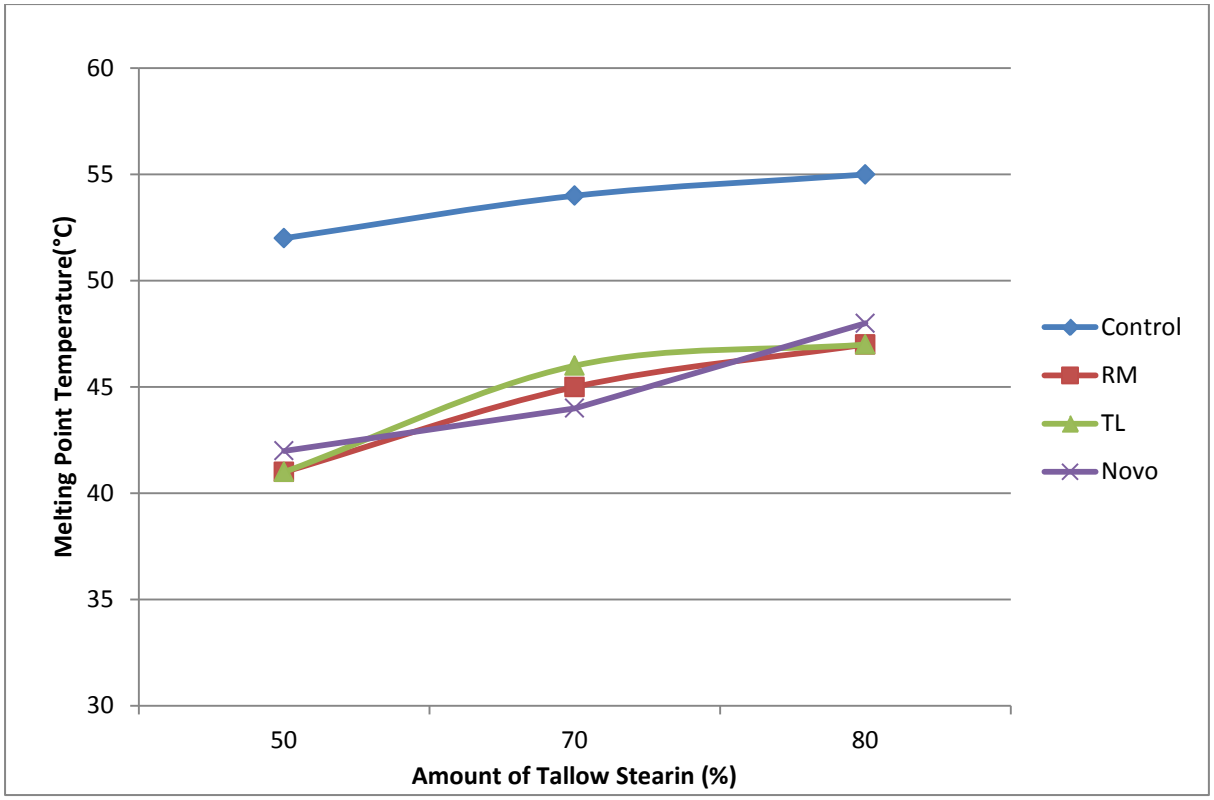

Figure 25. Changes in the melting points of tallow stearin and fully hardened coconut oil after interesterification for 8 hours at 65 ±1 ℃ with various commercial lipase enzymes. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The data points are means of duplicate trials with each trial tested in quadruplicate analysis ± standard deviation (n = 8). Note standard deviations values are small and not visible.

For the tallow stearin and fully hardened coconut oil mix (TS:FHCO), the melting point for interesterified fats with lipase enzymes Lipozyme RM (RM), Lipozyme TL (TL) and Novozyme 435 (Novo) were shown in Figure 25, all enzyme treated TS:FHCO fat blends had lower melting points than the control fat blend, after 8 hours. The drop in melting point was a result of the rearrangement of fatty acids within the triglyceride (TAG) molecule itself and also between other TAG molecules. With the increase in the percentage composition of tallow stearin from 50% to 80%, the melting point of the interesterified hard stock also increased by 7°C. All three enzymes showed an increase in melting point with increasing concentration of tallow stearin. The TS:FHCO treated with TL enzyme had a higher melting point of 46 ± 1°C at 70% tallow stearin compared to the other two enzymes. On the contrary, the Novo enzyme had higher melting point then the other two enzyme processed fats at both 80% and 50% tallow stearin but lower melting point at 70% tallow stearin at 44°C.

4.2.2 Tallow stearin and palm stearin (TS:PS)

The impact of increasing the tallow stearin content in the TS:PS hard stock fat blends on the melting point are shown in Figure 26 for all three commercial lipase enzymes tested.

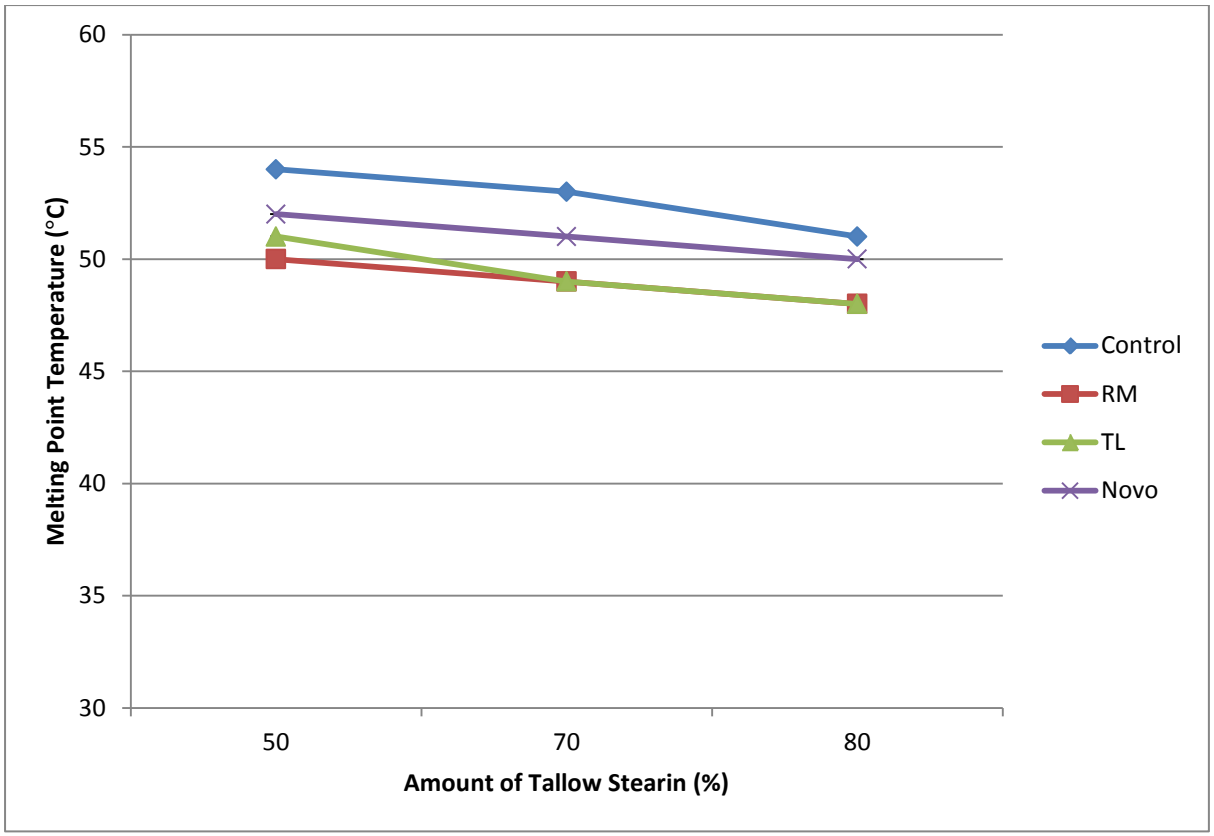

Figure 26. Changes in the melting point of tallow stearin and palm stearin after interesterification for 8 hours at 65 ±1 ℃ with various commercial lipase enzymes. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The data points are means of duplicate trials with each trial tested in quadruplicate analysis ± standard deviation (n = 8). Note standard deviations values are small and not visible.

For the fat blend TS:PS, the increase in the content of tallow stearin resulted in a decrease in the melting point temperature for both interesterified hard stocks and the control (non-interesterified) hard stock. In Figure 26 the melting point decreased from 54 to 51°C as the ratio of tallow stearin was increased form 50% to 80% in the control fat. The melting point temperatures for TS:PS fat blends with the Novo enzyme was significantly higher than the blend interesterified with the RM and TL enzymes, the Novo treated blend still had lower melting points than the control (non-interesterified) fat blend. The melting point

temperatures for TS: PS with RM and TL enzymes were the same at ratios of 50:50 and 70:30 tallow stearins: palm stearin.

4.2.3 Palm stearin and fully hardened coconut oil (PS:FHCO)

The impact of increasing the palm stearin content on melting point of the PS:FHCO hard stock blends is shown in Figure 27 for all three commercial lipase enzymes tested.

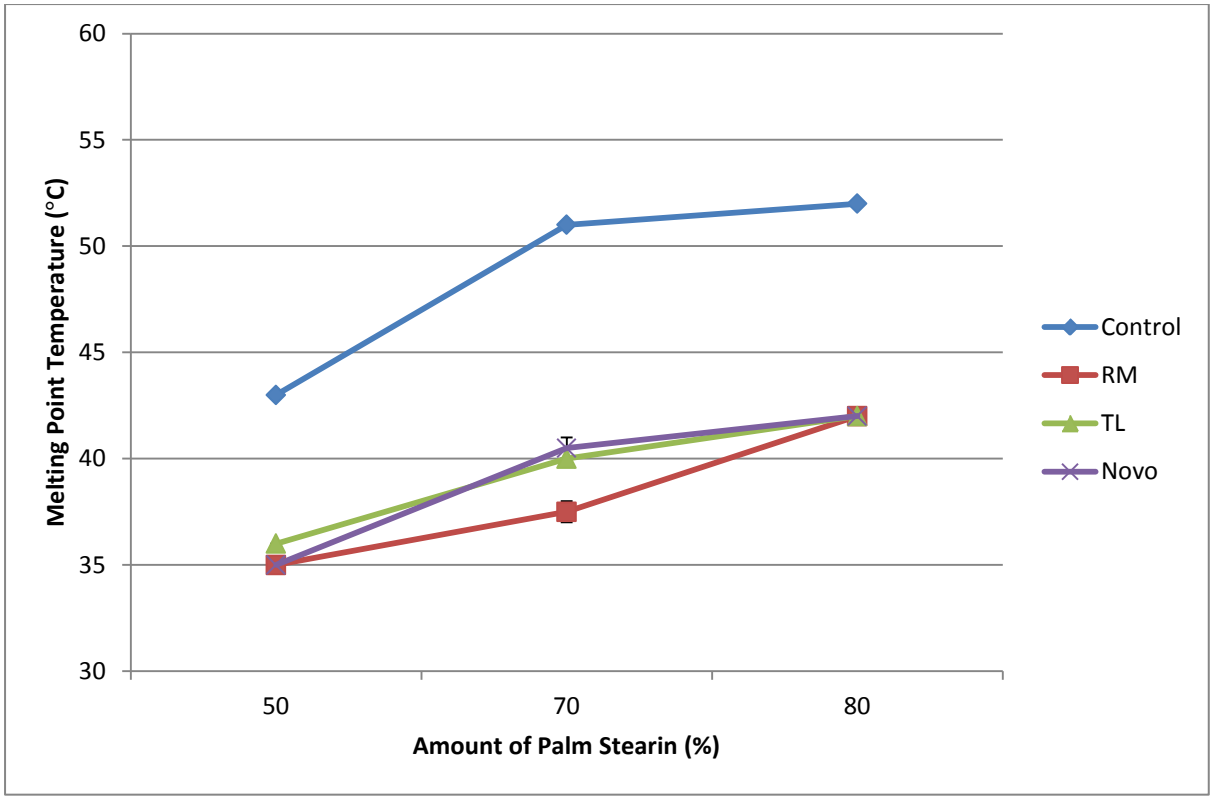

Figure 27. Changes in the melting point of palm stearin and fully hardened coconut oil after interesterification for 8 hours at 65 ±1 ℃ with various commercial lipase enzymes. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The data points are means of duplicate trials with each trial tested in quadruplicate analysis ± standard deviation (n = 8). Note standard deviations values are small and not visible.

The difference in melting points between interesterified and non-interesterified (control) hard stock blends of palm stearin and fully hardened coconut oil was found to be approximately 10°C (Figure 27). By increasing the ratio of palm stearin the melting point temperature, for the interesterified palm stearin and fully hardened coconut oil (PS:FHCO) hardstocks, increased by less than 5°C. At 50% palm stearin, all enzymatically interesterified hard stocks exhibited the same melting point, yet PS:FHCO blends interesterified with Novo and TL enzymes had slightly higher melting point temperatures at 70% palm stearin than the blends interesterified with the RM enzyme. The error bars on the 70% palm stearin fat blends indicates small differences within the mean of the melting point but significance differences still exist between the melting points of Novo and TL with RM at 70% palm stearin.

4.3 Triglyceride (TAG) changes after interesterification

The percentage composition of triglycerides (TAG) of both interesterified and non-interesterified (control) fats was monitored by HPLC. The TAGs were represented with three letters, with each letter representing each of the fatty acids attached to the glycerol backbone of the TAG molecule. The TAGs were ordered according to their equivalent carbon number (ECN) which also corresponded to their retention time and was based on the degree of unsaturation and increasing molecular weight. Each treatment was coded with their fat blend combination, enzyme added and their fat ratio code (Table 12). The unidentified TAGs were coded as unknown (RT) followed by the retention time at which the TAG eluted.

4.3.1 Tallow stearin and fully hardened coconut oil (TS:FHCO)

The TAG composition for the hard stock blends of tallow stearin and fully hardened coconut oil after interesterification with the three commercial enzymes, after 8 hours at 65°C, is shown in Table 18.

Table 18. TAG composition of tallow stearin and fully hardened coconut oil after interesterification, after 8 hours at 65℃. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The TAG results displayed are means of duplicate trials ± standard deviation (n=2). The fatty acid abbreviations for TAG names are mentioned page ix in the index section of the report.

		Percentage of individual TAG/total TAGs present											
ECN	Tag names	Control8.2(TS:FHCO)	Control7.3(TS:FHCO)	Control5.5(TS:FHCO)	Novo8.2(TSFHCO)	Novo7.3(TSFHCO)	Novo5.5(TSFHCO)	RM8.2(TSFHCO)	RM7.3(TSFHCO)	RM5.5(TSFHCO)	TL8.2(TSFHCO)	TL7.3(TSFHCO)	TL5.5(TSFHCO)
24	CpCpCp				6.5±0.9	5.0±1.3	3.4±0.1	4.7±0.1	4.3±1.0	2.8±0.6	5.0±0.1	4.9±1.3	3.4±1.1
	RT3.8				3.8±0		3.4±0.4	4.1±0.7	2.9±0	1.2±1.6	4.6±0.9	2.6±0	2.7±0
	RT4.8				3.2±1.2	6.0±0.7	5.1±0.9	3.4±0.1	3.8±0	1.7±1.0	4.0±0.9	2.2±0.5	1.2±0
	RT5.9				1.5±0.3	2.4±0.3	2.5±0.1	1.5±0.4	2.2±0.1	2.4±0	1.7±0	1.8±0.0	2.3±0
30	CCC	2.1±0.1	2.7±0.2	3.3±0.4	2.4±0.9	4.2±0.1	4.2±0.7	2.9±0.3	3.6±0.3	3.0±0.1	2.6±0.2	2.9±0.2	2.6±0
	RT9.5							1.1±0					
32	CCL	6.1±1.8	9.2±1.0	10.4±3.4	3.4±3.0	10.3±0.2	7.9±2.1	3.8±0.0	4.6±0.0	6.3±1.2	3.5±0.1	4.2±0.1	6.3±0.8
	RT10.8							1.4±0		2.8±0			
34	CLL	14.4±0.8	11.8±0.5	10.2±2.4	5.1±2.7	9.5±0.2	8.8±1.6	5.6±0.	5.6±0.4	6.8±1.4	4.7±0.1	4.2±0.1	3.8±0.4
	RT14.1				2.1±0			1.9±0				1.1±0	3.2±0.5
	RT16.4				2.2±0			1.1±0.3	2.0±1.0	3.8±0.6	1.9±0		
36	LLL	11.9±2.2	15.5±0.9	16.6±3.4	9.4±0.5	9.3±2.3	7.0±0.7	4.2±1.3	6.7±0.7	5.3±2.1	7.8±0.8	6.8±0.8	6.4±0.6
	RT18.6	2.5±0			1.9±0			1.6±0			2.1±0		
38	LLM					5.5±0		2.9±0		3.2±0.7	2.4±0	1.4±2.5	5.1±0.6
38	LLO	10.7±1.8	14.4±0.9	16.7±5.9	5.2±3.8	13.0±0.2	11.6±2.2	5.1±0.9	6.7±0.4	4.9±1.3	5.7±0.8	7.1±0.4	8.8±2.6
38	RT24.4								2.3±0.3	2.310		1.6±0.03	1.7±0.39
40	LMM	7.3±0.6	9.7±0.25	11.3±2.9	5.1±1.4	7.6±0.8	7.4±1.0	7.4±0.4	9.4±0.6	6.5±0.3	6.1±1.3	8.0±0.5	10.4±0.7
40	RT29.6				2.5±0			1.3±0					
40	RT36.4				2.5±0			1.3±0.2	1.0±0.1	1.8±0	2.1±0		
40	LMO	4.4±0.7	6.2±0.1	7.3±0.8	6.6±0.2	6.8±0.6	8.7±1.8	6.5±0.6	8.2±0.3	10.5±2.6	7.1±0.4	8.1±0.4	8.5±0.4
	RT40.1									1.6±0.2	1.7±0		
	RT49.2	2.8±0			3.8±0			1.4±0.9	3.4±0.8		2.1±0.2	2.4±0.2	2.0±0
42	LMP		2.3±0		8.5±0		5.4±1.9	8.5±2.1	10.1±0.6	9.3±0.4	8.9±1.2	11.9±1.5	8.7±0.1
44	PLO						3.4±0.4	4.3±0.9	3.2±0.6	4.8±0.9	5.2±2.1	2.5±1.4	
44	PPL						4.3±0			2.8±0			
	RT76.1	2.9±0.2	2.6±0.2	2.6±0.6								3.4±0	
48	POO	17.3±1.9	12.9±0.9	11.1±0	11.9±0.9	10.4±2.2	8.2±1.5	11.4±0.5	10.4±0.4	9.2±1.5	12.9±0.5	12.9±2.9	9.2±1.4
48	PPP	15.8±1.7	12.4±0.9	9.1±1.3	10.8±0.6	9.3±1.0	7.4±2.1	12.4±1.5	10.7±0.3	9.4±1.2	12.6±1.2	11.8±1.7	8.2±0.1
50	POS											2.2±0	

The non-interesterified TS:FHCO fat blends in Table 18 show that as the percentage composition of fully hardened coconut oil is increased in the blend, the amount of high molecular weight TAGs like POO and PPP decreased and a percentage of the TAGs originating from coconut oil increased, though not significantly, such as LLL and CLL. It was also observed that medium molecular weight TAGs such as PLO and PPL were not found in non-interesterified fats.

For TS:FHCO interesterified hard stock, the blends Novo7.3, RM7.3 and TL7.3 had higher percentages of low molecular weight TAGs, such as CCC, CpCpCp, than the control (non-interesterified) 7.3 blend. Unknown low molecular weight TAGs, RT3.7, RT4.8 and RT5.8 were found in interesterified TS: FHCO fat blends and decreased percentages of TAGs CCL, CLL, LLO and LLL was also observed. These observed changes in the TAG composition was a result of the changes to the fatty acid composition within the TAGs as the lipase enzymes converted the CCL, CLL, LLO and LLL to CCC, LLM, LMM and LMO. The LMP peak was only seen in one control TS: FHCO fat blend but found present at 8 to 11% in the interesterified TS:FHCO blends. The PPL and PLO TAGs were also not present in the control TS:FHCO blends (non-interesterified) but were seen in the interesterified fats at different percentages hence were identified as a newly formed TAGs by interesterification. The percentage of the high molecular weight TAGs; PPP and POO were reduced as the fats were interesterified. The TAG composition for TS:FHCO blend with RM7.3 had a similar composition as the TL7.3 treated blend. A higher percentage of CLL and CCL were found in the TS:FHCO Novo7.3 blend indicating that this enzyme was not as effective at modifying the TAG composition on the low molecular weight TAGs compared to the enzymes Lipozyme RM and TL in the 70:30 blend under the same conditions.

4.3.2 Palm Stearin and fully hardened coconut oil (PS:FHCO)

The TAG composition for the hard stock blends of palm stearin and fully hardened coconut oil after interesterification with the three commercial enzymes, for 8 hours at 65°C, is shown in Table 19.

Table 19. TAG composition of palm stearin and fully hardened coconut oil after interesterification, after 8 hours at 65℃. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The TAG results displayed are means of duplicate trials ± standard deviation (n=2). The fatty acid abbreviations for TAG names are mentioned page ix in the index section of the report.

ECN	Tag names	Control8.2(PSFHCO)	Control7.3(PSFHCO)	Control5.5(PSFHCO)	Novo8.2(PSFHCO)	Novo7.3(PSFHCO)	Novo5.5(PSFHCO)	RM8.2(PSFHCO)	RM7.3(PSFHCO)	RM5.5(PSFHCO)	TL8.2(PSFHCO)	TL7.3(PSFHCO)	TL5.5(PSFHCO)
24	CpCpCp			3.8±0	5.0±0	4.4±0.5	3.3±0.8	3.9±0.8	4.3±0.3	3.3±0.5	3.9±1.4	4.0±0.7	3.2±1.1
	RT5.3					1.3±0				1.3±0			1.7±0.5
	RT6.3	1.1±0.0	1.1±0	2.3±0	1.0±0	1.4±0	1.1±0	1.2±0	1.7±0		1.2±0	1.8±0	2.1±0.8
	RT6.6			1.8±0.6	1.1±0			1.4±0		1.9±0.2	1.6±0.2		
30	CCC	1.3±0	1.2±0.2	3.2±1.2	2.7±0.1	4.1±0	3.1±0.1	2.8±0.6	2.7±0.5	3.5±0.5	2.0±0.6	2.9±0.7	3.1±0.5
32	CCL	3.5±0.1	4.7±0.6	8.6±0.16	2.9±0.6	2.9±0.7	3.1±0.1	3.1±0	3.4±0.5	2.1±0	3.2±0.7	2.6±0.1	2.9±0.5
	CLL	5.2±0.2	6.2±0.1		4.2±0.1	4.3±2.0	4.3±1.9	4.3±0	4.5±0.12	4.6±1.2	3.9±0.3	4.1±0.5	4.7±1.5
34	RT10.8				1.3±0	3.7±0	2.1±0.89	1.3±0.1	2.2±0	2.2±0	1.1±0.1	1.3±0	
	RT13.6				4.4±0	1.2±0	3.4±0.2			2.7±0			1.9±0
	LLL	4.1±0.1	5.5±0.9	8.8±0.43	1.8±0.4	3.6±1.3	3.8±0.4	1.9±0	3.6±0.7	3.9±0.5	2.1±0.1	2.8±0.5	4.2±0
36	RT17.3	1.5±0.1	1.5±0	1.3±0			1.9±0.7			1.9±0.2		1.3±0	2.5±0.4
	LLM	3.1±0.2	3.4±0.8	4.5±2.1	3.4±0	3.1±0.3	5.2±0	3.6±0.6	3.0±0.7	3.8±0	3.5±0.2	2.6±0.5	5.9±0.7
38	RT24.9	1.6±0.2	1.7±0.5	4.4±0	1.2±0	1.9±0.7	3.9±1.2	2.3±0.9	2.0±0.3	4.3±1.5	2.4±0.9	2.2±0.3	4.5±0.5
38	LLO	3.2±0	3.9±0.4	4.7±0.7	3.9±1.4	4.1±0.2	7.8±0.2	3.1±0.8	3.8±0.5	8.0±0.6	3.9±0.8	3.9±0.4	7.4±0.9
38	RT25.3			2.1±0	2.6±0	1.3±0	1.7±0.4	2.6±0.6	1.6±0	1.4±0	2.6±0.3		
40	RT30.7				2.2±0	2.1±0.1	1.8±0.6	2.8±0	2.2±0.1	2.8±0.6	1.8±0.6	1.8±0.4	1.8±0.6
40	LMM	3.2±0.1	4.3±1.0	5.3±0.1	3.3±0.2	3.9±0.5	6.5±0.3	0.34±0	3.6±0	6.2±0.8	3.6±0.4	3.4±0.4	6.7±0.1
40	RT35.5			1.5±0	2.1±0.5			2.1±0.2	1.3±0		2.1±0.1	2.4±1.2	3.4±1.49
40	RT41.1	1.1±0	1.12±0.1			1.2±0.1		1.4±0	3.2±1.0		1.3±0	2.5±1.1	
	LMO	2.3±0	2.6±0	3.8±0	6.7±0.1	3.9±0.7	5.3±0.3	6.9±0.7	3.9±0.7	6.2±0.4	6.5±0.9	7.2±0.5	5.2±0.2
	LMP				6.5±0.9	5.3±2.0	8.4±0.4	7.9±0.8	7.3±0.2	9.1±0.1	8.4±0.7	8.3±1.1	8.1±0.5
42	PLO	4.2±0.1	3.6±0	2.6±0	4.8±0.4	5.2±0.9	3.4±0.2	3.7±0.6	6.9±0.5	3.9±0.2	5.7±0.2	4.3±1.3	2.2±0.6
44	PPL	4.9±0.2	4.4±0.1	2.9±1.8	6.9±0.1	3.8±0.6	6.1±0.1	4.9±1.7	3.8±0	5.1±0.2	6.5±0.9	4.0±0	4.7±0.1
44	RT60.2	2.1±0.1	2.67±0.3	2.1±0	2.8±0	4.5±1.2			6.2±0			4.4±1.4	
	RT63.0		1.4±0	2.9±0	5.4±0	4.7±0		4.8±0	5.9±0		6.8±0	4.3±1.0	
48	POO	11.2±0.9	10.1±1.1	6.1±0.4	6.3±0	5.7±0.7	3.8±0.6	6.6±0	6.1±0.7	3.9±0	6.4±0.5	5.5±0.2	3.9±0.4
48	POP	24.6±0.4	21.5±0.1	15.7±0.3	15.0±0.8	11.6±0.2	10.5±1.6	16.5±1.5	10.8±0.1	11.2±1.2	14.7±1.4	11.9±0.1	10.9±0.3
48	PPP	22.0±0.1	20.1±0.3	14.9±0	12.9±1.5	10.9±0.5	9.3±0.9	12.3±1.6	11.2±0	9.0±0.9	12.5±1.3	10.9±0.4	9.4±0.9
50	POS												
52	PSS		3.1±0	2.9±0		6.4±0			6.3±0	4.7±0		5.9±0	

The percentage composition of high molecular weight TAGs of POP, POO and PPP decreased as the composition of palm stearin decreased from 80% to 50% in the control (non-interesterified) for the PS:FHCO fat blends. While an increase in the percentage composition of CCL, CLL and LLL was also observed with the decrease of palm stearin in the PS:FHCO control fat blends. In the 80% palm stearin control blend, the percentage of the high molecular weight TAGs, POO, POP and PPP contributed to close to 50% of the total TAGs found in the blend. Even as the amount of palm stearin was reduced to 50%, the percentage composition of these high molecular TAGs still contributed over 30% of total TAGs in the PS:FHCO blends. The PS:FHCO fat blend interesterified by Novo 8.2 enzymes had a higher percentage of PPL than the other enzymes with the 80% palm stearin blends. The RM8.2 and TL8.2 enzyme interesterified fat blends had higher percentages of LMP TAGs than the Novo8.2 fat blends. The decrease of low molecular weight TAGs of CCL, CLL and LLL was observed for all the interesterified fat blends. Unknown TAG RT 60.2, 63.0 and PSS were observed after interesterification. When the percentage of palm stearin in the fat blend was reduced to 50%, reduction was observed in the high molecular TAGs of POP, POO and PPP. An increase in medium molecular weight TAGs of LLM, LLO and LMM was also observed with the decrease of palm stearin to 50% in the PS:FHCO fat blends. The TAG composition in Table 19 suggests that the decrease in the percentage composition of high molecular TAGs for the interesterified 80% palm stearin was caused by the increase in the medium molecular weight TAGs of PPL, PLO and LMP.

4.3.3 Tallow stearin and Palm Stearin (TS:PS)

The TAG composition for the hard stock blends of tallow stearin and palm stearin after interesterification with the three commercial enzymes, for 8 hours at 65°C, is shown in Table 20.

Table 20. TAG composition of tallow stearin and palm stearin after interesterification, after 8 hours at 65℃. Novo – Novozyme 435, RM – Lipozyme RM IM, TL – Lipozyme TL IM, Control – no enzyme. The TAG results displayed are means of duplicate trials ± standard deviation (n=2). The fatty acid abbreviations for TAG names are mentioned page ix in the index section of the report.

ECN	TAG names	Control8.2 (TSPS)	Control7.3(TSPS)	Control5.5(TSPS)	Novo8.2(TSPS)	Novo7.3(TSPS)	Novo5.5(TSPS)	RM8.2(TSPS)	RM7.3(TSPS)	RM5.5(TSPS)	TL8.2(TSPS)	TL7.3(TSPS)	TL5.5(TSPS)
24	CpCpCp				6.7±0.4	9.5±1.4	10.5±0.4	7.3±0.1	9.6±1.3	10.1±0.8	7.9±0.3	9.1±0.3	10.2±0
	RT4.6				4.2±0	4.7±0.5	4.3±0.3	4.6±0.2	4.6±0.4	4.2±0.4	4.6±0.1	4.1±0.1	4.0±0.2
30	CCC	1.3±0.1	1.3±0.1	1.9±0.2									
32	CCL	3.1±0.1	3.6±0.4	6.8±0.2	3.3±0.2	5.7±0.2	6.8±0.4	3.9±0.4	5.6±0.3	7.7±0.2	3.2±0.4	4.4±0.3	7.0±0.2
34	CLL	4.8±0.1	6.1±0.2	8.4±0.4	5.7±0.5	7.2±0.2	9.1±0.1	6.1±0.2	7.6±0.2	9.4±0.4	5.7±0.3	7.6±0.2	9.3±0.1
	RT13.6					2.7±0.4	3.1±0.6		3.3±0.2	3.6±0.2		3.5±0.2	3.2±0.1
36	LLL	4.5±0.3	6.4±0.1	9.2±0.6	9.1±0	9.1±0.1	9.1±0.1	9.1±0	9.1±0.1	9.1±0	9.1±0.1	9.1±0.1	9.1±0.1
38	LLM	3.3±0.1	4.8±0.1	6.8±0.4	3.7±0.1	3.2±0.2	3.4±0.4	3.5±0.1	3.3±0	3.6±0.3	3.3±0.1	2.8±0.2	2.9±0.5
40	LLO				3.2±01	3.4±0	3.8±0.1	3.3±0.2	3.5±0.1	3.7±0.1	3.6±0.1	3.6±0.4	3.8±0
40	LMM	4.4±0.2	4.7±0.1	5.3±0.3									
42	LMO	1.6±0.2	2.3±0.2	3.5±0.4									
42	LMP												
44	PLO	3.8±0.6	3.8±0.1	2.9±0.3									
44	PPL	4.5±0.4	4.7±0.1	3.2±0.1	3.1±0	3.1±0.1	3.54±0.28	3.1±0.1	3.0±0.1	3.2±0.1	3.1±0	3.1±0	3.3±0.1
	RT82.5	4.1±0.1	5.5±0.1	4.1±0.1									
48	POO	13.2±0.7	9.7±0.1	7.3±0.2	8.5±0.1	7.6±0.3	5.8±0.6	8.0±0.2	6.3±1.1	4.9±0.1	7.7±0.3	6.2±0.9	5.1±0.9
48	POP	23.8±0.4	22.1±0.1	17.5±1.8	10.3±0	8.6±0.4	7.2±0.2	9.9±0.3	8.1±0.1	7.2±0.4	9.9±0.4	9.1±0.1	7.1±0.1
48	PPP	27.1±0.3	24.6±0.5	21.1±1.1	12.8±0.1	11.7±0.3	10.9±0.7	12.8±0.4	11.5±0.3	10.1±0.5	12.4±0.4	12.0±0.4	10.8±0.1
52	PSS			2.6±0	28.6±0.3	23.5±1.0	21.7±0.3	28.1±0.1	24.2±0.5	22.2±0.7	28.8±0.9	24.6±0.3	23.0±1.7

For TS:PS fat blends, the TAG composition for the non-interesterified blend (control) showed a high percentage of high molecular weight TAGs like POP and PPP as both fats (tallow stearin and palm stearin) contained large amounts of the long chained fatty acids. When the fats were interesterified fewer changes in the TAG composition were observed compared to the interesterification of TS:FHCO (Table 18) or the interesterification of PS:FHCO (Table 19). The LMP and PLO TAGs were not found in the interesterified TS:PS fat blend as found in the earlier blends with fully hardened coconut oil. The TAG PPL found after interesterification of TS: FHCO and PS:FHCO was also found in the interesterified TS:PS blends. Over 20% of TAGs present in the interesterified TS:PS fat blends were found to be the TAG PSS, which was triggered by the exchange of fatty acids between the high molecular weight TAGs. The PSS TAG was found in the composition of raw material tallow stearin (Table 15). The high melting points were due to the presence of the large quantities of high molecular weight TAGs as the blending of two high molecular fats did not deliver fats with desired physical characteristics.

4.4 Differential scanning calorimetry

The change in heat capacity for the fat blends was monitored during crystallization of the interesterified and non-interesterified fats using differential scanning calorimeter (DSC) as described in Section 3.12. The temperature was reduced from 80°C to -60 °C at a rate of 10 °C min and the change in the specific heat capacity was registered in the form of a cooling curve.

4.4.1 Tallow stearin and fully hardened coconut oil (TS:FHCO)

The DSC cooling curves in Figure 28 indicates the change of heat occurred during crystallization of tallow stearin and fully hardened coconut oil with no enzyme at the ratios of 80%, 70% and 50% tallow stearin.

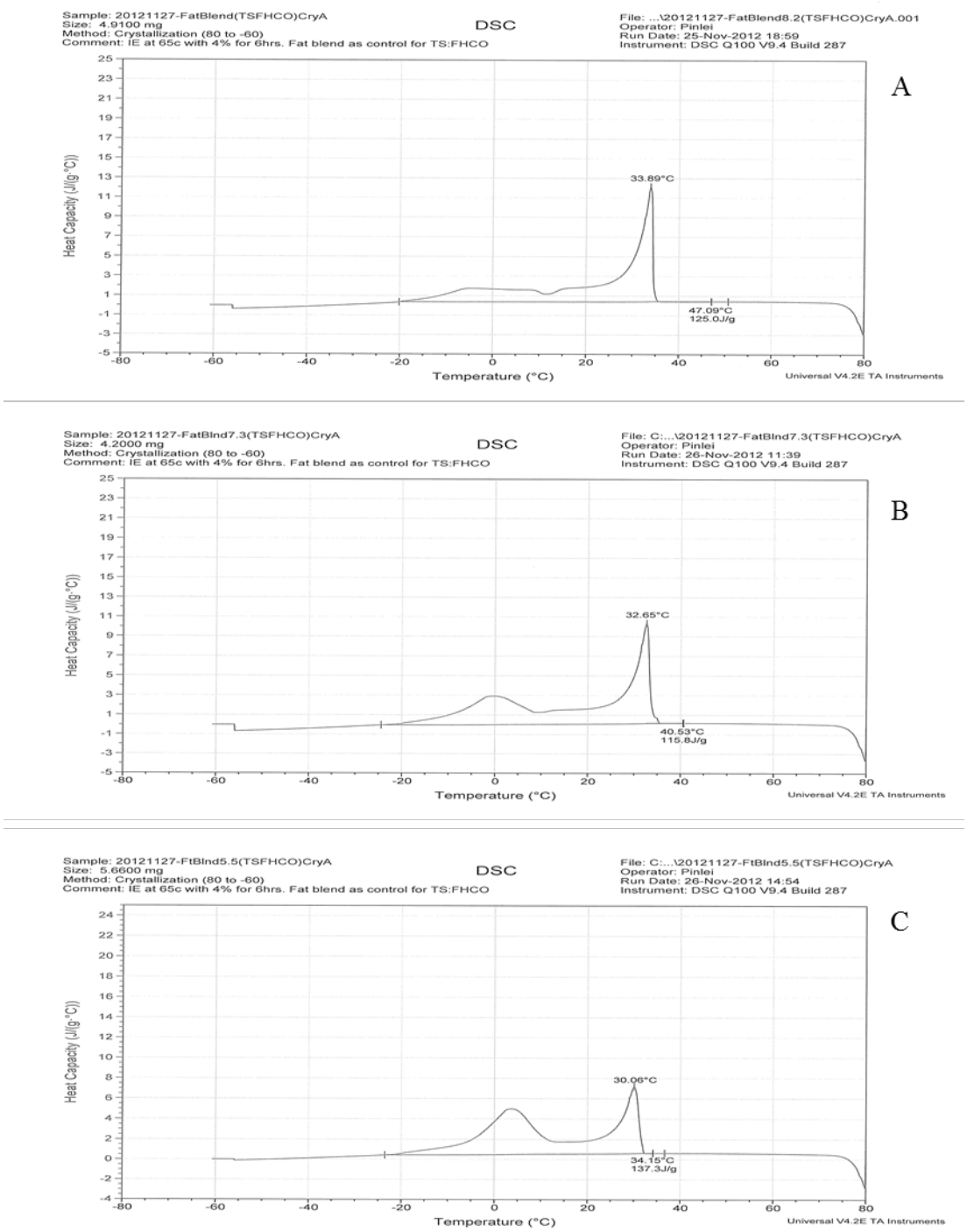

Figure 28. DSC cooling (crystallisation) curves for TS: FHCO blends with no added enzymes (Control, non-interesterified). A. 80:20 TS: FHCO, B. 70:30 TS: FHCO, C. 50:50 TS: FHCO.

The series of DSC heat curves shown in Figure 28 correspond to the heat released during crystallization of the non-interesterified fat blends of tallow stearin and fully hardened coconut oil (TS:FHCO) at different fat ratios. As the temperature was lowered, heat was released from the solid fat sample and two heat peaks were observed from fat blends of TS: FHCO. In Figure 28A the first large peak of 13 J/g°C occurred in the temperature range of 40 to 20°C whereas the second small peak of 4 J/g°C occurred between the temperatures of -20 to 10°C. The smaller peak between -20 to 10°C increased from 2 J/g°C up to 4 J/g°C while the peak during 40 to 20°C decreased, as the ratio of tallow stearin to fully hardened coconut decreased from 80:20 to 50:50 (Figure 28).

The amount of heat released for the -20 to 10°C peak in Figure 28C for the 50% tallow stearin was greater than the 70% tallow stearin (Figure 28B) fat blend due to the higher percentage of low molecular weight TAGs.

In the non-interesterified fat blends of TS: FHCO, the increase in the percentage of fully hardened coconut oil caused a rise in the heat released at lower temperature range (-20 to 10°C) while the high temperature peak (40 to 20°C) decreased as the amount of tallow stearin was reduced. The increase in the low temperature peak can also be explained by the HPLC analysis on TAGs, as the percentage of fully hardened coconut oil was increased, the amount of low molecular weight TAGs like CLL, LLL and CCL was increased which corresponded to the larger low temperature heat peak.

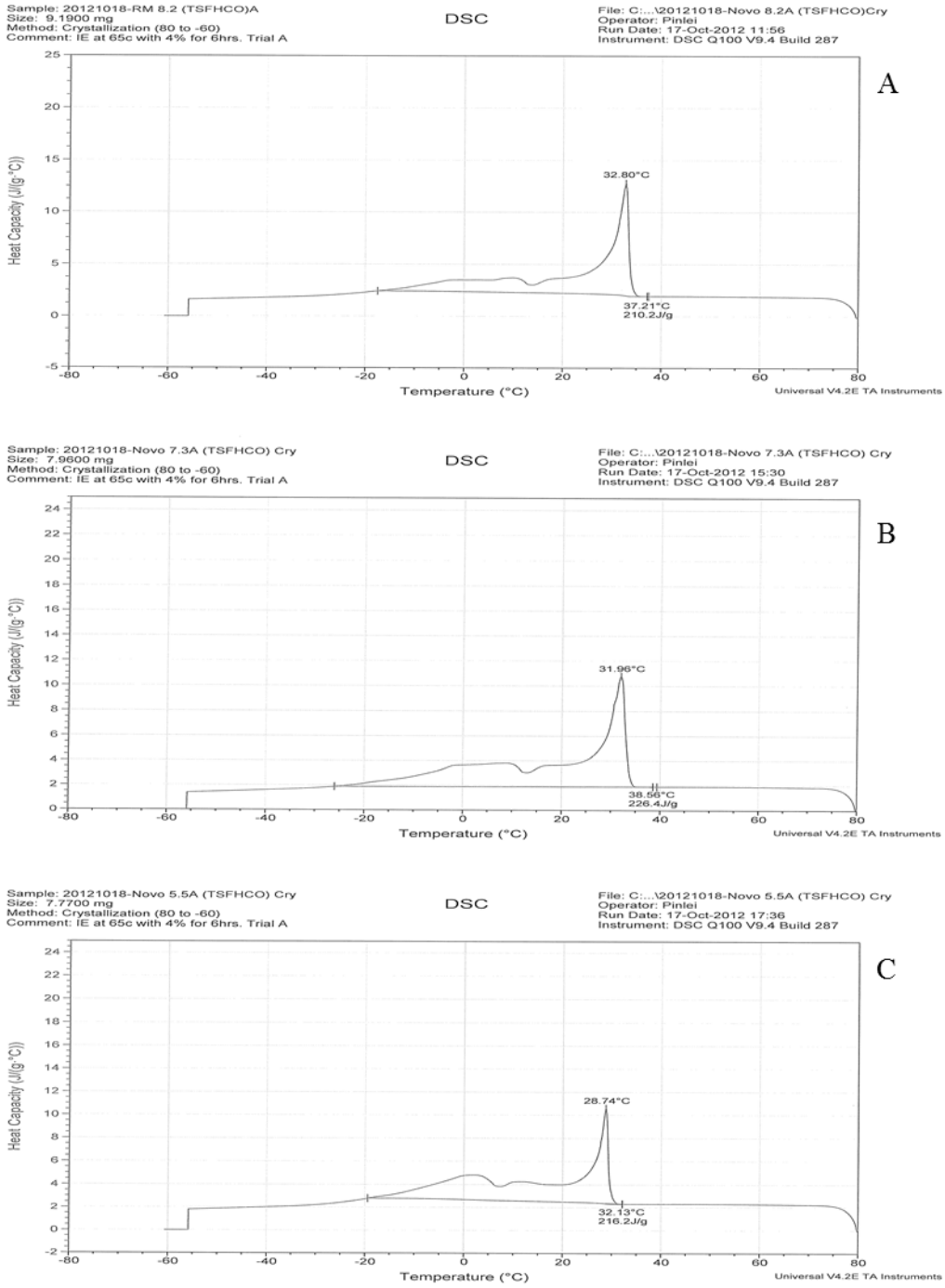

Figure 29. DSC cooling (crystallisation) curve for TS: FHCO blends with Novozyme 435 (added at 4% w/w), after 8 hours at 65℃. A. 80:20 TS: FHCO, B. 70:30 TS: FHCO, C. 50:50 TS: FHCO.

In the TS: FHCO fat blends interesterified with Novo enzyme differences in the heat release were found between these interesterified fat blends and the control non-interesterified fat. In the TS:FHCO blend with 80% tallow stearin the -20 to 10°C peak of the Novo interesterified fat was higher (4 J/g°C) than that of non-interesterified control fat blend (2 J/g°C) (Figure 29A). The 40 to 20°C peak for the Novo interesterified TS: FHCO blend was also higher, at 13 J/g °C compared with 11 J/g°C in the control sample (Figure 28A). This change can be explained by the increase in CpCpCp and CCC TAGs in the interesterified TS: FHCO blends (Table 17). The shoulder formed as the fat was cooled from 25 to 15°C between the 40 to 20°C and -20 to 10°C peaks in the interesterified fats increased from 1 J/g°C to 4 J/g°C compared to the non-interesterified control TS:FHCO fat blend. This change can be explained by the formation of newly formed interesterified TAGs in the medium molecular weight range like PPL, PLO and LMP (Table 18).

As the percentage of tallow stearin was lowered from 80% to 50%, changes in the specific heat released can be observed in -20 to 10°C peaks. In the interesterified 70% TS:FHCO blend, the height of the 40 to 20°C peak remained between 12 to 13 J/g°C after interesterification while the -20 to 10°C peak increased to above 4 J/g°C. In the 50% TS:FHCO blend there was no observed change in the height of the -20 to 10°C peak but an increase was observed in the shoulder between 25 to 5°C. This is the result of the formation of LMP TAGs that occurred during interesterification. In contrast with TS:FHCO Novo8.2 and Novo7.3, the 40 to 20 °C peak of Novo5.5 was much lower due to the lower percentage of high molecular weight TAGs, less tallow stearin. The duplicate trial results from the DSC are in the appendix (Appendix D) indicated similar heat releases.

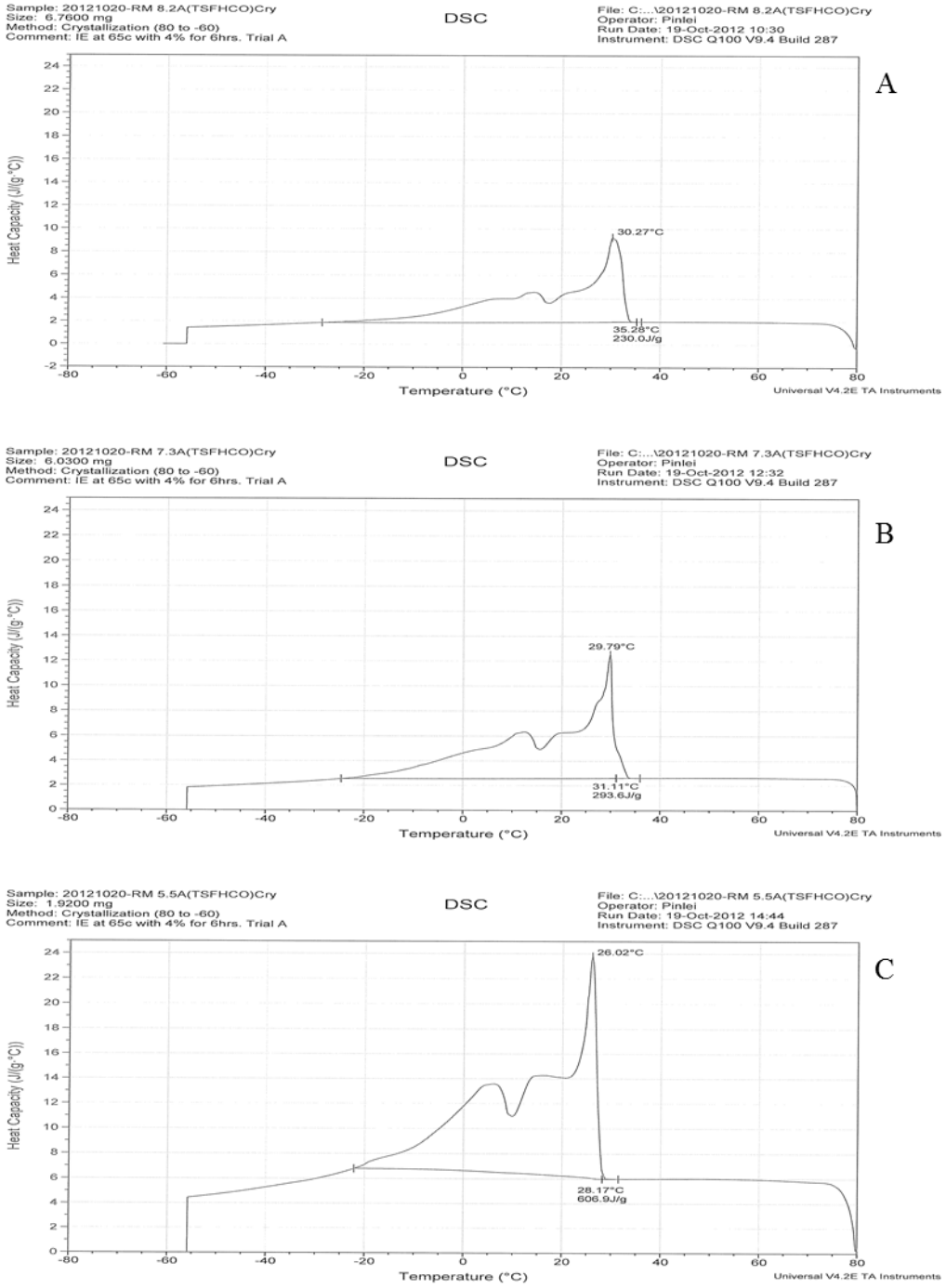

Figure 30. DSC cooling (crystallisation) curve for TS: FHCO blends with Lipozyme RM IT (added at 4% w/w), after 8 hours at 65℃. A. 80:20 TS: FHCO, B. 70:30 TS: FHCO, C. 50:50 TS: FHCO.

In the TS: FHCO blends interesterified with Lipozyme RM enzyme the heat released on cooling is shown in Figure 30. In the TS:FHCO blend with 80% tallow stearin (Figure 30A) small changes were observed in the -20 to 10°C peak but the height of the 40 to 20°C peak was reduced from 13 J/g°C to 10 J/g°C. This was caused by the slight decrease in the high molecular weight TAGs, PLO and PPL; in the RM interesterified TS: FHCO fats as shown in Table 17. For TS:FHCO with RM7.3, the higher shoulder between the -20 to 10°C and 40 to 20°C peaks, between 25 to 20°C at 6 J/g°C was due to the presence of LMP and PLO TAGs as well as Unknown RT 49.17 TAG . There was no difference in the heat released by the TS: FHCO RM enzyme interesterified fats and the Novo enzyme interesterified fats at the 40 to 20°C peak as TAG composition was similar in these two interesterified blends. When the percentage of tallow stearin was reduced to 50%, a larger shoulder was observed in the RM enzyme interesterified fats than the Novo enzyme interesterified fats during 25 to 10°C. This observation can be explained by the newly formed LMP, PPL and PLO TAGs indicated in Table 17. Large peaks were observed at -20 to 1 0°C and 40 to 20°C in the TS: FHCO RM5.5 interesterified fat blends at 25 and 15 J/g°C. Figure 30C indicates that most of the heat released during crystallization occurred between temperatures of 25 to -20°C while all previous interesterified blends showed that major heat released temperature was between 35 to -20°C.

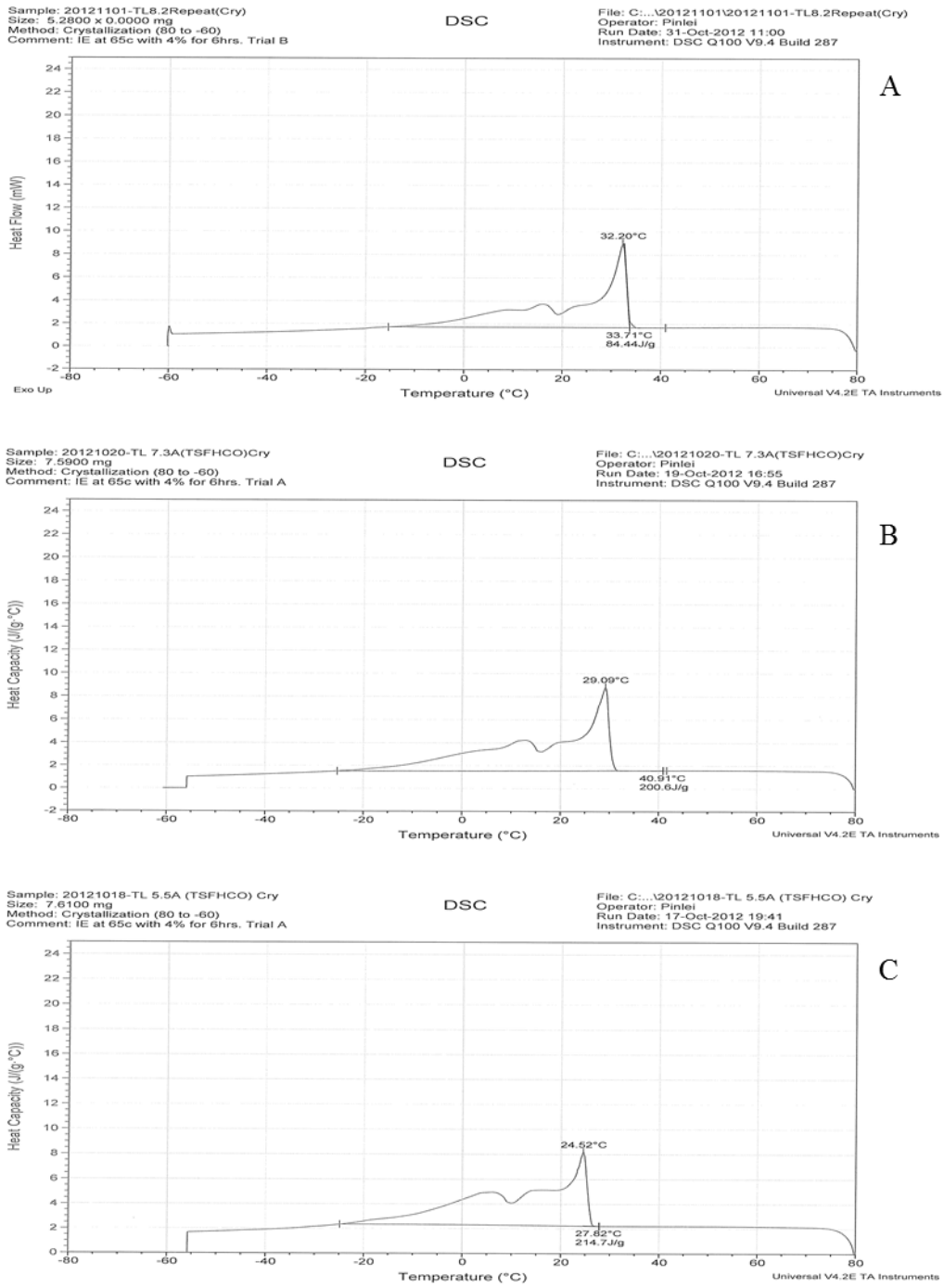

Figure 31. DSC cooling (crystallisation) curve for TS: FHCO blends with Lipozyme TL IT (added at 4% w/w), after 8 hours at 65℃. A. 80:20 TS: FHCO, B. 70:30 TS: FHCO, C 50:50 TS: FHCO.

For TS: FHCO blends the TL enzyme interesterified fats showed similar heat release (Figure 31) as the RM enzyme interesterified fats (Figure 30). In the TS: FHCO blends interesterified with TL enzyme with 80% tallow stearin (Figure 31A), similar results were obtained as observed for the RM enzyme (Figure 30A). The shoulder between 25 to 20°C was present in both enzyme treated samples as the TAG composition between the RM and TL enzymes was very similar (Table 18). For the TS: FHCO blends, the only differences between the TL interesterified fats and RM interesterified fats was that most of the heat released by the TL enzyme interesterified fat blend was between -10 to 35°C while heat released by RM enzyme occurred between -20 to 35°C. The TL 7.3 (TS:FHCO) interesterified fat showed a similar peak structure as the RM 7.3 (TS:FHCO) interesterified fat but the height of peaks between both -10 to 20°C and the 20 – 40°C reduced from 9 and 4 J/g°C and from 13 and 6 J/g°C, respectively. The crystallization temperature for both RM (TS: FHCO) and TL (TS: FHCO) interesterified fats were between -20 to 35°C. The TL 5.5 (TS:FHCO) interesterified fat blend showed a crystallization curve similar to other TL interesterified fats as most of the heat peaks were released between the temperatures of -20 to 25°C. The TL enzyme interesterified fats had lower heat release at both -20 to 10°C and 40 to 20°C peaks as well as the shoulder between these peaks. The high temperature peak of the TL interesterified fats was at 8 J/g°C instead of 24 J/g°C as found in RM interesterified fats and the heat released by the low temperature peak was at 5 J/g°C. The duplicated results of the TL enzyme interesterified fats showed similar heat release profiles during crystallization for all 80%, 70% and 50% interesterified tallow stearin fat blends.

4.4.2 Palm stearin and fully hardened coconut oil (PS:FHCO)

Non- interesterified solid fats of palm stearin and fully hardened coconut oil was first blended and tested as a control to the interesterified hard stocks. Higher ratios of palm stearin was used and the blend ratios were 80% palm stearin with 20% fully hardened coconut oil, 70% palm stearin with 30% fully hardened coconut oil and 50% palm stearin and 50% fully hardened coconut oil.

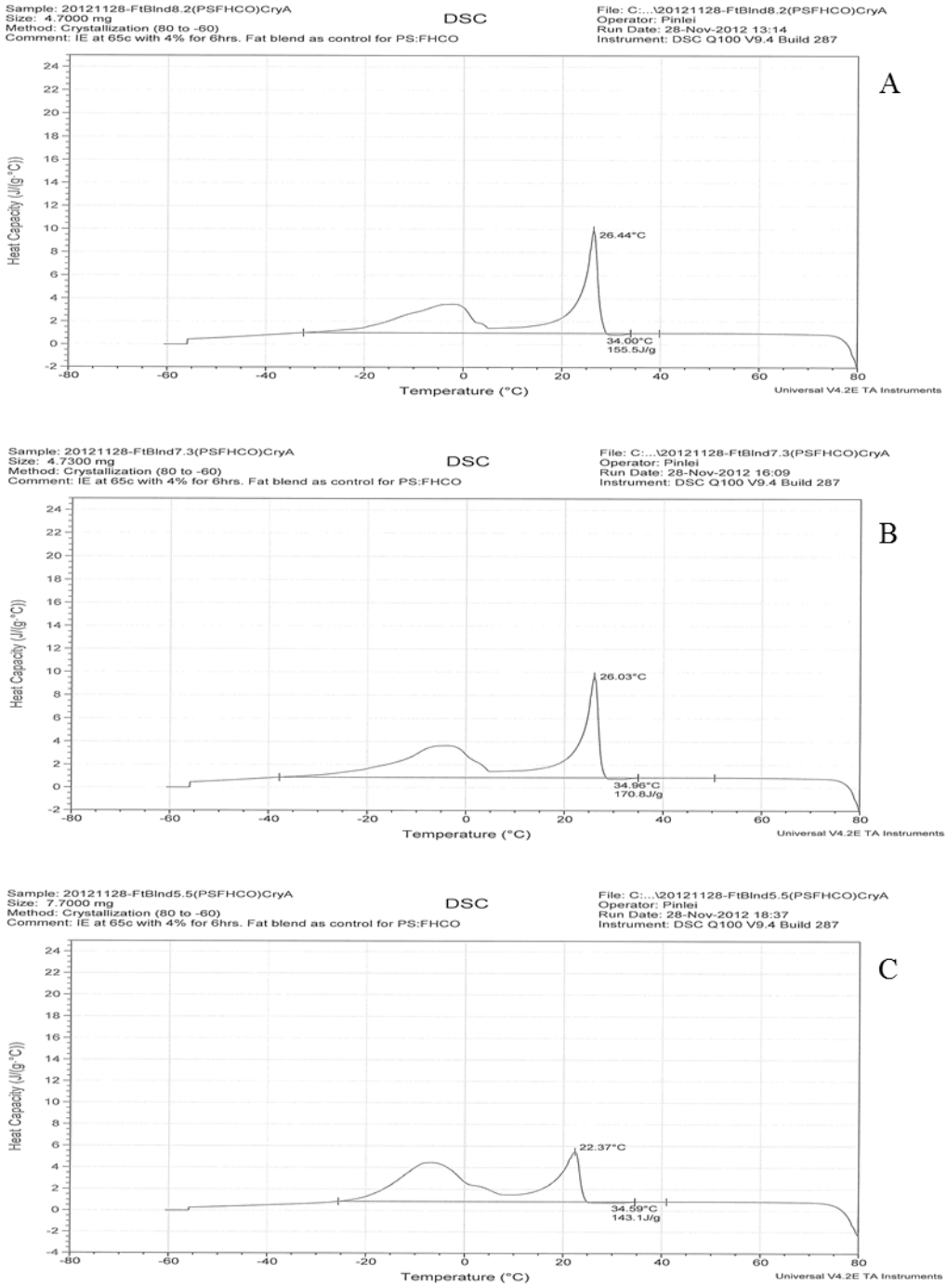

Figure 32. DSC cooling (crystallisation) curve for PS:FHCO blends with no added enzymes (Control, non-interesterified) A 80:20 PS:FHCO, B. 70:30 PS:FHCO, C. 50:50 PS:FHCO.

Two very distinct peaks can be observed in Figure 32A, which represent the heat released by the 80% palm stearin fat blend during crystallization. The high temperature peak can be observed at temperatures between 30 to 20°C which represents the heat given off by the high molecular weight TAGs such as POO, POP, PPP and PSS (Table 19). The low temperature peak between 5 to -20°C represents the low molecular weight TAGs that were mainly made up of CCL, CLL, LLL and CCC. As the amount of palm stearin was decreased to 70% (Figure 32B), small changes were observed in the height of both the high and low temperature peak, this is supported with the TAG composition between the 70% palm stearin blend and the 80% palm stearin blend were very similar. There was no shoulder observed in all non-interesterified palm stearin blends between the temperatures of 20 to 5°C, hence two separate peaks were observed. When the amount of palm stearin was reduced to 50%, a reduction in the high temperature peak can be seen in Figure 32C. The peak reduced to below 6 J/g°C from 10 J/g°C in the 70% and 80% palm stearin blends. The low temperature peak was increased to above 4 J/g°C in the 50% blend whereas previously it was below 4 J/g°C. This change can also be observed in the change of TAGs in Table 19. The percentage of POP, PPP and POO reduced as the amount of palm stearin was decreased in the blend and the typical TAGs for coconut related fats such as LLL increased as the amount of fully hardened coconut fats increased to 50%.

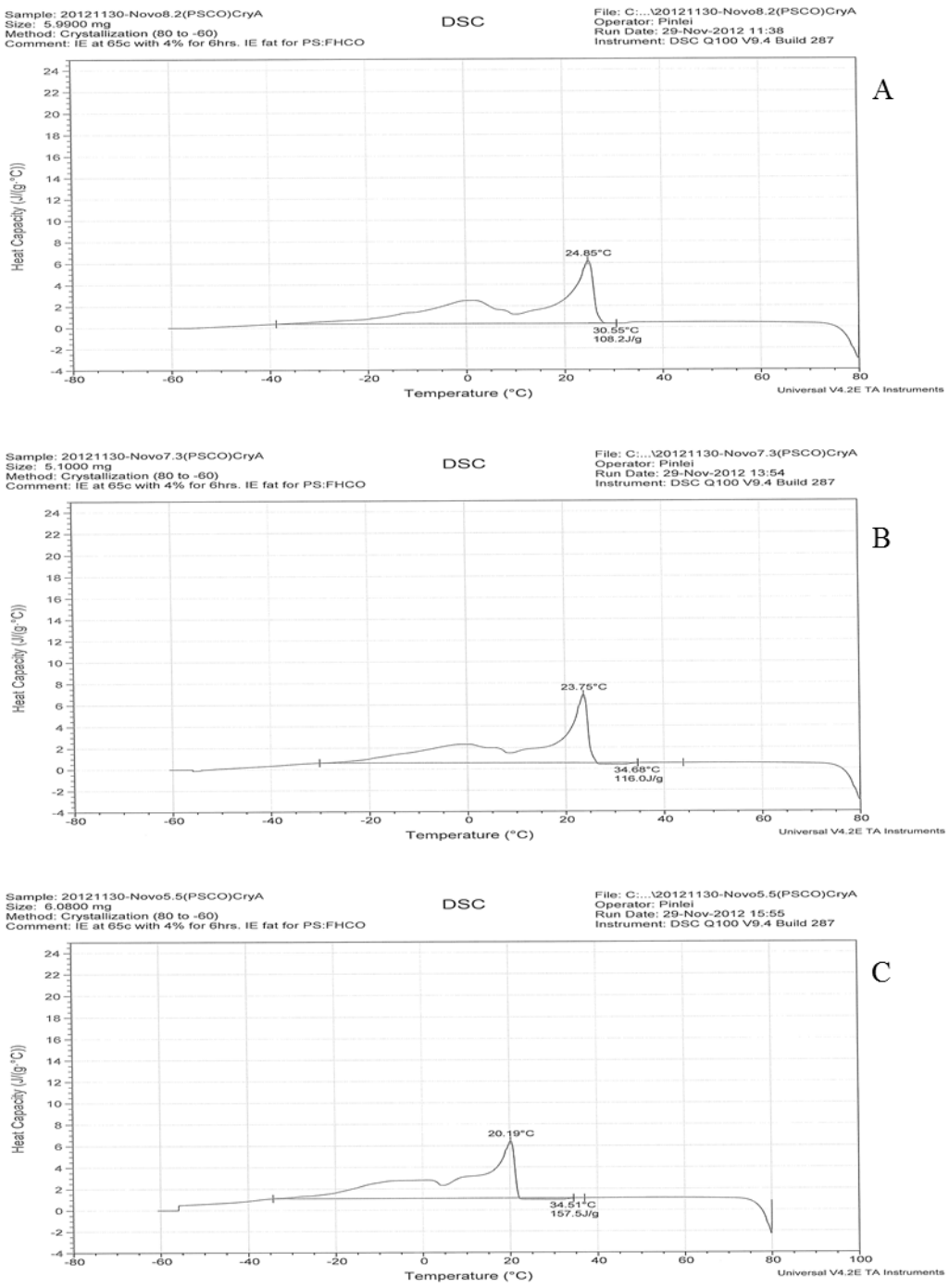

Figure 33. DSC cooling (crystallisation) curve for PS: FHCO blends with Novozyme 435 (added at 4% w/w), after 8 hours at 65℃. A. 80:20 PS: FHCO, B. 70:30 PS: FHCO, C. 50:50 PS:FHCO.

In the 80% palm stearin fat blend interesterified by Novo enzymes, a decrease in the height of both the high and low temperature peaks was observed (Figure 33). The high temperature peak decreased to 7 J/g°C from 10 J/g°C while the low temperature peak decreased to 2 J/g°C from 4 J/g°C. Like most interesterified fats from the tallow stearin and fully hardened coconut oil blend (Section 4.4.1), a shoulder was observed at temperatures between 10 to 20°C though the shoulder only reached the height of 2 J/g°C in Figure 33A. The crystallization curve for Novo enzyme interesterified fats with 70% palm stearin had a similar heat release profile as the interesterified 80% palm stearin and fully hardened coconut. Most heat released in this fat blend occurred over a large temperature range between temperatures of 25 to -25°C. The 50% palm stearin blend had the lowest high temperature peak at 6 J/g°C compared to the 70% and 80% palm stearin blends. The shoulder which appeared between 15 to 10°C, at a height of 4 J/g°C can be explained by the increase of the newly formed LMP and PPL TAGs. The low temperature peak in the interesterified fats was lower than that of non-interesterified fats at 2 J/g°C instead of 5 J/g°C due to the drop in the percentage of low molecular weight TAGs such as CCL, CLL and LLL.

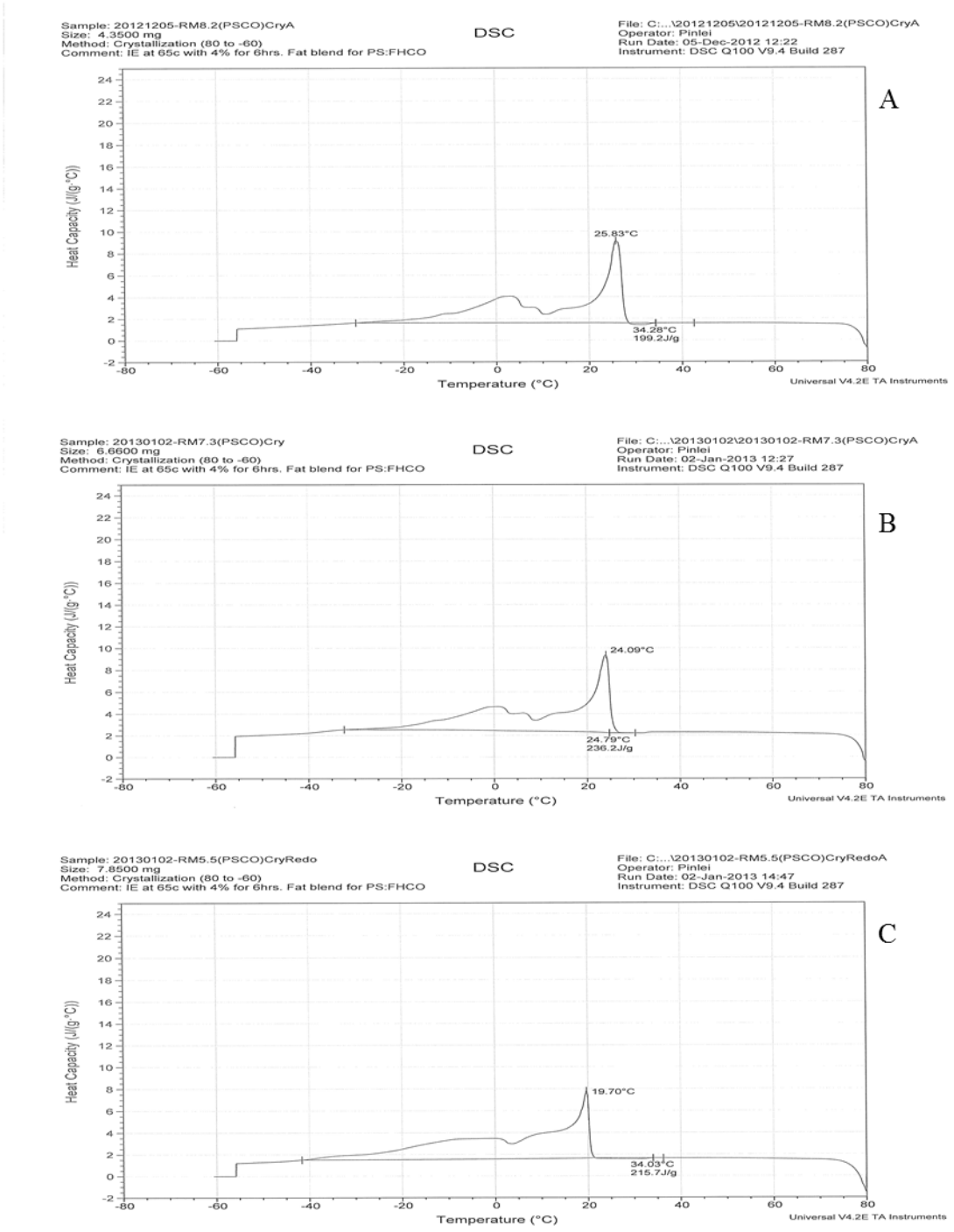

Figure 34. DSC cooling (crystallisation) curve for PS:FHCO blends with Lipozyme RM IT (added at 4% w/w), after 8 hours at 65℃. A. 80:20 PS:FHCO, B. 70:30 PS:FHCO, C. 50:50 PS:FHCO.

The RM enzyme interesterified hard stock at 80% palm stearin showed larger peaks of 9 J/g°C between 30 to 15°C in Figure 34A compared to the Novo interesterified hard stocks of the same blend with peaks at 6 J/g°C (Figure 33A). Higher low temperature peak was also observed in all the RM enzyme interesterified fats compared to the Novo enzyme interesterified fats with the same shoulder peak between 20 to 10°C (4 J/g°C). Like the Novo enzyme treated hard stock, the heat released by RM enzyme treated hard stock occurred mostly between temperatures of 30 to -20°C. The RM enzyme interesterified 70% palm stearin hard stock showed similar heat release profile as the 80% palm stearin hard stock. The temperature range for the majority of the heat released by the RM enzyme hard stock at 70% palm stearin was shorter than the Novo enzyme, between 30 to -15°C. In the RM interesterified 50% palm stearin blend, the high temperature peak was at 8 J/g°C which was higher than the Novo enzyme interesterified fats at 6 J/g°C. The low temperature peak of the 50% palm stearin RM enzyme interesterified fats was higher than the 50% palm stearin Novo enzyme interesterified fats. The temperature range for heat release by the 50% palm stearin RM enzyme interesterified fats was similar to the 50% palm stearin Novo enzyme interesterified fats at between 20°C to -20°C.

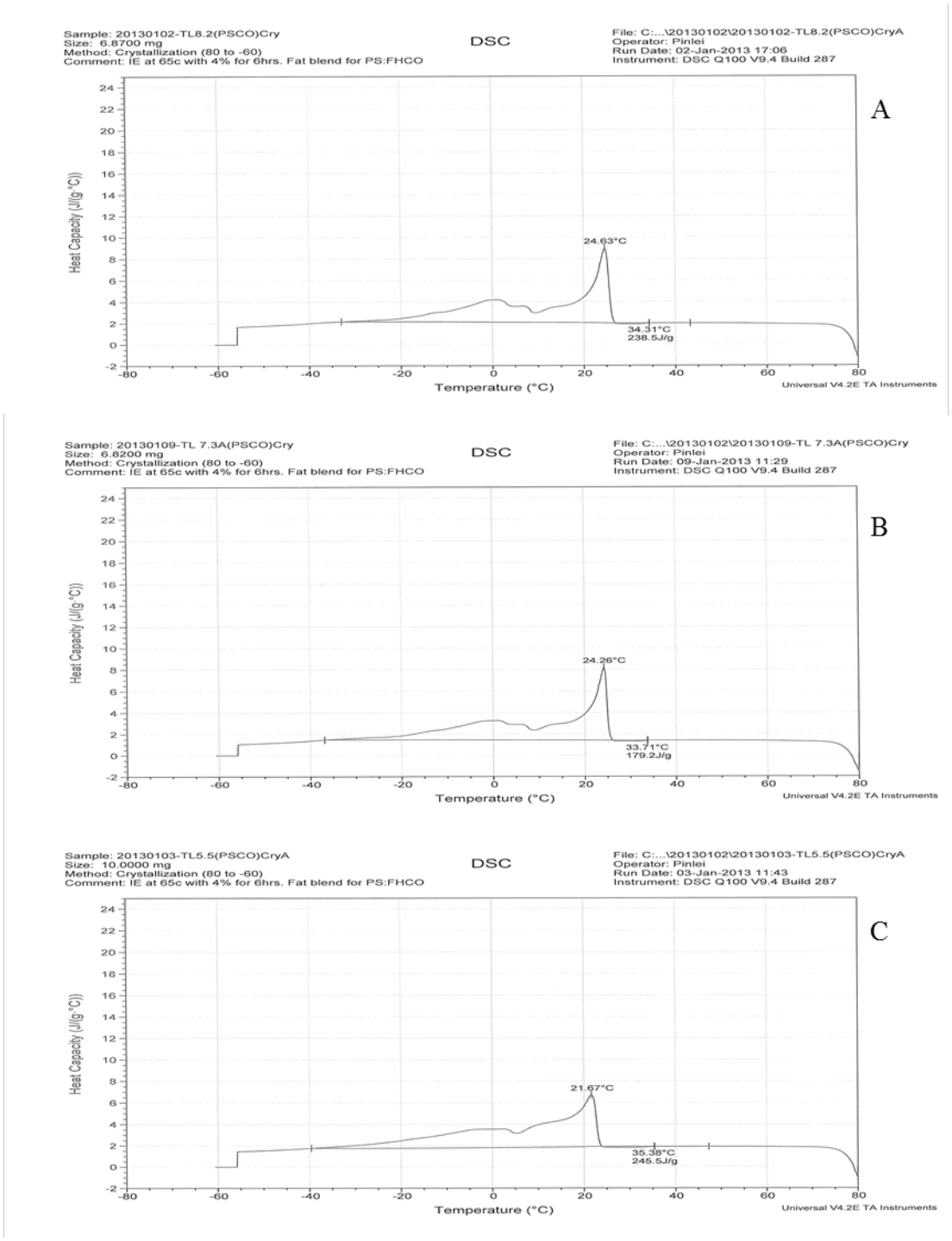

Figure 35. DSC cooling (crystallisation) curve for PS:FHCO blends with Lipozyme TL IT (added at 4% w/w), after 8 hours at 65℃. A. 80:20 PS:FHCO, B. 70:30 PS:FHCO, C. 50:50 PS:FHCO.

In the TL enzyme interesterified palm stearin and fully hardened coconut fat blends, the heat release profile of the 80% palm stearin during crystallization was similar to the RM enzyme interesterified fat with the same ratio of palm stearin. Both high and low temperature peaks for this blend were at 9 J/g°C and 4 J/g°C, respectively, while the shoulder peak between 20 to 10°C was also at the same height as for the same RM interesterified fat blends. In the 70% palm stearin blend, the heat released by the TL enzyme was observed to be less than that released in the RM enzyme interesterified fat blends, with a lower high temperature peak between 25 to 20°C. The low temperature peak from the TL enzyme interesterified fats was also lower than the RM interesterified fats while the shoulder peak between 20 to 10°C was still observed. The concentration for LMP and PPL TAGs in both the TL and RM enzyme interesterified fats was found to be similar. The crystallization temperature range for the 70% palm stearin TL enzyme interesterified fats was between 25 to -20°C which was the same as the RM enzyme interesterified fats. When the amount of palm stearin was lowered to 50%, the shape of the heat release peak was similar to the RM enzyme interesterified fats. For 50% palm stearin, the high temperature peak of the TL enzyme interesterified fats (7 J/g°C) was lower than the RM enzyme interesterified fats (8 J/g°C) Similar shoulder peaks was also observed between the 50% palm stearin RM and TL enzyme interesterified fats and crystallisation was observed over a similar temperature range.

4.4.3 Tallow stearin and palm stearin (TS:PS)

Tallow stearin and palm stearin were blended and interesterified during this stage of trials at the same ratios of 80:20, 70:30 and 50:50 with tallow stearin been the higher proportion of these blends.

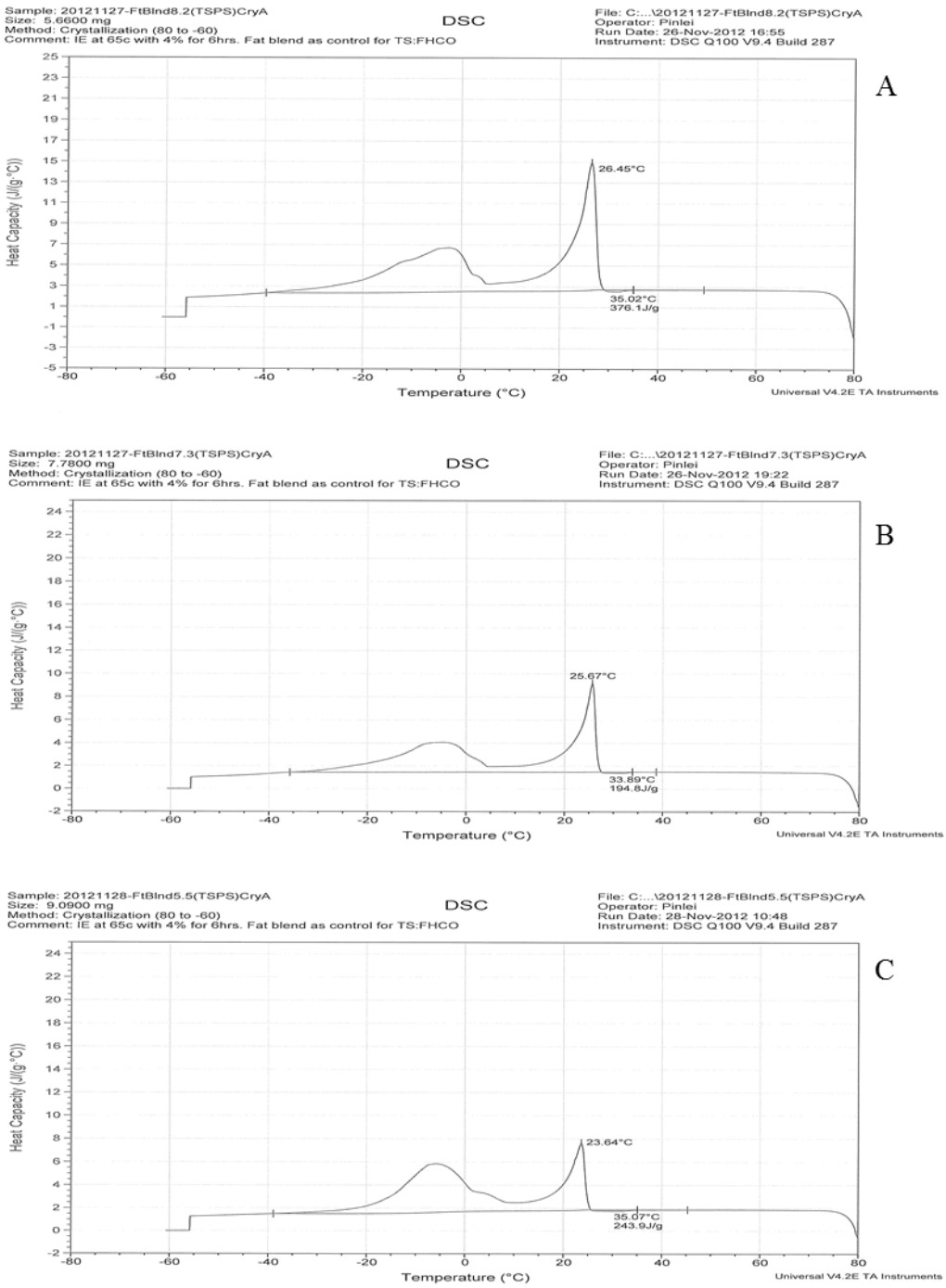

Figure 36. DSC cooling (crystallisation) curve for TS: PS blends with no added enzymes (Control, non-interesterified). A. 80:20 TS:PS, B. 70:30 TS:PS, C. 50:50 TS:PS.

Non-interesterified solid fats of tallow stearin and palm stearin were blended and the crystallization analysis of these fat blends was conducted as reference for the interesterified fats (Figure 36). Higher ratios of tallow stearin were always used in this blend and the percentage ratio was reduced from 80% to 70% and 50% as the amount of palm stearin was increased from 20% to 30% and 50%. In the non-interesterified fat blend with 80% tallow stearin as shown in Figure 36A, a large high temperature peak between 25 and 20°C was observed reaching 15 J/g°C and the low temperature peak was between 5 and -30°C. The high and low temperature peaks were well separated as the shoulder peak was very low at 2 J/g°C. Non-interesterified 70% tallow stearin blend in Figure 36B had a lower high temperature peak at 9 J/g°C than the 80% blend. As the fat blend was further lowered to 50% tallow stearin, the high temperature peak was further lowered to below 8 J/g°C while the low temperature peak showed similar height as the 80% tallow stearin and 70% tallow stearin blends. The temperature range for heat release during crystallization was between 25 to -25°C this blend.

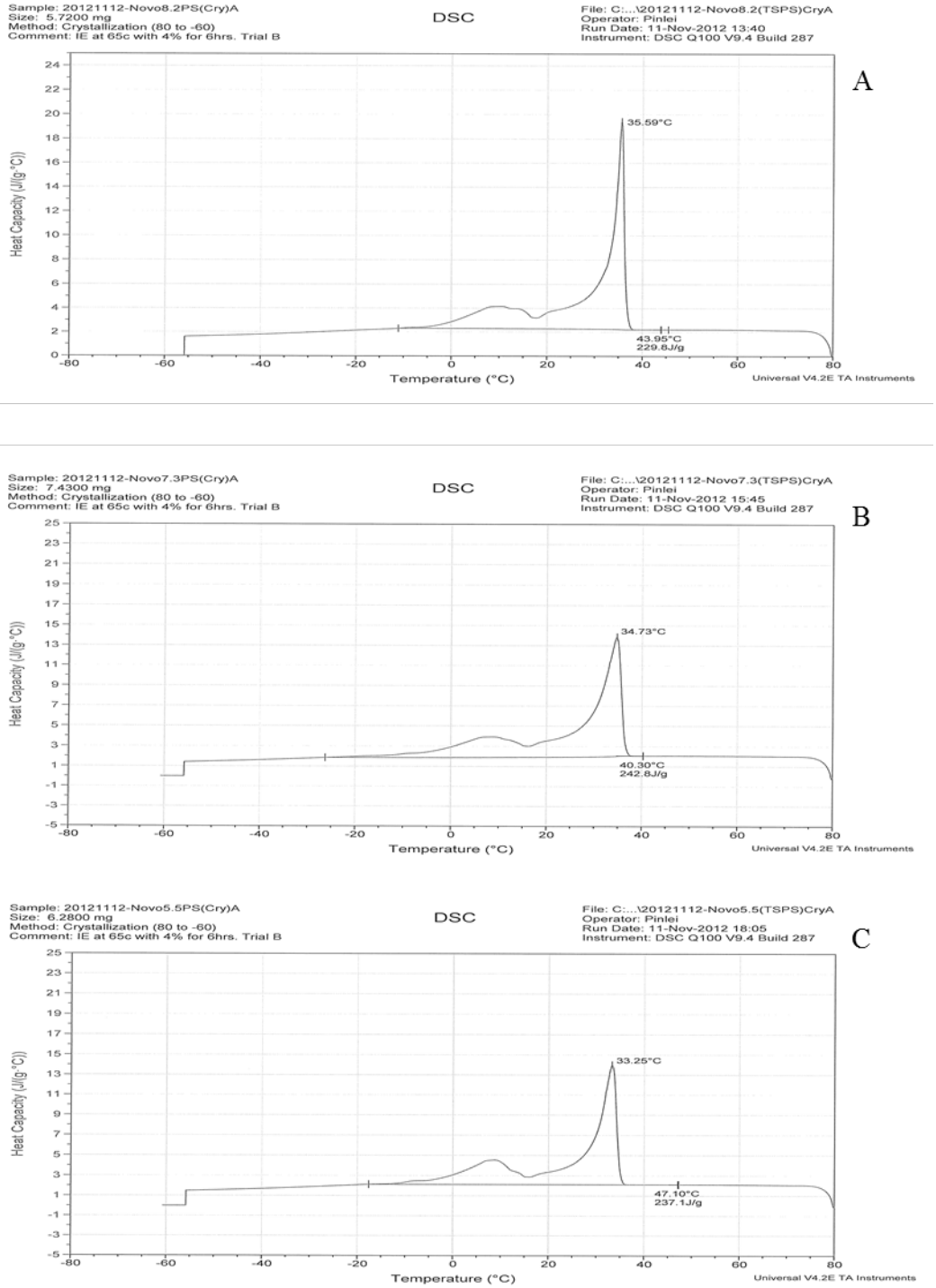

Figure 37. DSC cooling (crystallisation) curve for TS:PS blends with Novozyme 435 (added at 4% w/w), after 8 hours at 65℃. A. 80:20 TS:PS, B. 70:30 TS:PS, C. 50:50 TS:PS.

In the Novo enzyme interesterified hard stock at 80% tallow stearin, the high temperature peak between 40 and 30°C was observed to reach 20 J/g°C which was higher than the non-interesterified hard stock at 15 J/g°C (Figure 37A). The low temperature peak between 15 and 0 °C was at 4 J/g°C and was lower than the non-interesterified 80% tallow stearin and palm stearin fat blend at 7 J/g°C. The shoulder peak between the high and low temperature peak at temperatures 30 to 20°C showed a small increased to 4 J/g°C from 2 J/g°C in the non-interesterified hard stock. The temperature range for heat released during crystallization in Figure 37 showed that the majority of the heat was released between temperatures of 40 to 0°C which is higher than the previous blends in Figures 30A and 33A as the blend of tallow stearin and palm stearin contained a high percentage of two high molecular weight TAGs than the combination of tallow stearin with FHCO or palm stearin with FHCO.As the percentage of tallow stearin was reduced to 70% in the interesterified fat blend with Novo enzyme, the high temperature peak was also observed to decrease while the height of the low temperature remained at the same size of 4J/g °C. No difference was observed in the shoulder peak between the high and low temperature peak as neither LMP and PLO peaks were observed in the TAG composition (Table20). The amount of PPL TAG remained the same in all the Novo enzyme interesterified fats in the tallow stearin and palm stearin blends. The temperature range for the heat released by the 70% tallow stearin was between 35 to -5°C, this decrease in temperature range compared to the non-interesterified blend can be explained by the decreasing amount of high molecular weight TAGs. In the 50% tallow stearin hard stock interesterified by Novo enzyme, the high temperature peak reached 15 J/g°C which was higher than non-interesterified fats at 8 J/g°C. The low temperature peak remained at the same height and no differences were observed in the height of the shoulder peak between temperatures of 30 to 20°C. The increase in the size of high temperature peak in all the Novo enzyme interesterified fats can be explained by the formation of the high molecular weight TAG PSS as indicated in Table 20. The presence of PSS resulted in a large heat release and also shifted the temperature range for heat release during crystallization.

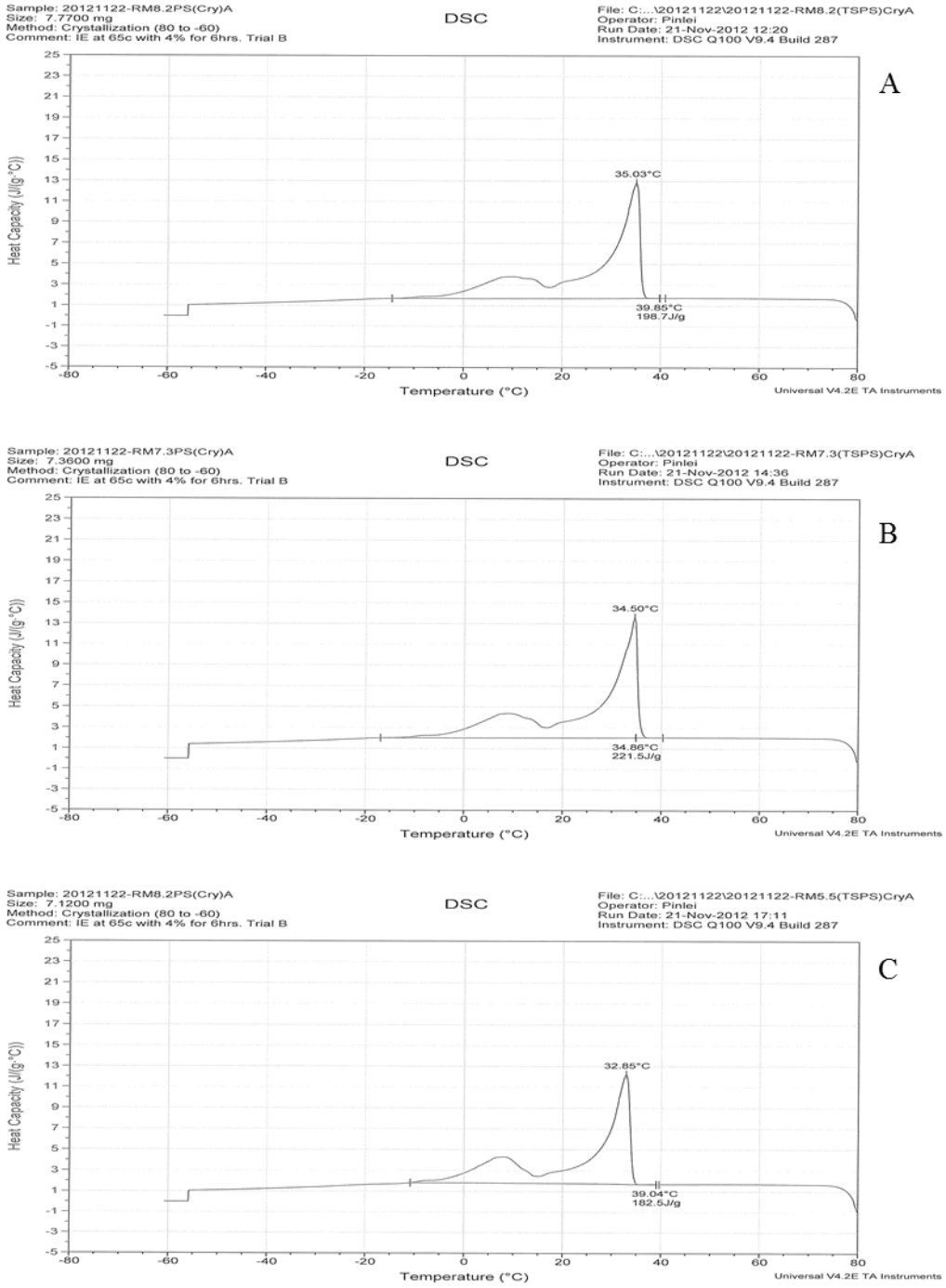

Figure 38. DSC cooling (crystallisation) curve for TS:PS blends with Lipozyme RM IT (added at 4% w/w), after 8 hours at 65℃. A .80:20 TS:PS, B. 70:30 TS:PS, C. 50:50 TS:PS.

The RM enzyme interesterified hard stock at 80% tallow stearin showed smaller high temperature peak at 13 J/g°C then 15 J/g°C observed for the Novo enzyme interesterified hard stocks (Figure 38A). The size of the low temperature peak remained unchanged at 4 J/g°C. The shoulder peak between the high and low temperature peaks also remained unchanged from the Novo enzyme interesterified while the temperature range of the heat released by the fat blend was between 40 to 0°C. The RM enzyme interesterified hard stock at 70% tallow stearin showed a similar heat release profile as the Novo enzyme interesterified hard stock and the RM enzyme interesterified hard stock at 80% tallow stearin. The size of the high temperature peak remained at just below 14 J/g°C while the low temperature peak was still at 4 J/g°C. The crystallization temperature range remained at between 35 to 0°C similar to the Novo enzyme interesterified fats. As the percentage of tallow stearin was reduced to 50% in the RM enzyme interesterified hard stock, the shape of the heat release peak remained the same as Novo enzyme interesterified hard stock. The height of both low and high temperature peaks remained similar to that of the Novo enzyme interesterified hard stock and the same as the 80% tallow stearin RM enzyme interesterified hard stock.

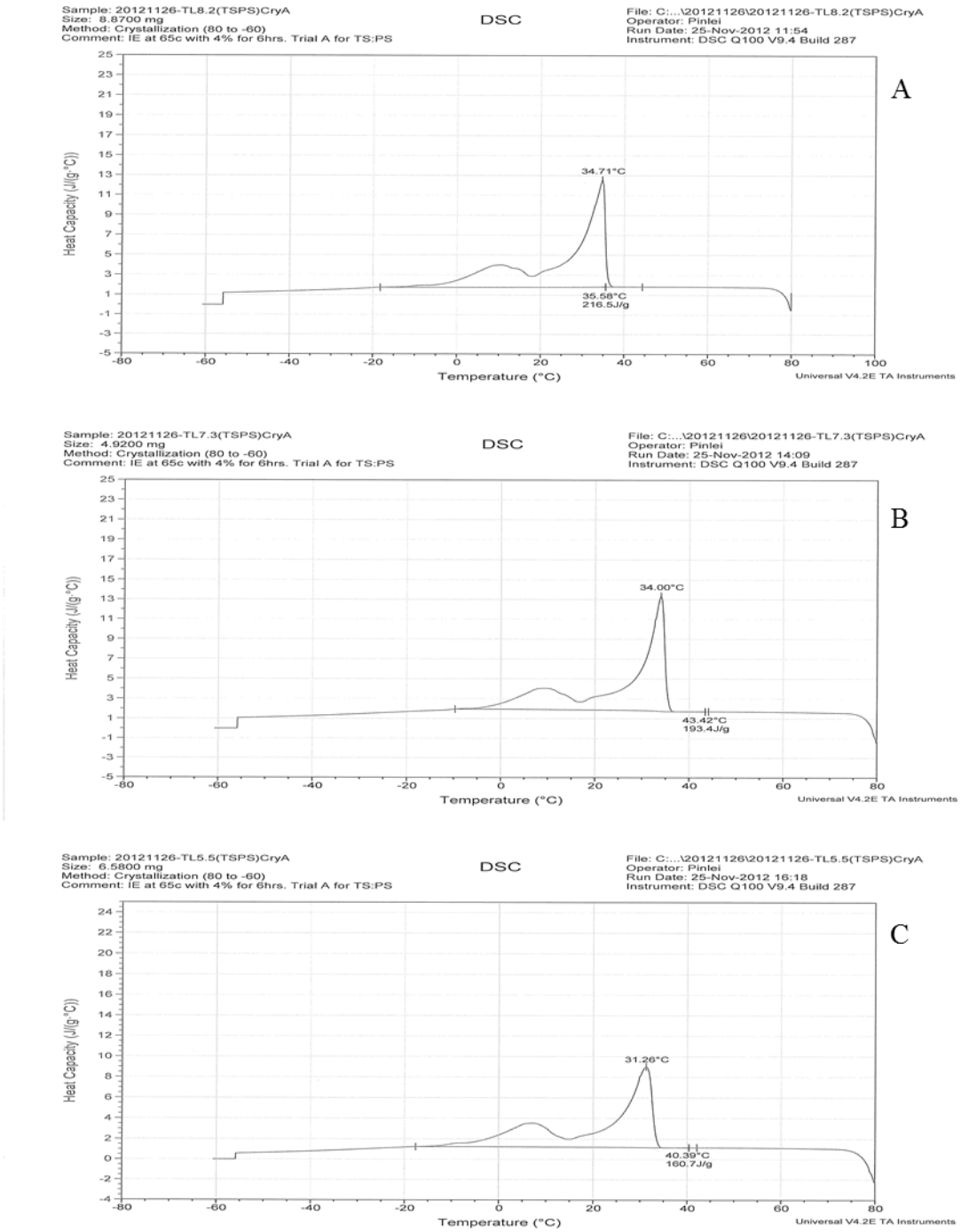

Figure 39. DSC cooling (crystallisation) curve for TS: PS blends with Lipozyme TL IT (added at 4% w/w), after 8 hours at 65℃. A .80:20 TS:PS, B. 70:30 TS:PS, C. 50:50 TS:PS.

The heat changes in the crystallization profile of the three fat blends with TL enzyme showed similar sized high and low temperature peak as the Novo and RM enzyme interesterified fats, the temperature range for heat released by TL enzyme interesterified fats occurred between 35 to 0°C (Figure 39).

Small changes were observed in the heat released during crystallization of the tallow stearin and palm stearin fat blends overall. Both tallow stearin and palm stearin consist of high percentages of high molecular weight TAGs although the TAG composition of tallow stearin and palm stearin was different but the heat change during crystallization of the high molecular TAGs was similar during crystallization. The interesterification process rearranges the fatty acid composition of fatty acids within the TAG molecule but when the long chain high molecular weight fatty acids are exchanged with similar molecular weight fatty acids between the same TAG molecules or with other TAG molecule, little change can be observed in heat differences released during crystallization. From Table 20 it was observed that TAGs such as LMP and PLO were not formed during interesterification as found in tallow stearin or palm stearin blends with fully hardened coconut oil. The level of PPL remained the same in all three enzyme treatments and in all three fat blend ratios indicating the minimal effect of interesterification on a blend with two fats with similar molecular weight TAGs.

4.5 Solid fat content of different fat blends after interesterification

Solid fat content was determined in both interesterified and non-interesterified (control) fat blends and the ratio of solid to liquid fat was determined at the temperatures of 0, 10, 20, 30 and 40°C according to the method described in Section 3.1.4.

4.5.1 Tallow stearin and fully hardened coconut oil (TS:FHCO)

The solid fat content was reported as a percentage of the total fat sample at each temperature tested. The preliminary results from Table 16 suggests that the interesterified fats should have a solid fat content of 61.5% at 30 °C like the IPL hard stock.

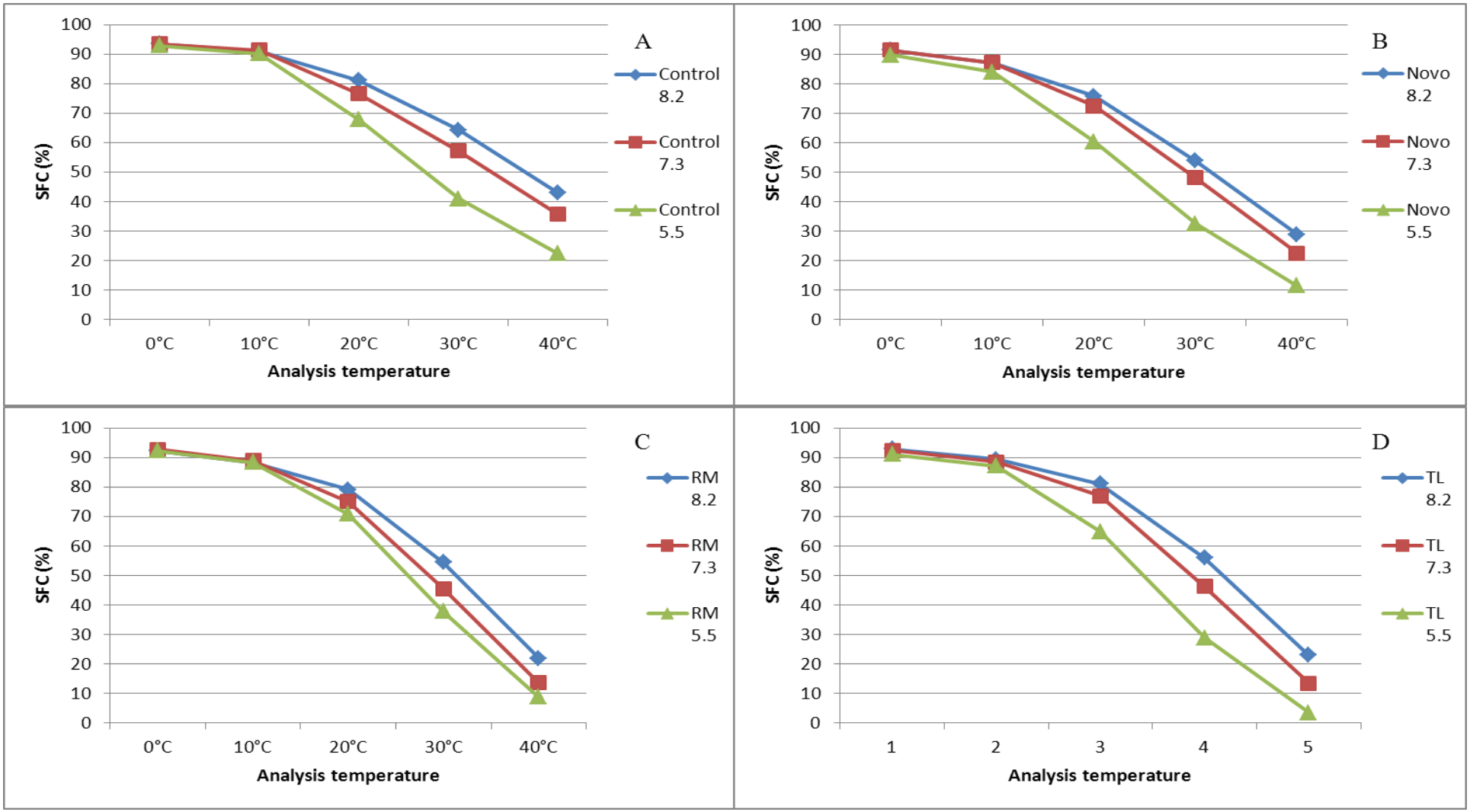

Figure 40. Percentage solid fat content in tallow stearin and fully hardened coconut oil fat blends A non-interesterified fats (control), B Novo enzyme interesterified fats, C RM enzyme interesterified fats, TL enzyme interesterified fats(mean ± standard deviation, n =2)

In the TS: FHCO fat blend, non-interesterified fats (control) with tallow stearin compositions of 80%, 70% and 50% all had higher solid fat content than the interesterified fats with the Novo, RM and TL enzymes at temperatures ranging from 10 to 40°C as shown in Figure 40. This observation can be linked to the decrease in melting point of these hard stocks in Figure 23. As the percentage of low temperature melting TAGs of fully hardened coconut oil was increased to 50%, the solid fat content of both interesterified and non-interesterified fats decreased (Figure 40). At 0°C, the solid fat content of the TL enzyme interesterified fats behaved much like non-interesterified fats while RM and Novo enzyme interesterified fats had lower solid fat contents. At 10°C, there were very little differences observed between the 80% and 70% tallow stearin fat blend but as the holding temperature was increased differences in solid fat content was observed between the interesterified and non-interesterified fats. The changes in the solid fat content changed drastically between the 20 and 30°C holding temperature in the interesterified fats as it dropped from 81.0% to 55.8% in the TL enzyme interesterified fats at 80% tallow stearin. But as the content of tallow stearin was decreased to 50%, the change of solid fat content was much greater than the 80% tallow stearin blend between the temperatures of 20 to 30°C from 64.9 to 28.9%. This observation indicates the change in fat structure also resulted in definite changes in the melting profile of the fat blends.

4.5.3 Tallow stearin and palm stearin (TS:PS)

The solid fat content for tallow stearin and palm stearin is shown in Figure 41. The results displayed in this figure show the percentages ratio of solid to liquid in the interesterified and non-interesterified fat sample.

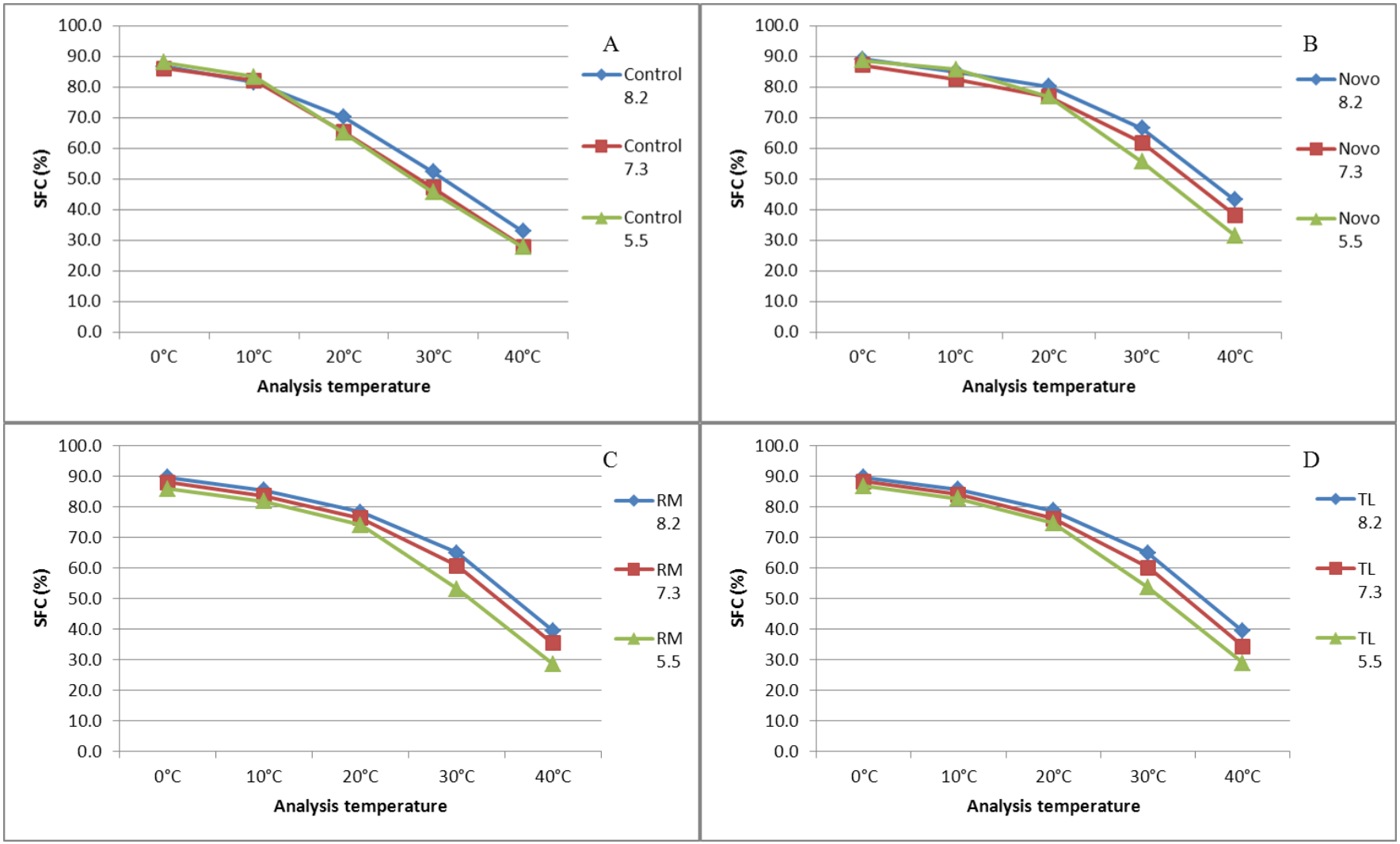

Figure 41. Percentage solid fat content in tallow stearin and palm stearin fat blends A non-interesterified fats (control), B Novo enzyme interesterified fats, C RM enzyme interesterified fats, TL enzyme interesterified fats(mean ± standard deviation, n =2)

The solid fat content of the interesterified fats of tallow stearin and palm stearin was higher than non-interesterified fats (Figure 41). We can see from Figure 41 that the change of solid fat content of tallow stearin and palm stearin blend was much smaller than the tallow stearin and fully hardened coconut oil and this was due to the mixture of the two high melting point solid fats. The high solid fat content of 28.9 to 33.1% in the 40°C analysis shown in Figure 41 indicates that the blends were still not fully melted at this temperature.

4.5.2 Palm stearin and fully hardened coconut oil (PS:FHCO)

The solid fat content for palm stearin and fully hardened coconut oil blend is shown in Figure 42 which includes both interesterified and non-interesterified fats.

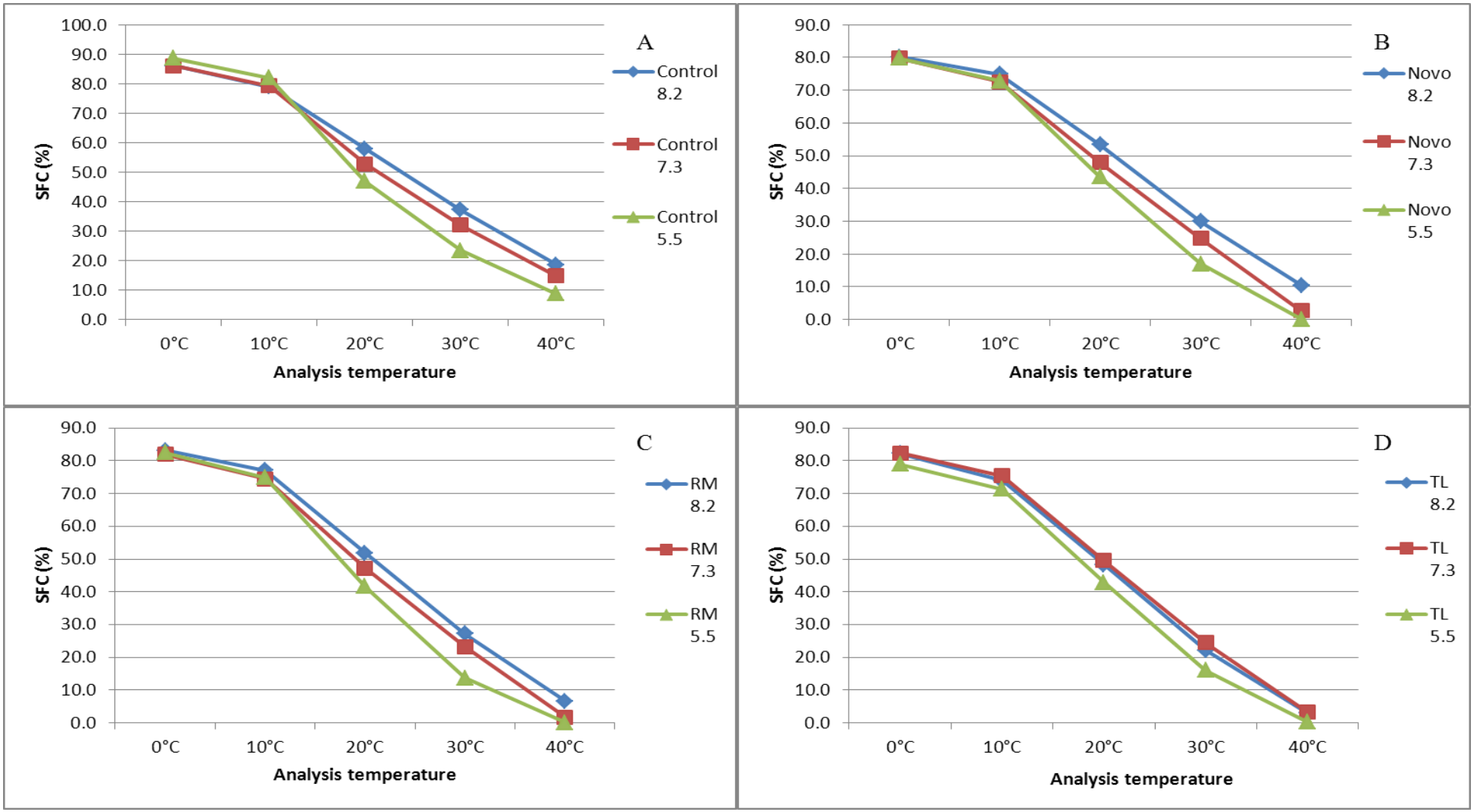

Figure 42. Percentage solid fat content in palm stearin and fully hardened coconut oil fat blends A non-interesterified fats (control), B Novo enzyme interesterified fats, C RM enzyme interesterified fats, TL enzyme interesterified fats(mean ± standard deviation, n =2)

In the palm stearin and fully hardened coconut oil blends, the solid fat content was 6 to 10% lower than that of the tallow stearin and fully hardened coconut oil in the 0°C analysis (Figure 42). When the percentage composition of palm stearin was decreased to 50%, the solid fat content for both interesterified fats and non-interesterified fats was reduced; this observation confirms the observed decrease of melting point in both non-interesterified and interesterified fats (Figures 27). Non-interesterified fat blends showed higher solid fat content at 0°C at 88.6to 86.1% than the interesterified fat blends of 80.2%, 83.1% and 82.2% for the interesterified fats at 80% palm stearin at 0°C. As the holding temperature was increased, the decrease in the percentage of solid fat content occurred more rapidly in the interesterified fats than in the non-interesterified fats. The largest change in the percentage of solid fat content occurred between temperatures of 10 to 20°C in both interesterified and non-interesterified fats. The solid fat content of the Novo and TL enzyme interesterified fats showed virtually no solid fat at 40°C for the 50% palm stearin fat.

4.6 Time of crystallization

The rate of crystallization analysis was conducted on the NMR instrument as described in Section 3.1.5. This method monitored the changes of solid fat content (SFC) over a crystallization period when the melted sample was kept in a 0°C an ice water bath and the SFC reading was taken at regular intervals. The time interval for SFC reading was set at 2 minute intervals for the first 20 minutes as the fats crystallizes most rapidly in this period. After this the time interval was extended to 10 minutes. The SFC reading for the crystallizing fats stopped once two or more of the SFC readings were constant. At this point the time it took for the fats to crystallize was then recorded. Table 21 indicates the time of crystallization for both interesterified and non-interesterified fats at different fat ratios and the translation for the abbreviated trial names can be found in page ix in the index section of the report.

Table 21. Time taken for hard stocks to have constant SFC values during crystallization from 60℃ and cooling at 0°C

TS:FHCO	Time for crystallization (min)	PS:FHCO	Time for crystallization (min)	TS:PS	Time for crystallization (min)
Fat blend 8.2	60	Fat blend 8.2	70	Fat blend 8.2	60
Fat blend 7.3	60	Fat blend 7.3	70	Fat blend 7.3	70
Fat blend 5.5	60	Fat blend 5.5	70	Fat blend 5.5	70
NOVO8.2	18	NOVO8.2	50	NOVO8.2	70
NOVO7.3	40	NOVO7.3	40	NOVO7.3	70
NOVO5.5	30	NOVO5.5	50	NOVO5.5	70
RM8.2	18	RM8.2	60	RM8.2	60
RM7.3	16	RM7.3	16	RM7.3	70
RM5.5	18	RM5.5	16	RM5.5	60
TL8.2	18	TL8.2	40	TL8.2	70
TL7.3	16	TL7.3	50	TL7.3	60
TL5.5	16	TL5.5	14	TL5.5	50

The results in Table 21 show that that the non-interesterified fats took 60 to 70 minutes to completely crystallize, whereas the interesterified fats took 16 to 20 minutes to crystallize. The change in the crystallization time was caused by the change in TAG composition of the fats. The tallow stearin and palm stearin (TS:PS) fat blend had the longest crystallization time period, this was because both fats contained relatively larger amounts of high molecular weight TAGs and when interesterified minimal changes occurred in the TAG composition as mentioned in Table 19.

4.7 Discussion for fat blending trials

The objective of the fat blending trial was to determine and select the commercial lipase enzymes and the blend of interesterified hard stocks from tallow stearin, palm stearin and fully hardened coconut oil as well as the ratio of their combination. The physical parameters of the raw material and some of the current hard stocks including the benchmark IPL hard stock was established during the preliminary trial. The target melting point temperature of IPL was at 44 °C (Figure 20) with rapid crystallization time at 16 minutes (Table 16). The heat release profile of IPL differed from other commercial hard stocks by showing a high shoulder peaks between temperatures of 25 to 10 °C. The SFC results suggests that IPL initially had high percentage of solids to liquid ratio at 95.5 % in the 0°C analysis and the largest changes occurred during the 30 to 40°C analysis when the SFC value decreased from

61.5 to 14.1 % (Figure 24). The rapid change in the solid to liquid ratio suggests that IPL melts quickly in the temperature range between 30 to 40°C.

The fat blend of tallow stearin and palm stearin was not selected for the next stage of trials. The crystallization time of this fat blend was long and ranged between 50 to 70 minutes (Table17) rather than 16 minutes for the IPL hard stock. The reason for this long crystallization time was due to the composition of high molecular weight TAGs in both fats (Table 19) the interesterification reaction re-arranges the fatty acids of the TAG molecule and re-combines the fatty acids from both fats to form new TAG molecules. Small changes were observed when two fats that both contain large amounts of high molecular TAG molecules such as POO, POP, POS and PPS undergoes an interesterification reaction. The compositional change in the blends of tallow stearin and palm stearin has led to restricted changes in physical parameters like melting point temperature, for tallow stearin and palm stearin blends interesterified the melting point was between 50 to 54°C. The slow crystallization time can also be observed from the heat release profile in the DSC analysis. The shape of the interesterified hard stock was very similar to the non-interesterified hard stock and no shoulder peak was observed in the interesterified hard stock. This observation confirms the lack of compositional changes and the lack of change in melting point temperature of the tallow stearin and palm stearin fat blend hence making it unfit for the objective of this project and was not continued to further trials.

The lipase enzyme Novo (Novozyme 435) was also not continued through to the time and concentration stage of trials. This was due to the reaction of Novo enzyme was not as efficient as the RM and TL enzyme. Figure 26 and 27 shows that the change in melting point by Novo enzyme interesterified hard stock had higher melting points than the RM and TL enzyme treated fat blends. This was because the enzyme was not position Sn1, 3 specific like the RM and TL enzyme (Appendix M2 and M3) although it has wider optimum temperature range and was reported at 40 to 60°C (Appendix M1) but did not suit the purpose of this project hence was rejected for the next stage of trials.

The physical changes in the hard stock between interesterified and non-interesterified were significant in the palm stearin to fully hardened coconut oil. The melting point temperature dropped from 43 to 35°C after interesterification for the 50 to 50% (w/w) blend but this fat blend had lower melting point than hard stocks produced using tallow stearin and fully hardened coconut oil. When the palm stearin composition was increased to 80%, the

interesterified hard stock had a melting point at 42°C (Figure 27) which was still lower than the IPL hard stock at 44°C. This fat blend was selected for the next stage of trials due to the rapid crystallization time at 14 to 16 minutes but the solid fat content (Figure 42) was much lower than tallow stearin and fully hardened coconut oil (Figure 41) and IPL (Table Figure 43). The drop in the melting point can be explained by the conversion of high molecular TAGs like POO, POP and PPP and low molecular TAGs like LLL, LL< and LMO into medium weight TAGs like PPL, PLO and LMP.

4.8 Conclusion

Based on results obtained from physical and compositional analysis, the RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% ratios, TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30%, TL enzyme interesterified palm stearin and fully hardened coconut oil at 50 to 50% were selected for the next stage of trials. All three hard stocks were selected due to their rapid crystallization time and similar melting point to the benchmark IPL hard stock. The Novo enzyme interesterified hard stock was not selected because it's less efficient than the RM and TL enzyme and the tallow stearin and palm stearin was not selected because the impact of interesterification reaction could not occur due to the high molecular weight TAGs present in both fats.

Chapter 5 Impact of enzyme concentration and time on interesterification

The objective of the next set of trials was to investigate the appropriate amount of lipase enzyme and the minimum amount of time required for the interesterification process. Experiments to determine the effect of enzyme concentration and interesterification time on the resulting fat blend were conducted on selected interesterified fat blends that included RM and TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70% and 30% and palm stearin and fully hardened coconut oil at 50% and 50%. The enzyme concentrations investigated were 0%, 2%, 4% and 8% (w/w) and each experimental trial was conducted for 8 hours. A sample was taken every two hours from 0 hour to 8 hours in order to monitor the changes in the composition of the fat blends. The most efficient processing time and enzyme concentration would be identified at the end of this stage of trials.

5.1 Melting points interesterified fat blends with different reaction time and enzyme concentration

The melting point analysis was conducted on samples taken every two hours. The changes in melting point were affected by any changes in the TAG composition of fats during interesterification. Each trial was not replicated but analyses were carried out in duplicate or quadruplicate as stated. Figure 43 shows the melting point temperature for the TL and RM enzyme interesterified tallow stearin and fully hardened coconut oil and palm stearin and fully hardened coconut oil blends.

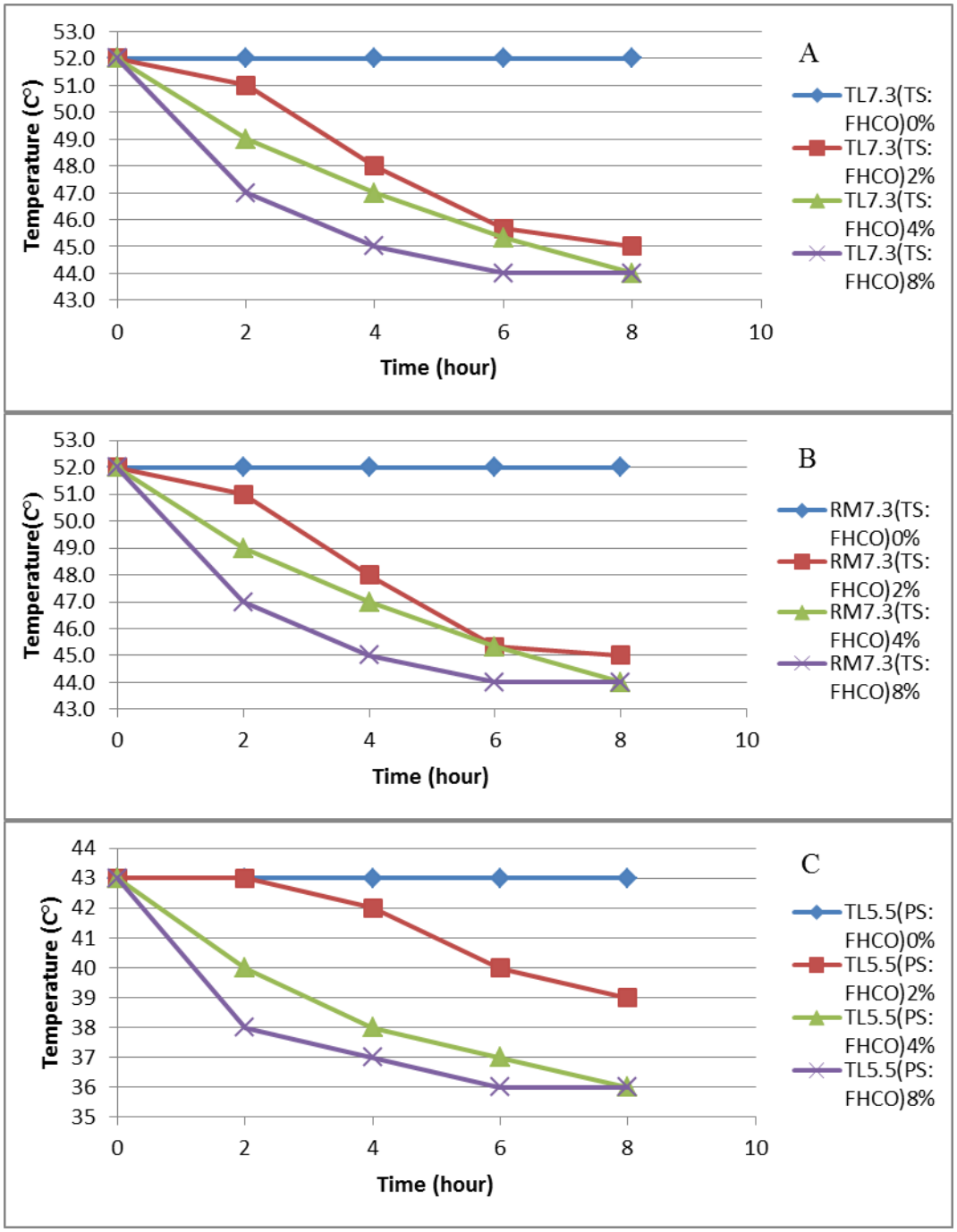

Figure 43. The change in melting point with TL enzyme interesterified TS:FHCO at 70:30% (A) and RMenzyme interesterified TS:FHCO at 70:30% (B) and TLenzyme interesterified PS:FHCO at 50:50% (C) with enzyme concentrations 0 to 8% over 8 hours at 65±1 ℃. The data points are mean± standard deviation (n =4). Note standard deviations values are small and not visible. Note standard deviations values are small and not visible.

In Figure 43A, when no enzyme was added, no changes in the melting point temperature was observed over the 8 hour period but with 2% TL enzymes, changes occurred between 0 hour to 8 hours as the melting point temperature dropped from 52 to 45°C. In the 4% and 8% concentration enzyme treatments, similar trends were also observed, the melting point temperature dropped most rapidly between 0 to 6 hours. At the end of 8 hours, the 8% TL enzyme interesterified fat blends had a melting point of 44°C which was lower than the 2% TL enzyme interesterified fats; this showed that addition of 2% TL enzyme was not able to achieve the same melting point as 4% enzyme in 8 hours.

In Figure 43B, tallow stearin and fully hardened coconut oil at 70 to 30% (w/w) ratio was interesterified by the RM enzyme and the results for the change of melting point temperature was very similar to hard stocks interesterified by the TL enzymes on the same fat blend and ratio. The change in melting point temperature occurred much faster when 8% (w/w) of enzyme was added than the 4 and 2% (w/w) of enzymes. This is shown by the change in melting point temperature within first 2 hours of reaction, the 8% RM enzyme interesterified fats had melting point temperature of 47°C whereas the 4% RM enzyme interesterified fats had melting points temperature of 49 °C and the 2% RM enzyme interesterified fats only had melting point temperature of 51 °C. But the changes in melting point temperature slows down after the first 4 hours and reaches 44 °C by the end of the 8 hour reaction like the hard stocks interesterified with 4% of TL enzymes.

The melting point temperatures in Figure 43C were lower than Figure 43A and Figure 43B because the palm stearin was used instead of tallow stearin and the ratio for the palm stearin and fully hardened coconut oil was at 50 to 50% (w/w). When 2% TL enzymes was added, a change in the melting temperature was observed only after 2 hours of interesterification reactions. In the 4% enzyme trial, there was a steady drop in the melting point over the 8 hour reaction period from 43 to 36°C. In the 8% TL enzyme trial, the rate of change in melting point temperature observed was similar to the 4% enzyme interesterified fats but reached the melting point temperature of 36°C within 6 hours and remained at 36°C after 8 hours of reaction.

The change in the rate of interesterification reaction can be observed in the change in melting point analysis and the change in rate of change for melting point analysis was depended on the amount of enzymes added to the reaction.

5.2 Triglyceride composition

The triglyceride composition of the three selected fat blends was investigated in this experiment with different enzyme concentration and reaction time. The change in the TAG composition indicates the interesterifying effects of the enzymes. The three treatments of TS:FHCO with RM 7.3, TL 7.3 and PS:FHCO with TL 5.5 were sampled at 2 hour intervals. The TAG composition of the tallow stearin and fully hardened coconut oil interesterified by TL and RM enzyme at 70 to 30% (w/w) are shown in Table 22 and Table 23 for 2% and 8% enzyme added. TAG composition of palm stearin and fully hardened coconut oil with TL enzyme at 50 to 50% (w/w) ratios are shown in Table 24 for 2% and 8% enzyme added.

Table 22. TAG composition for 2% to 8% TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70% to 30%

Percentage of individual TAG/total TAGs present											
ECN	Name	TL7.3(TSFHCO)2%0hr	TL7.3(TSFHCO)2%2hr	TL7.3(TSFHCO)2%4hr	TL7.3(TSFHCO)2%6hr	TL7.3(TSFHCO)2%8hr	TL7.3(TSFHCO)8%0hr	TL7.3(TSFHCO)8%2hr	TL7.3(TSFHCO)8%4hr	TL7.3(TSFHCO)8%6hr	TL7.3(TSFHCO)8%8hr
24	CpCpCp	4.6	4.2	3.9	3.7	3.7	4.4	3.9	3.8	3.3	3.3
	RT5.6	1.8	1.5	1.3	1.1		1.5	1.4	1.1		
30	CCC	4.6	4.4	3.9	3.8	3.7	4.6	3.9	3.5	3.4	3.4
	RT9.3	1.2	1.1	1.2			1.0	1.5	2.2	1.7	1.2
	RT10.5					4.9					2.8
32	CCL	5.9	5.2	5.4	5		5.3	3.7	3.7	3.7	3.4
	RT11.8					3.9	1.6	1.8	1.4	1.3	1.1
34	CLL	6.4	5.9	5.7	4.4		6.5	5.1	5.5	5.3	5.6
	RT15.2					6.9		1.2	1.8	1.7	1.6
36	LLL	8.2	7.4	7.1	7.1	3.9	7.6	6.9	6.5	6.6	6.9
	RT16.2		4.4	4.2	4.1			1.6	1.3	1.1	1.4
	RT22.7		1.9	1.1		6.8			2.1	1.7	
38	LLM	7.5	7.4	7.2	7.1		7.7	6.3	6.1	6.3	6.3
	RT26.3					6.9		2.1			
	RT28.6					6.9		1.4			
38	LLO	8.3	7.9	7.5	7.8		8.7	7.5	6.3	6.2	6.2
	RT36.6					2.8		1.5	1.1	1.3	1.2
	RT39.3					3.9		1.8			
	RT41.6					2.3		1.2		2	1.9
	RT45.7					3.3		1.9			
40	LMM	8.8	7.9	7.7	7.2	3.5	8.5	7.6	6.1	6.3	6.3
	RT51.3	2.9			1.9		3.1		1.9	2.9	2.7
42	LMP		2.7	2.9	3.6	3.6		3.1	7.3	7.3	7.4
	RT71.8	1.6		1.2	1.9		1.5	2.7	2.5	2.7	2.5
44	PLO		1.5	1.9	2.9	2.1		2.9	4.3	4.4	4.2
44	PPL			2.1	2.8			2.1	5.6	5.7	5.3
48	POP	16.8	15.9	15.7	15.9	14.9	17.1	12.9	12.5	12.4	12.2
48	PPP	16.6	15.9	15.1	14.9	14.2	16.6	12.8	12.4	12	12.8

The changes in the TAG composition of TL enzyme interesterified hard stock at 70 to 30% (w/w) ratio at the same reaction time differed when different concentration of enzymes between 2 to 8% (w/w) was added in the reaction. The results in Table 22 shows that at 0 hours when no enzymes were added, the TAG composition between the 2 and 8% TL enzyme treatment was similar. The differences in the TAG composition between the two treatments occurred after the first 2 hours of reaction. As we can see from Table 22 the composition of high molecular TAGs POP and PPP for the 2% enzyme treatment was at 15.9 and 15.9% and higher than the 8% enzyme treatment at 12.8 and 12.9%. This decrease in high molecular TAGs was accompanied by the increase of medium molecular TAGs as the results from Table 22 shows that the composition of LMP, PLO and PPL TAGs were higher for the 8% enzyme treatment at 3.1, 2.7 and 2.9% than the 2% enzyme treatment where the percentage concentration of the same TAGs were 2.7, 1.5 and the PPL TAG was not yet formed. After 4 hours of reaction, a big increase was observed in the LMP, PLO and PPL TAGs was observed for the 8% enzyme treatment as they increased to 7.3, 4.3 and 5.6%. The rapid change of high molecular TAGs into medium molecular TAGs by the 8% enzyme treatment caused the drop in melting point temperature indicated in Figure 43A as medium molecular weight TAGs require less energy to melt than the high molecular weight TAGs hence lowering melting point temperature. The TAG composition for the 8% enzyme treatment remained stable after 6 hours of reaction as no big changes were observed in the transition of high molecular weight TAGs to medium molecular weight TAGs, this result was also confirmed by the melting point analysis from Figure 43A as the change in melting point temperature only decreased by 1°C between 4 to 8 hours of reaction.

Table 23. TAG composition for 2% to 8% RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70% to 30%

Percentage of individual TAG/total TAGs present											
ECN	Name	RM7.3(TSFHCO)2%0hr	RM7.3(TSFHCO)2%2hr	RM7.3(TSFHCO)2%4hr	RM7.3(TSFHCO)2%6hr	RM7.3(TSFHCO)2%8hr	RM7.3(TSFHCO)8%0hr	RM7.3(TSFHCO)8%2hr	RM7.3(TSFHCO)8%4hr	RM7.3(TSFHCO)8%6hr	RM7.3(TSFHCO)8%8hr
24	CpCpCp	4.5	4.3	4.1	3.9	3.5	4.3	4.1	4.0	4.0	3.9
	RT5.5	1.6	1.5	1.8			1.2	1.1	1.1	1.1	
30	CCC	4.5	4.3	4.4	4.6	3.9	4.5	3.8	3.7	3.6	3.6
32	CCL	5.5	5.2	5.0	4.3	4.6	5.3	4.3	3.9	3.6	3.7
	RT12.6	1.8					1.7	1.4		1.6	1.8
34	CLL	7.0	6.8	6.4	5.6	5.2	6.7	5.3	5.2	5.3	5.3
36	LLL	7.4	7.1	7.0	5.7	5.6	8.0	6.4	6.0	5.9	6.4
	RT15.6		4.0	4.0	4.3	4.3					
38	LLM	7.1	7.4	5.8	5.8	5.4	7.4	6.7	6.7	6.1	6.0
	RT20.7				1.8	1.9					
	RT21.1		1.7		3.2	2.2				3.7	1.7
	RT22.0			2.0	1.4						
38	LLO	8.6	8.2	8.2	8.6	7.9	8.6	6.8	6.8	6.5	6.5
	RT31.9								1.2	1.8	1.0
40	LMM	9.5	7.0	10.1	10.7	8.9	9.2	6.1	6.1	6.1	6.1
40	LMO	4.5					4.2		2.0	1.1	
	RT50.8							3.7	3.5	3.3	1.7
42	LMP		3.0	2.9	3.4	3.7		6.8	7.8	7.8	7.7
	RT69.8							2.4	2.5	2.5	2.7
44	PLO		1.4	1.7	2.5	3.0		5.0	4.9	4.9	4.9
44	PPL				1.6	3.8		5.7	5.9	5.1	5.1
	RT76.1					3.2					
48	POO				1.3	2.6					
48	POP	16.8	16.2	15.9	14.3	13.5	17.1	13.0	13.6	12.8	12.8
48	PPP	16.5	15.6	15.2	12.7	12.3	16.8	12.9	12.8	12.4	12.4
52	PSS	4.4	5.5	4.9	3.9	3.7	4.2	4.3			

The changes in TAG composition for RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% ratios between 2 and 8% (w/w) was shown in Table 23. The change in the TAG composition of the RM enzyme interesterified hard stock was very similar to that of the TL enzyme interesterified hard stock as shown in Table 22 with the difference that the PSS TAG was present in the 2% (w/w) RM enzyme interesterified hard stocks. The PSS TAGs was still present after 2 hours of reaction in the 8% (w/w) treatment but was missing in the 4th hour of reaction. This result was also accompanied by the decrease of other high molecular TAGs like POO, POP and PPP while medium molecular TAGs such as LMP, PLO and PPL increased. In the 2% RM enzyme interesterified hard stocks, we can see that the conversion of high molecular to low molecular weight TAGs was not very rapid as the percentage amount of LMP, PLO and PPL TAGs were at 3.7, 3.0 and 3.8% after 8 hours of reaction but the percentage composition for the 8% RM enzyme interesterified hard stock had 7.7, 4.9 and 5.1% of the same TAGs. These observations suggest that the interesterification reaction occurs more rapidly and completely with higher amount of enzymes. These results can also be observed in the melting point analysis in Figure 43B. The rapid change in TAG composition with the 8% of RM enzymes has led to change in the physical parameters like melting point temperature and it can be seen that the decrease in melting point temperature after 2 hours of reaction can be associated with the formation of medium molecular TAGs as shown in Table 23.

Table 24. TAG composition for 2% and 8% TL enzyme interesterified palm stearin and fully hardened coconut oil (PS:FHCO 50:50).

Percentage of individual TAG/total TAGs present											
ECN	Name	TL5.5(PSFHCO)0hr2%	TL5.5(PSFHCO)2hr2%	TL5.5(PSFHCO)4hr2%	TL5.5(PSFHCO)6hr2%	TL5.5(PSFHCO)8hr2%	TL5.5(PSFHCO)0hr8%	TL5.5(PSFHCO)2hr8%	TL5.5(PSFHCO)4hr8%	TL5.5(PSFHCO)6hr8%	TL5.5(PSFHCO)8hr8%
24	CpCpCp	4.6	4.5	4.6	4.3	4.0	4.7	3.9	3.6	3.1	3.1
	RT4.4			1.1	1.2	1.3		1.3	2.2	2.3	2.4
	RT5.4	1.4	1.1	1.5	1.7	1.4			1.6	2.2	1.9
	RT6.9			2.1	1.5	1.9	1.4	1.4	1.1	1.6	1.5
	RT7.2				2.1	2.3		2.9	3.3	3.5	3.5
	RT7.9									1.3	1.3
30	CCC	5.8	4.9	4.3	3.8	3.1	5.3	4.7	3.9	3.3	3.2
	RT11.5				1.0			1.1	1.8	1.9	1.8
32	CCL	8.4	7.2	6.8	6.2	5.6	8.9	7.3	5.7	4.9	4.7
	RT15.4				1.2			1.3			2.1
34	CLL	7.9	7.1	6.4	6.1	5.4	7.6	6.6	4.3	4.0	2.1
36	LLL	8.3	8.1	7.7	7.0	6.1	8.4	6.1	5.7	4.3	4.6
	RT20.6				1.3						
38	LLM	7.5	7.2	6.8	6.6	5.0	7.5	4.7	4.8	4.8	4.5
	RT22.4			1.2	1.0						
38	LLO	3.1	3.1	2.3	2.3	2.4	3.8	2.6	2.4	2.3	4.2
	RT28.4					2.9				1.2	1.9
40	LMM	5.6	5.1	5.7	5.5	7.8	5.9	4.9	5.6	5.6	5.6
40	RT38.5					3.4			1.4	1.2	
40	LMO	3.7	4.3	5.2	4.9	6.6	3.9	4.9	5.0	4.9	5.2
	RT46.4										
42	LMP		1.6	3.6	4.5	5.1		5.4	6.6	7.7	7.8
	RT50.3		2.8	2.8	2.0						
44	PLO	1.4	1.5	1.6	1.7	2.9	1.2	2.0	4.7	5.8	5.7
44	PPL		1.5	2.7	2.7	3.2		4.1	5.9	6.3	6.2
	RT88.0	2.6	4.2		5.0	5.7	2.7	2.8	2.8	2.5	2.0
48	POO	6.6	6.1	5.8	3.2	3.5	6.5	5.8	5.4	4.9	4.7
48	POP	15.6	14.4	12.2	11.8	10.3	15.2	11.1	9.7	9.2	9.1
48	PPP	14.5	12.2	11.7	10.4	9.5	14.3	10.4	9.4	8.8	8.5
50	POS	2.9	2.9	2.9			2.3	2.1	2.2	2.0	2.0

When 0% of TL enzyme was added to the 50:50% palm stearin and fully hardened coconut oil, no change was observed in the TAG composition of the fat blend throughout the 8 hours. This tells us that the composition of the TAGs are not affected by heating the fat blend at 65°C for 8 hours and this trial was conducted as a reference for non-interesterified trials of this blend. There was no LMP and PPL TAGs present in non-interesterified fats and a high percentage of high molecular weight TAGs like POO, POP and PPP were detected as well as CCL, CLL, LLL and LLM TAGs in the low molecular weight range. The high percentage of these TAGs was due to the higher percentage of fully hardened coconut oil in the fat blend. Changes in the TAG composition were observed when 2% of TL enzyme was added to the fat blend Table 24. With 2% TL enzyme in the PS:FHCO 50:50 blend, the composition of low molecular TAGs like CCL, CLL, LLL and LLM decreased in the four samples over the 8 hour period. The high molecular TAGs of POO, POP and PPP were also reduced, the percentage of PPP decreased from 14% in non-interesterified fats to 9 % and the PSS TAG disappeared after 6 hours of reaction. New TAGs like LMP, PLO and PPL were formed that had molecular weights between the low and high molecular weight TAGs in the original fat blend. The LMP TAG increased to 5.1% of the TAGs present after 8 hours of reaction. The percentage composition of LMP increased from 0% to 5% over the 8 hours and the percentage of PLO and PPL also increased to 5%. When the amount of TL enzyme was increased to 8%, the percentage of POP and PPP TAGs reduced from 15% to 8% and the CLL, LLL and LLM TAGs reduced from 7%, 8% and 7% to 2%, 4% and 4%. The LMP, PPL and PLO TAGs increased from to 6%, 5% and 7%, respectively. The composition of TAGs sampled at 6 hours showed similar results from samples after 8 hours. This observation indicates that the fat blends are fully interesterified after 6 hours and reached a stable TAG composition with no further changes.

5.3 Differential scanning calorimetry

The DSC scans show the heat released by interesterified and non-interesterified hard stocks with different concentration of enzymes at different reaction times. The crystallization of solid fats was achieved by decreasing the fat sample temperature from 80 to -60°C and the heat released by the solid fat sample was registered at peaks during the change of temperature. The DSC scan for TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30%was shown in Figure 42. DSC scan for the RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% was shown in Figure 43 and DSC scan for TL enzyme interesterified palm stearin and fully hardened coconut oil at 50 to 50% was shown in Figure 44.

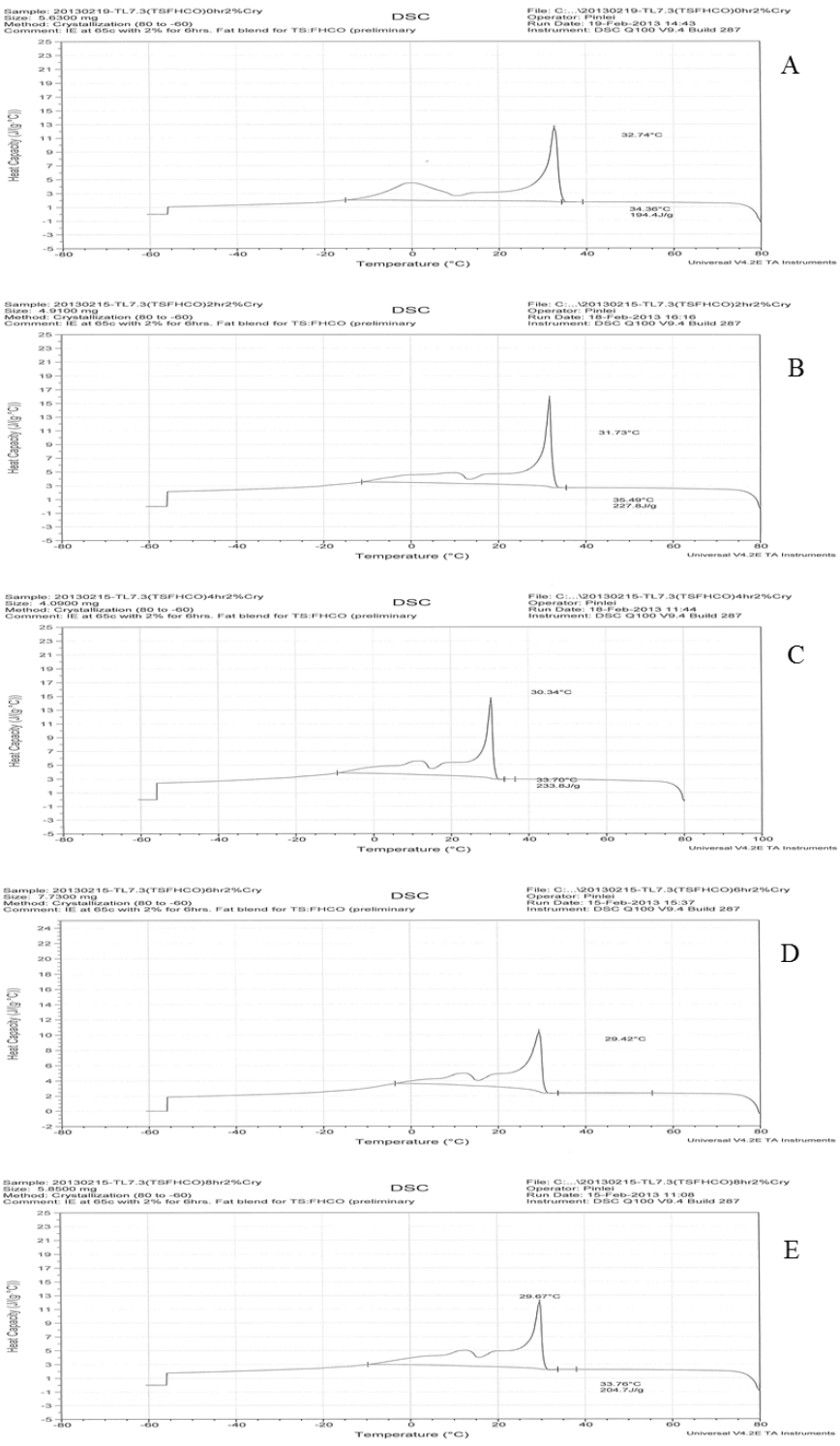

Figure 44. DSC cooling (crystallisation) curve for 70% to 30% TS: FHCO blends with Lipozyme TL IM (added at 2% w/w). A 2% TS: FHCO for 0 hours, B 2% TS: FHCO for 2 hours, C 2% TS: FHCO for 4 hours, D 2% TS: FHCO for 6 hours and E 2% TS: FHCO for 8 hours.

The DSC crystallization curves in Figure 44 shows the change in heat released during the crystallization of TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30%. Figure 44A shows that when no enzymes were added, two peaks can be observed with the tallow stearin and fully hardened coconut oil blend. The high temperature peak occurred at temperature between 35 and 10°C and the low temperature peak occurred at temperature between 10 to -15°C as the fat sample was cooled. The high and low temperature peak both represents the high molecular weight TAGs and low molecular TAGs as indicated in the TAG composition shown in Table 22.When 2% of the TL enzyme was added to the fat blend, the interesterification reaction was initiated and change in the heat release profile was observed as shown in Figure 44E. The crystallization curve for the 2% TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% (w/w) for 8 hours shows the formation of a shoulder peak at temperatures between 25 to 20°C. The formation of the shoulder peak was confirmed by the newly formed medium molecular TAGs mentioned in Table 22 and the presences of the shoulder peaks in crystallization curves shows that interesterification reaction did occur with this fat blend

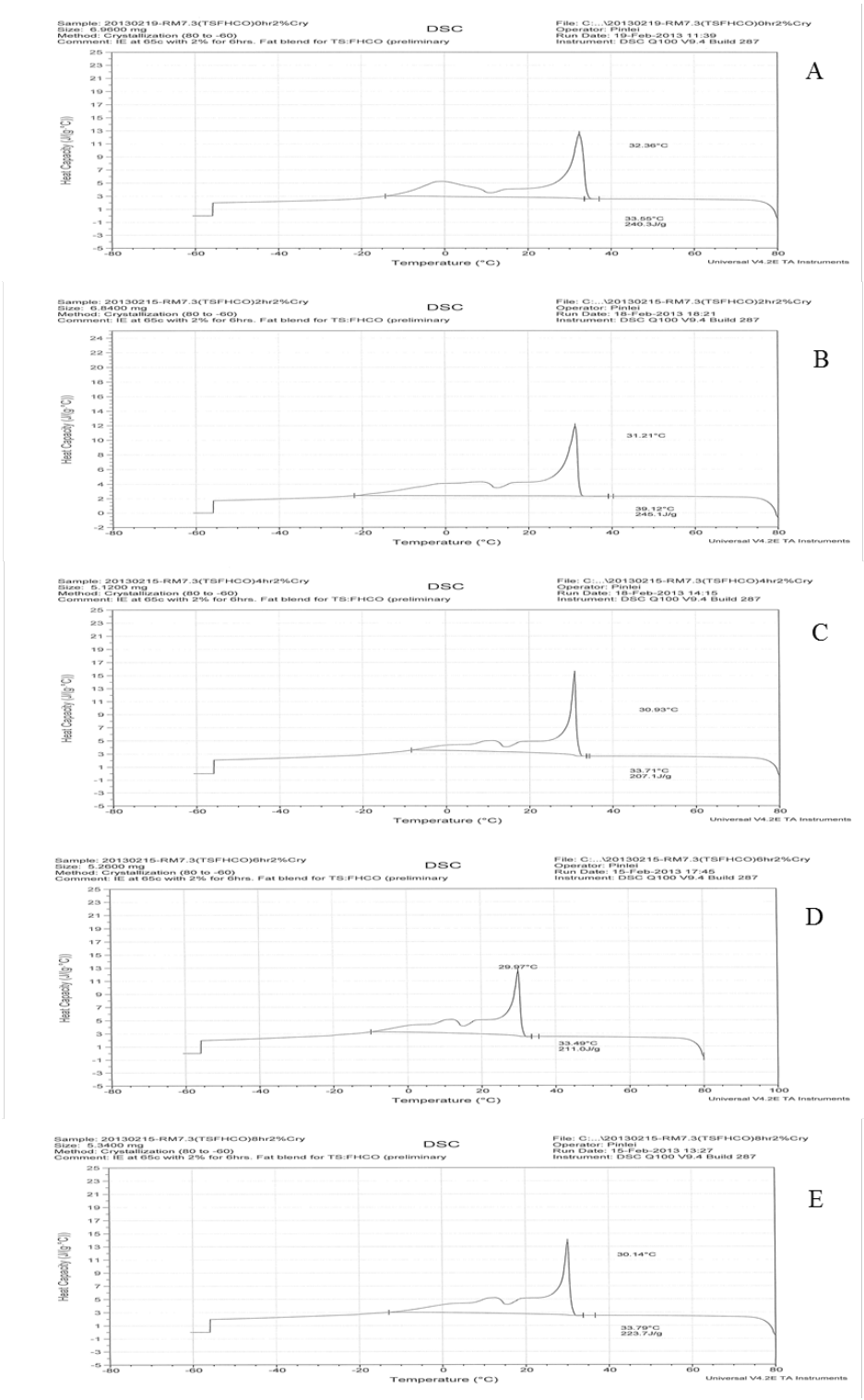

Figure 45. DSC cooling (crystallisation) curve for 70% to 30% TS: FHCO blends Lipozyme TL IM (added 2% w/w). A 2% TS: FHCO for 0 hours, B 2% TS: FHCO for 2 hours, C 2% TS: FHCO for 4 hours, D 2% TS: FHCO for 6 hours and E 2% TS: FHCO for 8 hours.

The DSC crystallization curve in Figure 45 indicates the heat release profile for RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% over 8 hours of reaction time. In Figure 45A, two separate peaks were also observed. The high temperature peak at temperature between 35 and 10°C shows the heat release profile by the high molecular TAGs of the hard stock whereas the low temperature peak between 10 to -15°C represents the low molecular TAGs indicated in Table 23. The change in the heat release profile for the RM enzyme interesterified was similar to that of the TL enzyme interesterified fats. This is due to the similar TAG composition of the two hard stocks as mentioned in Table 22 and Table 23. After 8 hours of reaction, the shoulder peak representing medium weight molecular TAGs was seen in Figure 45E at temperature between 25 and 20°C. The crystallization curve was different from the IPL benchmark in Figure 23C because tallow stearin was used instead of palm oil and 2% (w/w) of enzyme was not capable of reaching reaction end point in 8 hours.

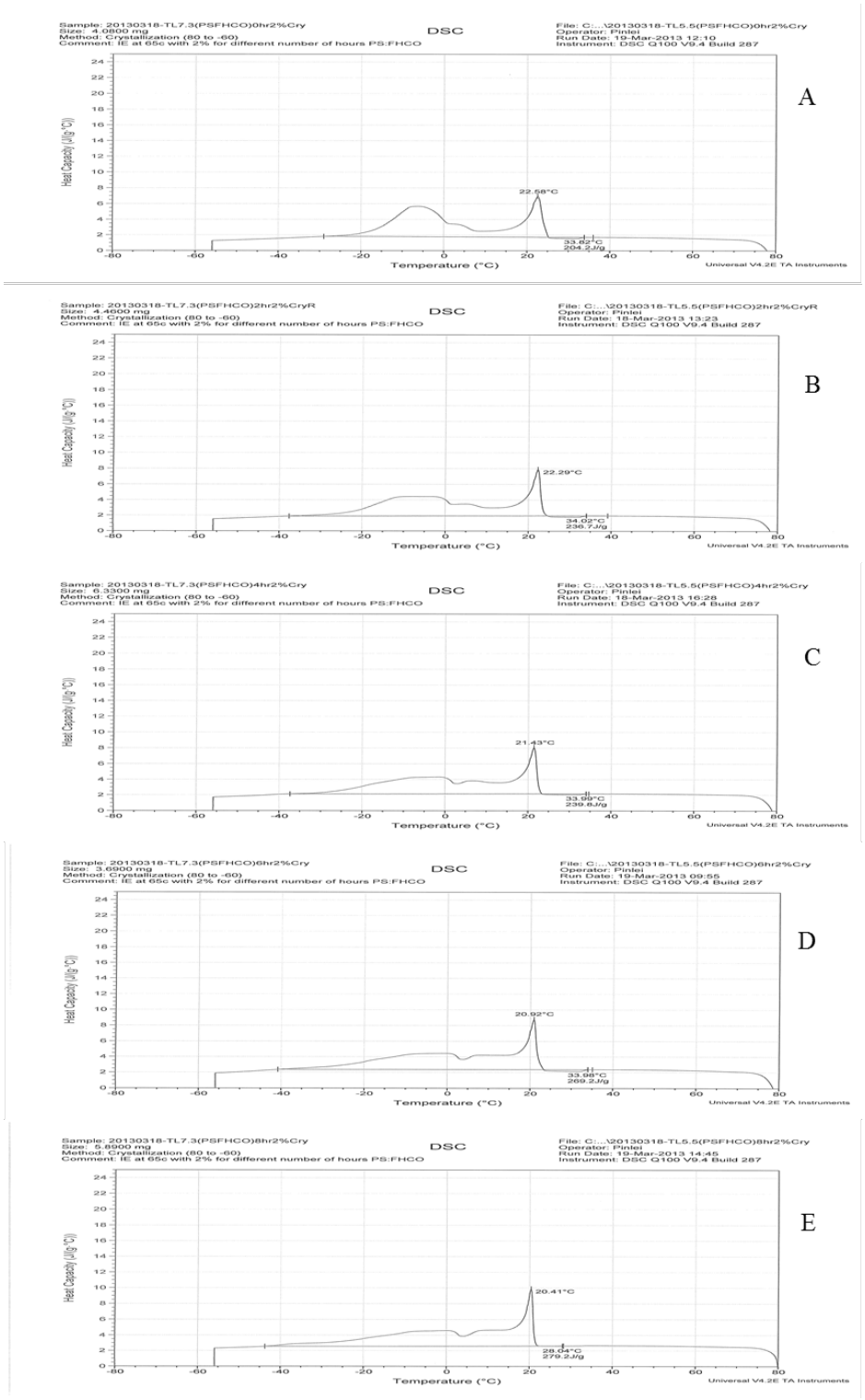

Figure 46. DSC cooling (crystallisation) curve for 50 to 50 % PS: FHCO blends with Lipozyme TL IM (added 2% w/w). A 2% PS: FHCO for 0 hours, B 2% PS: FHCO for 2 hours, C 2% PS: FHCO for 4 hours, D 2% PS: FHCO for 6 hours and E 2% PS: FHCO for 8 hours.

The crystallization curve in Figure 46 differs from Figure 45 and 44 because palm stearin was used as raw material instead of tallow stearin. Two peaks were shown in Figure 46A and the high temperature between 25 to 10°C shows the high molecular weight TAGs and the large low temperature peak between 10 to -20°C that represents low molecular weight TAGs. The reason for the small high temperature peak was due to the ratio of palm stearin and fully hardened coconut oil was adjusted to 50 to 50% and the increased amount of fully hardened coconut oil has also led to the increase of the low temperature peak between 10 to -20°C as shown in Figure 46A. When 2% of TL enzyme was added to the palm stearin and fully hardened coconut oil blend, the low temperature peak was reduced from 4 to 2 J/g°C while the shoulder peak was formed between 15 to 5°C. This change was due to the conversion of low and high molecular weight TAGs into medium molecular weight TAGs as indicated in Table 24.

5.4 Sold Fat Content (SFC)

The solid fat content was analysed on the three fat blends tested with different enzyme concentrations over 8 hours of reaction time. The solid fat content of TL enzyme interesterified tallow stearin and fully hardened coconut oil is shown in Figure 47 and the solid fat content of RM enzyme interesterified tallow stearin and fully hardened coconut oil is shown in Figure 48. Figure 49 shows the solid fat content for TL enzyme interesterified palm stearin and fully hardened coconut oil

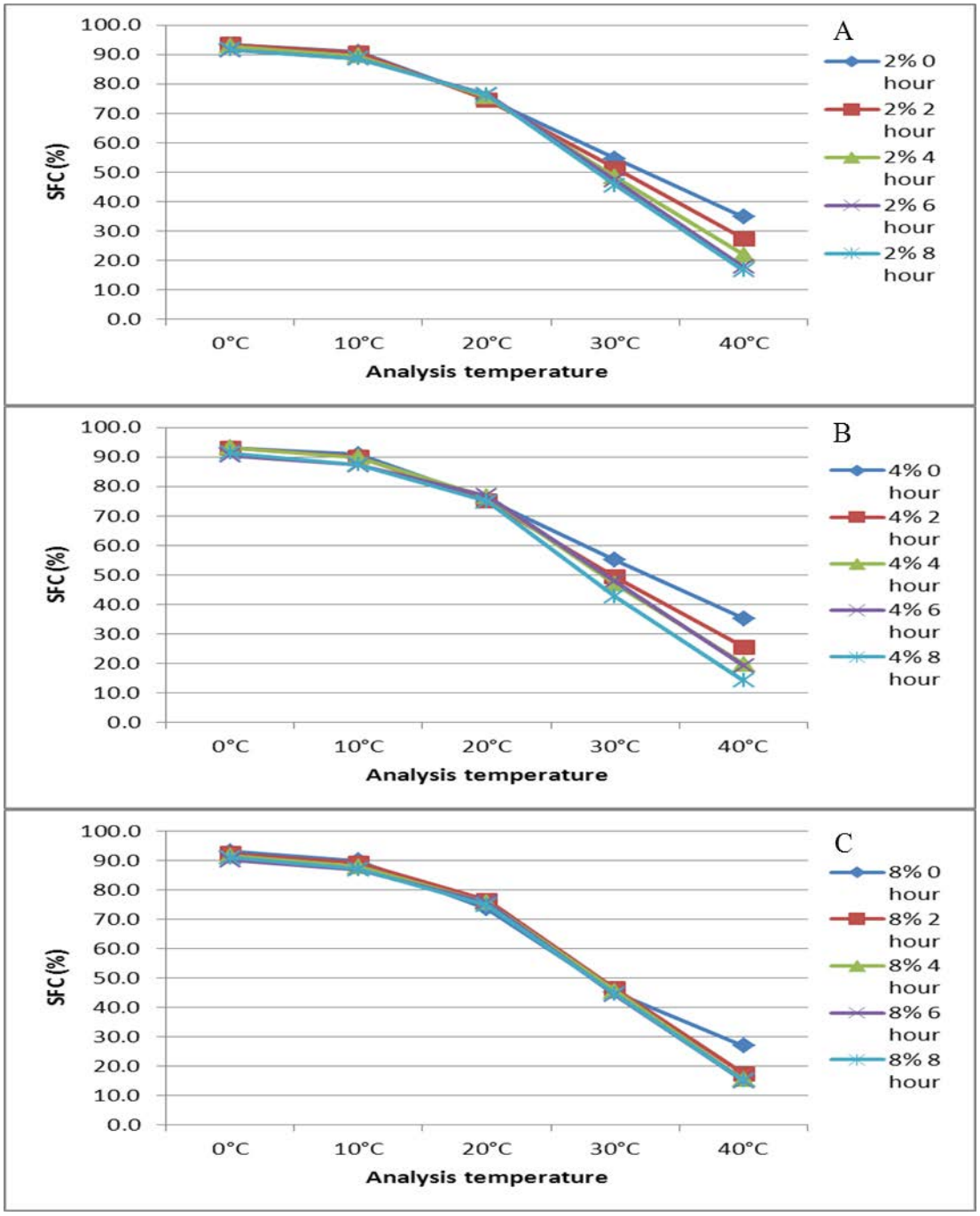

Figure 47. Percentage solid fat content of different concentration of TL enzyme interesterified tallow stearin and fully hardened coconut oil hard stock over 8 hours, A 2% enzymes added, B 4% enzyme added and C 8% enzyme added

The percentage of solid fat content on TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% was indicated in Figure 47. There were no obvious changes in percentage solid fat content in the 0 to 20°C analysis temperature over all three concentrations of enzymes but the changes were more apparent in the 20 to 40°C analysis temperatures. The solid fat content for the fats when no enzymes were added at 0 hours of reaction has shown higher solid fat content of 57 and 38% at 30 and 40°C instead of the hard stock interesterified by 2% of enzyme over 8 hours at 46 and 18% at 30 and 40°C in Figure 47A. At analysis temperatures of 30 and 40°C in Figure 47 B, the percentage of solid fat content decreased earlier in the reaction when 4% of the TL enzyme was added to the reaction. The percentage solid fat content was at 37, 28, 20, 20 and 18% in the order of 0, 2, 4, 6 and 8 hours of reaction time. This result confirms that the interesterification did have impact on decreasing the solid fat content of the hard stock and with the same concentration of enzymes, larger differences was observed in the percentage of solid fat content when the reaction time was increased. In Figure 47C, 8 % of enzyme was added to the hard stock and the solid fat content was similar between 0 to 30 °C analysis temperatures but changes occurred at the 40°C analysis temperature. The non-interesterified fats at 0 hour of reaction had high percentage solid fat content than the interesterified hard stocks and the solid fat content was the same for all hard stocks interesterified at 8% with different reaction times.

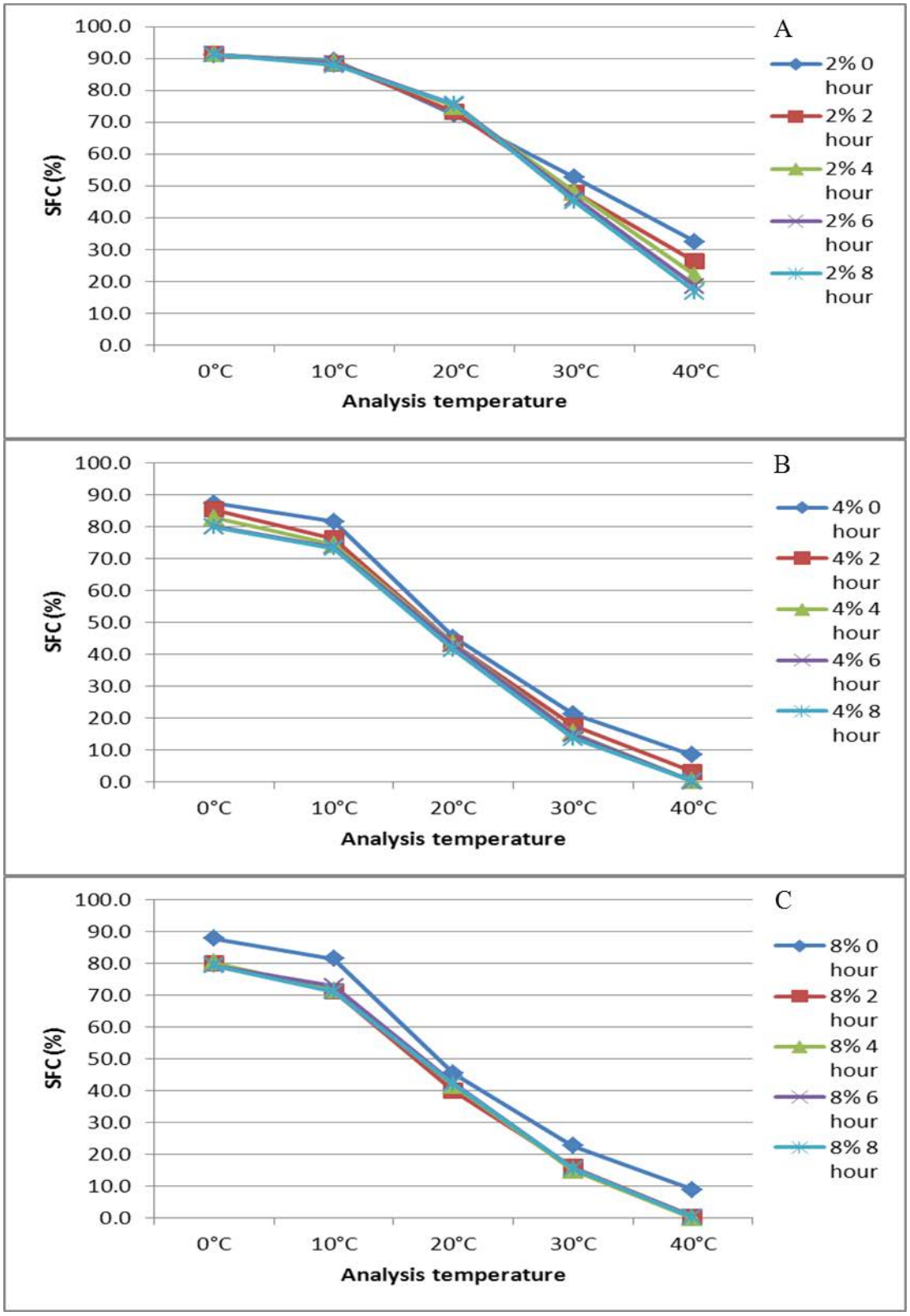

Figure 48. Percentage solid fat content of different concentration of RM enzyme interesterified tallow stearin and fully hardened coconut oil hard stock over 8 hours, A 2% enzymes added, B 4% enzyme added and C 8% enzyme added

The percentage of solid fat content with RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% was shown in Figure 48. There were no obvious changes in the percentage solid fat content in the 0 to 20°C analysis temperature when 2% of enzymes were added in Figure 48A. The changes in solid fat content became obvious between 30 to 40°C analysis temperatures. In the 40°C analysis, the solid fat content decreased from 36% for non-interesterified fats at 0 hour to 28, 22, 21 and 20% for 2, 4, 6 and 8 hours of reaction when the same concentration of enzymes were added. But when the concentration of enzyme was increased to 4% in Figure 48B, the changes in solid fat content can be seen from 10°C analysis temperature to 40°C analysis temperature. The interesterified hard stock had lower solid fat content than the non-interesterified hard stock at 0 hour reaction over all the analysis temperatures in the order of 2, 4, 6 and 8 hours of reaction time.

The differences in solid fat content between interesterified and non-interesterified hard stock was more obvious when 8% of enzymes was added to the hard stock as shown in Figure 48C. changes in the solid fat content was observed in all the analysis temperature even in the 0°C analysis, this results shows that the increase in enzyme concentration has significant impact on the solid fat content of the hard stocks. The solid fat content for the RM enzyme interesterified hard stock (Figure 48) differ from the TL enzyme interesterified hard stock (Figure 47) as the changes in solid fat content occurred at lower analysis temperature at 10°C and was more obvious over other analysis temperatures hence RM enzyme was more effective at manipulating solid fat contents of the fats than the TL enzyme.

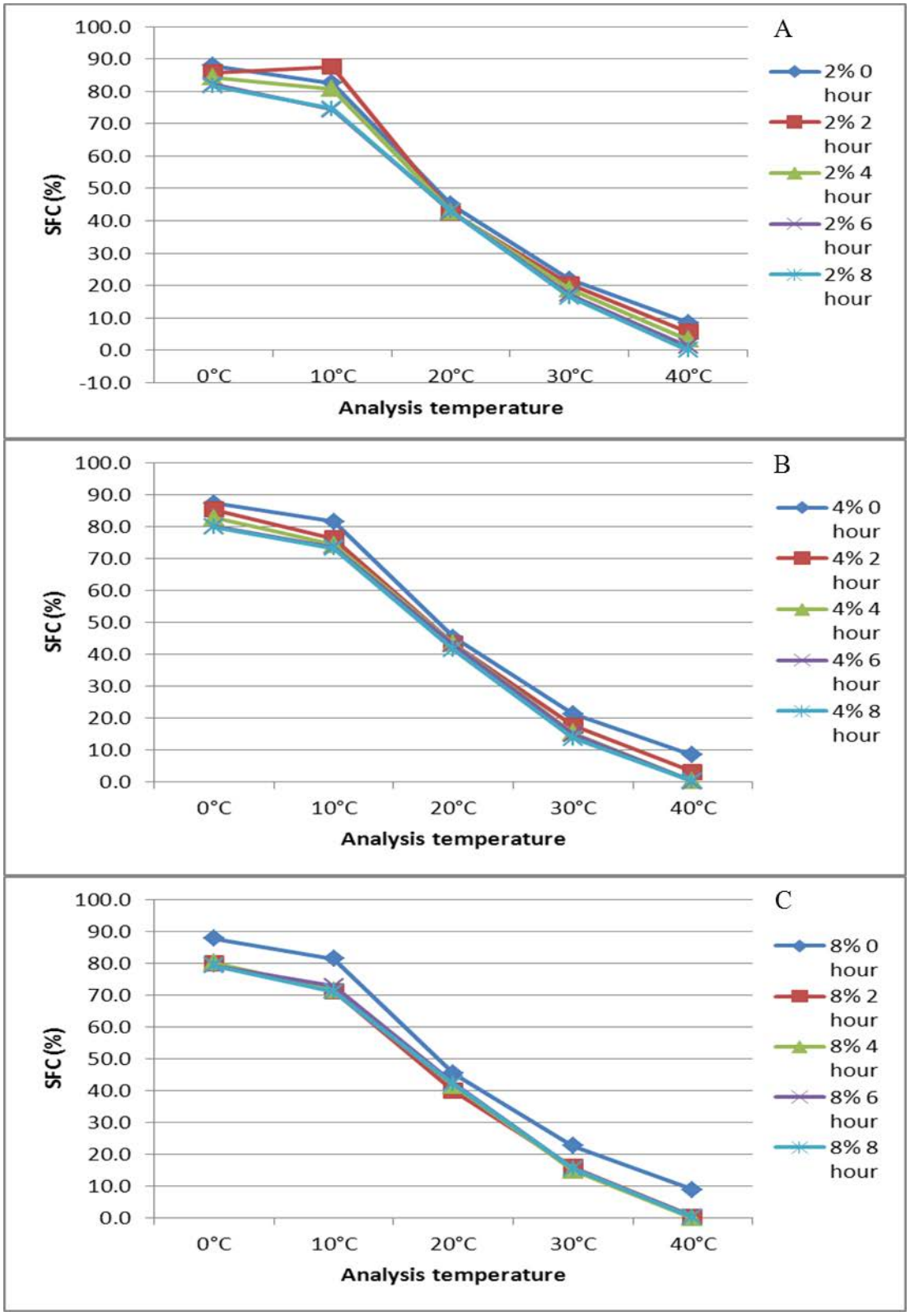

Figure 49. Percentage solid fat content of different concentration of TL enzyme interesterified palm stearin and fully hardened coconut oil hard stock over 8 hours, A 2% enzymes added, B 4% enzyme added and C 8% enzyme added

The percentage of solid fat content with TL enzyme interesterified palm stearin and fully hardened coconut oil at 50 to 50% was shown in Figure 49. The changes in the percentage of solid fat content with 2 % of TL enzyme can be seen in Figure 49A. Similar trends were also observed in Figure 49B and Figure 45C when the amount of enzymes added was increased to 4 and 8%. In the 20, 30 and 40°C analysis temperature for solid fat content in Figure 49C, the solid fat content curve for the interesterified fats overlapped when 8% of enzymes were added. This observation shows that there is no difference in the solid fat content for the hard stocks after 4 hours of reaction when 8% of enzymes were added to the reaction and indicates that the interesterification reaction was complete for this hard stock after 4 hours of reaction.

5.5 Time of crystallization

The time of crystallization analysis was conducted with a pulsed NMR instrument as described in Section 3.1.5. Table 25 indicates the time of crystallization for RM enzyme interesterified tallow stearin and fully hardened coconut oil, TL enzyme interesterified tallow stearin and fully hardened coconut oil and TL enzyme interesterified palm stearin and fully hardened coconut oil with different concentrations of enzyme over 8 hours of reaction period and the translation for the abbreviated trial names can be found in ix section of the report.

Table 25. The time taken for hard stocks to reach constant SFC values during crystallization from 60℃ and cooling at 0°C

RM7.3 TS:FHCO	Time for crystallization (min)	TL7.3 TS:FHCO	Time for crystallization (min)	TL5.5 PS:FHCO	Time for crystallization (min)
TL7.3 0%0HR	60	RM7.3 0%0HR	60	TL5.5 0%0HR	50
TL7.3 0%2HR	60	RM7.3 0%2HR	60	TL5.5 0%2HR	50
TL7.3 0%4HR	60	RM7.3 0%4HR	60	TL5.5 0%4HR	50
TL7.3 0%6HR	60	RM7.3 0%6HR	60	TL5.5 0%6HR	50
TL7.3 0%8HR	60	RM7.3 0%8HR	60	TL5.5 0%8HR	50
TL7.3 2%0HR	60	RM7.3 2%0HR	60	TL5.5 2%0HR	50
TL7.3 2%2HR	40	RM7.3 2%2HR	40	TL5.5 2%2HR	40
TL7.3 2%4HR	40	RM7.3 2%4HR	40	TL5.5 2%4HR	40
TL7.3 2%6HR	30	RM7.3 2%6HR	30	TL5.5 2%6HR	30
TL7.3 2%8HR	30	RM7.3 2%8HR	30	TL5.5 2%8HR	20
TL7.3 4%0HR	60	RM7.3 4%0HR	60	TL5.5 4%0HR	50
TL7.3 4%2HR	40	RM7.3 4%2HR	40	TL5.5 4%2HR	40
TL7.3 4%4HR	30	RM7.3 4%4HR	30	TL5.5 4%4HR	20

TL7.3 4%6HR	20	RM7.3 4%6HR	20	TL5.5 4%6HR	18
TL7.3 4%8HR	16	RM7.3 4%8HR	16	TL5.5 4%8HR	14
TL7.3 8%0HR	60	RM7.3 8%0HR	60	TL5.5 8%0HR	50
TL7.3 8%2HR	18	RM7.3 8%2HR	18	TL5.5 8%2HR	30
TL7.3 8%4HR	16	RM7.3 8%4HR	16	TL5.5 8%4HR	16
TL7.3 8%6HR	14	RM7.3 8%6HR	14	TL5.5 8%6HR	14
TL7.3 8%8HR	14	RM7.3 8%8HR	14	TL5.5 8%8HR	14

The results from Table 25 suggest that when the enzyme concentration or the reaction time was increased, the time for the hard stocks to crystallize was decreased. In the RM enzyme interesterified hard stock, the time for crystallization was 60 minutes when no enzymes were added but after 8 hours of reaction with 2% (w/w) of enzymes, the time for crystallization was decreased to 30 minutes. When the concentration of the RM enzyme was increased to 8% (w/w) the time for crystallization was further reduced to 16 minutes after 4 hours of reaction then 14 minutes after 6 hours of reaction. The time for crystallization remained constant after 8 hours of reaction and this observation further confirmed that the interesterification reaction was complete after 8 hours of reaction.

5.6 Discussion for time and enzyme concentration trial

The reaction time and the amount of enzymes used for the interesterification reaction were investigated in this stage of the trials. The hard stocks selected for this stage of trials were the RM enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% ratios, TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% ratios, TL enzyme interesterified palm stearin and fully hardened coconut oil at 50 to 50% ratio.

The objective for this stage of trials was to investigate the optimum amount of enzyme and time required to complete the interesterification reaction. The change in melting point analysis (Figure 38A and 38B) suggests that the reaction has reached the target melting point after 8 hours of reaction with 4% of enzymes. This observation was confirmed when the enzyme concentration was increased to 8% that the melting point remained constant at 44°C. It was also observed from all three hard stocks that when 4% of enzyme was added, it takes the enzymes 8 hours to produce hard stocks at their terminal melting point temperatures of 36 and 44°C. However, when the enzyme concentration was increased to 8%, the terminal melting point temperature was reached in 6 hours of the reaction. The change in the melting

point temperature can be explained by the change in the TAG composition. The formation of the medium molecular TAG LMP reached 3.6% after 8 hours of reaction when 2% of enzymes were added (Table 24) while the formation of the same TAG reached 7.3% after 4 hours of reaction when 8% of the TL enzyme was added during the reaction and remained at 7.3% throughout 8 hours of reaction. This observation confirms that when 8% of enzymes are added to the hard stock, the reaction occurs much faster. The TL enzyme interesterified palm stearin and fully hardened coconut oil at 50 to 50% ratio was not continued to the next stage of trials due to the lower melting point of 36 °C (Figure 43) from the IPL hard stock at 44°C (Figure 18) although this hard stock is a rapid crystallizer but has low solid fat content at 0% at 40°C (Figure 45). Though this blend of palm stearin and fully hardened coconut oil could be investigated further as a vegetable fat blend.

5.7 Conclusion for time and enzyme concentration trial

The enzyme concentration and reaction time trial was conducted in order to select and optimize the efficiency of the batch interesterification reaction. The hard stock selected for the reusability trial was the RM enzyme and TL enzyme interesterified tallow stearin and fully hardened coconut oil at 70 to 30% ratio. The reaction time and concentration of enzyme was determined at 8 hours and 4% (w/w) of enzymes at 65 ± 1°C although doubling the enzyme concentration to 8% can reduce the reaction time to 6 hours.

Chapter 6 Reusability Trials for Lipase Enzymes

The number of times a batch of RM or TL enzymes could be reused was investigated. . Literature suggested several organic solvents for washing the enzyme; ethanol, chloroform, acetone and isooctane (Kim. 2008; Ognjanovic et al. 2009; Souza. 2011; De Martini Soares et al., 2013). The enzymes were washed with these solvents then rinsed, any changes in degree of interesterification achieved was measured in the fresh fat blend with the recycled enzyme. The melting point temperatures and TAG compositions were monitored.

6.1 Melting point

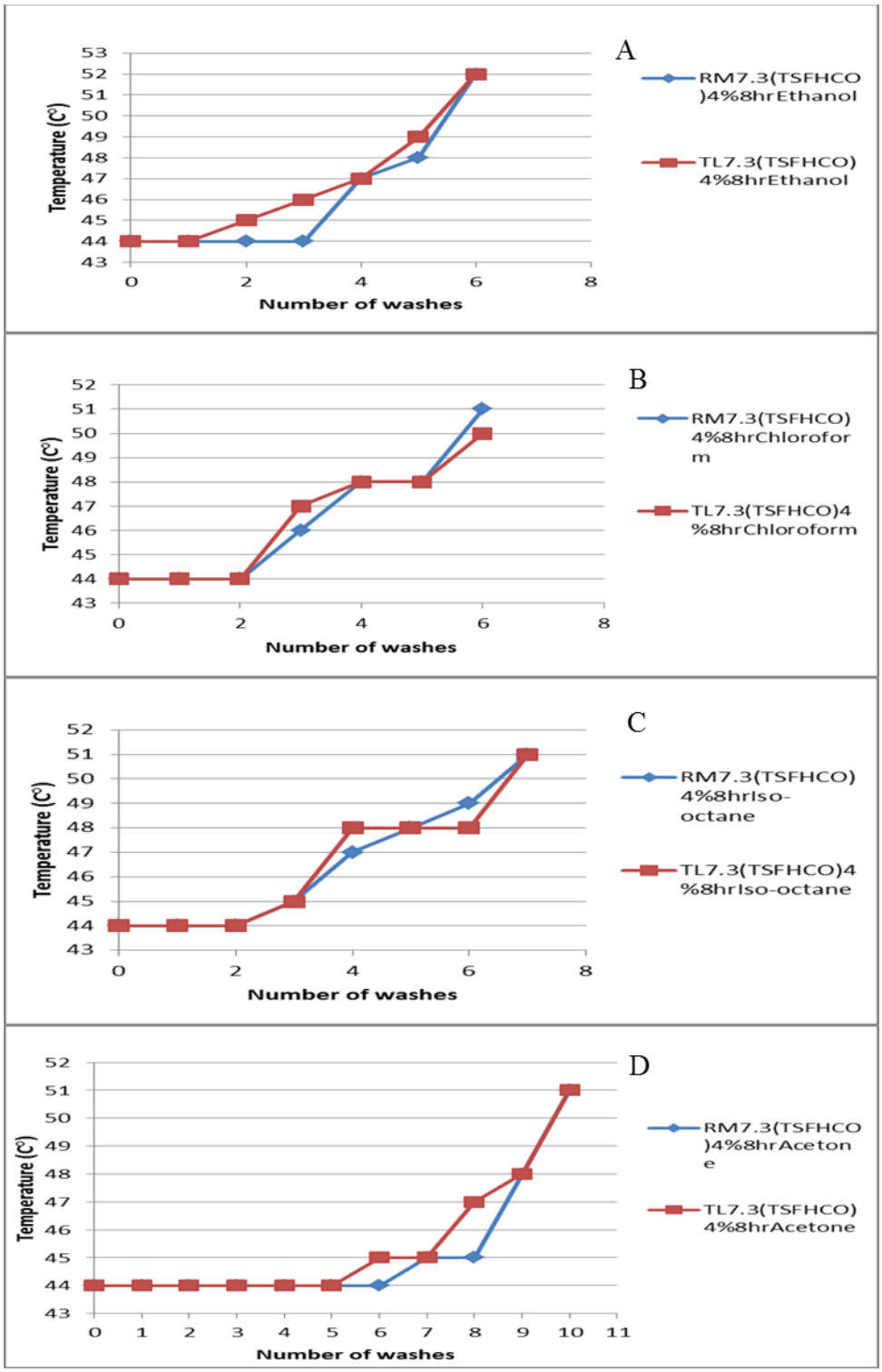

Figure 50. Melting point temperature for by RM and TL enzyme interesterified fats for reusability tests, A Ethanol washed enzymes, B Chloroform washed enzymes, C Iso-octane washed enzymes, D Acetone washed enzymes. The data points are mean± standard deviation (n=4). Note standard deviation values are small and not visible.

Figure 50 shows the change in melting point temperature for both RM and TL enzyme interesterified fats when the enzymes were reused after washing with organic solvents. In Figure 50A the melting point temperature after 8 hours of interesterification remained at 44 ± 1°C after the first three washes with ethanol for the RM enzyme. The TL enzyme produced fat blends with melting points of 44 ± 1°C only after the first wash, after this point the melting point of the interesterified fat blend after 8 hours kept increasing indicating a loss in its activity after that first wash. In Figure 50B both RM and TL enzymes were washed by the organic solvent chloroform. There was no change in the melting point for both RM and TL enzymes in the first two washes by chloroform but the melting point temperature rose to 47 ± 1°C by the third wash and continued to rise to 51 ± 1°C by the sixth wash. This result indicated the enzymes were losing activity after each wash.

The RM and TL enzymes after washing with iso-octane were shown in Figure 43C and similar changes in melting point temperature as ethanol and chloroform was observed. The melting point of the fat blend with TL enzyme increased after three washes with isooctane to 48 ±1°C and increased to 51± 1°C after seven washes.

The enzymes washed with acetone as indicated in Figure 50D was able to produce fat blends after 8 hours of interesterification with the same melting point temperature far better than when washed with chloroform, ethanol and isooctane. The melting point temperature of 44 ± 1°C was achieved in the fat blends after five washes for TL enzyme and six washes for the RM enzyme. The enzyme began to lose its activity after the sixth wash and had no effect on the melting point temperature by the tenth wash. The RM enzyme performed better than the TL enzyme when washed with acetone.

6.2 Triglyceride composition for reusability trials

The triglyceride composition checked after the reusability tests to determine if there were any changes in the TAGs of the interesterified fats by the recycled enzymes, washed by various organic solvents. The composition of TAGs from initial analysis prior to washing was used as control to monitor any changes. The TAG composition for the batches that achieved 44 ± 1 °C melting point temperature was compared to the first use of the enzyme. All interesterification trials were carried out for 8 hours.

Table 26. TAG content of RM7.3 TS:FHCO (70:30) with 4% enzyme interesterified at 65 ± 1°C then washed with ethanol.

ECN	NAME	Zero wash	First wash	Second wash	Third wash
24	CpCpCp	3.4	4.8	4.1	4.3
	RT7.1		1.2		1.3
30	CCC	3.4	4.3	4.2	4.7
	RT10.1	1.2	1.2	1.6	1.2
	RT11.2		2.3	1.4	
32	CCL	3.2	3.7	5.1	5.4
32	RT10.9	1.9	1.2	1.1	1.1
34	CLL	5.1	5.6	6.2	6.1
	RT13.9	1.4			
36	LLL	6.9	6.3	6.9	7.6
	RT20.1			1.0	
38	LLM	6.5	7.0	7.4	7.5
38	LLO	6.5	5.4	8.1	8.1
	RT37.2	3.6	3.9	1.1	
42	LMO	1.1	1.6		
40	LMM	6.3	7.1	9.3	9.0
42	RT50.8	2.9	3.9	4.2	4.3
42	LMP	7.1	6.2	2.7	
	RT74.2	2.3	1.2		
44	PLO	4.7	2.1		
44	PPL	5.3	2.1		
48	POO				
48	POP	12.6	14.6	16.2	17.3
48	PPP	12.7	13.3	15.3	16.2
50	POS			3.2	4.5

The changes in the TAG composition can be observed after the first wash. The percentage for both high and low molecular TAGs like CLL, CCC and POP, PPP began to rise after the first wash and the TAG composition became similar to non-interesterified fats after the third wash (Table 26). This observation illustrates that ethanol decreases the reaction ability of the enzyme hence only three washes was managed with this solvent.

Table 27. TAG content of RM7.3 TS:FHCO (70:30) with 4% enzyme interesterified at 65 ± 1°C then washed with chloroform.

ECN	NAME	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash
24	CpCpCp	3.2	3.2	3.2	3.3	3.0	3.9	4.2	4.3
	RT6.8								1.5
30	CCC	3.4	3.4	3.4	3.6	4.2	4.2	4.3	4.4
	RT9.4	1.4	1.5	1.6	1.1	1.9	2.2	1.8	1.1
	RT10.2	2.2	2.2	2.2	1.3				
32	CCL	3.4	3.4	3.4003	3.3	3.7	3.8	4.6	5.2
32	RT12.1	1.4	1.4	1.5	4.7	1.1	1.9	1.0	1.1
34	CLL	5.2	5.1	5.2	4.7	5.3	5.7	6.2	6.3
	RT16.5	1.4	1.3						
36	LLL	7.1	7.2	7.2	6.1	6.3	6.3	7.2	7.4
	RT20.0				1.8				
38	LLM	6.2	6.1992	6.2	6.5	6.9	7.0	7.2	7.4
38	LLO	6.0	6.1	6.2	6.5	7.5	7.9	7.9	8.7
	LMM	3.2	3.2	3.2	2.8	2.3	1.5	1.0	
42	RT40.9	1.6	1.5	1.4	1.3				
42	LMO	1.1	1.1	1.2	1.2	1.9	3.7		
40	RT41.6							2.4	
42	LMM	6.1	6.1	6.2	6.7	7.4	8.2	8.7	9.2
42	RT51.2	2.7	2.7	2.6	3.4	3.6	4.0	4.2	4.5
44	LMP	7.8	7.7	7.6	6.2	5.1	3.3	1.6	
44	RT75.7	2.2	2.2	2.3	1.2	4.5	1.1		
44	PLO	4.2	4.3	4.1	3.9	3.2	2.4	1.1	
48	PPL	5.1	5.2	5.2	2.1	1.2			
48	POO								
48	POP	12.3	12.3	12.2	13.9	15.8	16.8	17.0	18.0
48	PPP	12.2	12.2	12.1	13.7	14.9	15.8	16.6	16.2
50	PSS							2.2	4.2

The TAG composition of the TS:FHCO 70:30 fat blend with RM enzyme that was washed with chloroform showed similar results for washed enzymes as the other solvents presented (Table 27). The level of POP and PPP TAGs remained unchanged after the second wash, while the formation of LMP, PLO and PPL TAGs was reduced or not detected t after the seventh wash. This result confirms the change in melting point temperature from Figure 43B, as when no change was observed in the melting point temperature the fats are fully interesterified but once the enzyme begins to lose its activity after several washes, the melting point was also affected as smaller changes in the TAG composition was observed. The PSS was no longer lost after the fifth wash indicating that the reaction was slowing down and the enzyme was losing its ability to interesterify.

Table 28. TAG content of RM7.3 TS:FHCO (70:30) with 4% enzyme interesterified at 65 ± 1°C then washed with isooctane .

ECN	NAME	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash
24	CpCpCp	3.1	3.2	3.3	3.4	3.1	2.9	5.3	4.3
	RT6.1							1.3	1.0
30	CCC	3.0	3.1	3.3	3.5	3.5	4.7	4.9	4.9
	RT9.9	1.0	1.1	1.1		1.8	1.9	1.0	1.8
	RT10.5	2.2	2.1	2.3	4.4	4.3	3.2	1.0	
32	CCL	3.7	3.5	3.1	4.1	4.7	4.9	5.1	5.1
32	RT13.1	1.6	1.3	1.1	1.0	1.0			1.5
34	CLL	5.2	5.1	5.6	5.5	1.5	1.4	5.2	5.9
	RT15.3	1.3	1.3	1.3	2.6	1.9			
36	LLL	7.4	7.4	7.6	7.1	7.2	6.8	6.8	7.5
	RT22.2	1.3	1.2	1.3		3.5			
38	LLM	6.7	6.8	6.9	7.1	7.1	7.6	7.6	7.5
38	LLO	6.7	6.7	6.8	7.3	7.7	8.1	8.0	8.0
	RT35.9	3.6	3.3	3.0	2.6	2.0	1.1		
42									
42	LMO	1.2	1.3	1.3	1.1				
40	LMM	6.2	6.1	6.0	6.5	7.2	8.6	9.2	9.2
42	RT51.1	2.5	2.6	2.6	3.0	3.8	4.3	4.2	4.1
42	LMP	7.2	7.1	7.2	6.5	4.7	3.9		
44	RT73.4	1.1	1.5	1.9					
44	PLO	4.1	4.2	4.4	2.9	2.4	1.0		
44	PPL	5.2	5.3	5.3					
48	POO								
48	POP	12.4	12.6	12.1	15.2	16.4	17.5	17.9	17.6
48	PPP	12.0	12.0	11.2	14.9	15.1	16.18	16.2	16.7
50	PSS						3.1	3.9	4.2

The TAG composition for the 4% RM enzyme interesterified tallow stearin and fully hardened coconut oil showed that after the first two washes with iso-octane (Table 27) similar TAG profiles to the first unwashed batch of enzyme was observed. When the amount of LMP, PLO and PPL TAGs produced was less than the fresh enzyme treated fat blend, this it showed that the enzyme's activity was decreasing and reaction conditions may need to be changed in order to produce the fats of the same consistency or a new batch of enzymes used. Some interesting findings from Table 28, shows that the PSS TAG was not lost after the fourth wash, the PSS TAG only exists in non- interesterified fats so could be used as an indicator to show the enzyme was no longer able to interesterify the fats.

Table 29. TAG content of RM7.3 TS:FHCO (70:30) with 4% enzyme interesterified at 65 ± 1°C then washed with acetone.

ECN	Name	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash	Eighth wash	Ninth wash	Tenth wash
24	CpCpCp	3.2	3.2	3.4	3.3	3.3	3.4	3.3	3.4	3.7	4.1	4.4
	RT6.6											1.5
30	CCC	3.2	3.4	3.6	3.6	3.5	3.5	3.3	3.8	4.1	4.1	4.4
	RT9.4	1.3	1.3							1.0	1.2	1.2
	RT10.5	2.4	2.3	2.5	2.3	2.3	2.5	2.5	2.6	1.8	1.2	
32	CCL	3.5	3.6	3.6	3.5	3.7	3.4	3.6	3.3	4.7	5.1	5.3
32	RT11.2	1.2	1.3	1.3	1.9	2.0	2.2	2.4	2.0	1.5	1.1	1.0
34	CLL	5.3	5.3	5.1	5.2	5.3	5.1	5.2	5.2	4.5	5.5	6.3
36	RT15.7	1.2	1.2	1.2	1.3	1.3	1.2	1.2	1.3			
36	LLL	7.1	7.0	6.9	6.4	6.8	6.7	6.7	6.5	6.8	7.3	7.6
	RT19.6									1.1	1.2	
38	LLM	6.2	6.2	6.1	6.2	6.2	6.2	6.2	6.8	6.9	7.2	7.7
38	LLO	6.4	6.1	6.3	6.6	6.3	6.2	6.3	6.5	7.3	8.1	8.4
	RT36.2	3.4	3.3	1.4	1.6	1.7	1.7	1.7	1.6	1.3	1.1	
42	RT40.5	1.9	1.9	1.8	1.9	1.9	2.0	1.9	2.2	1.5		
42	LMO	1.1	1.1	1.1	1.1	1.0	1.0	1.1	1.1	1.1		
40	RT43.4			1.6	1.8	1.7	1.7	1.7	1.7	1.1		
42	LMM	6.1	6.1	6.4	6.4	6.4	6.5	6.5	6.4	6.9	8.1	9.3
42	RT50.6	2.7	2.8	2.7	2.7	2.5	2.4	2.4	2.2	2.6	3.5	4.4
44	LMP	7.3	7.5	7.6	7.2	7.7	7.5	7.6	7.1	3.9	2.0	
44	RT75.9	2.2	2.2	2.2	2.2	2.4	2.2	2.3	2.2	1.6	1.1	
44	PLO	4.2	4.2	4.1	4.4	4.5	4.2	4.2	4.0	1.3		
48	PPL	5.3	5.1	5.3	5.1	5.3	5.1	5.4	5.2	3.4	1.9	
48	POO									2.5		
48	POP	12.5	12.2	12.4	12.3	12.0	12.4	12.5	11.8	12.9	15.4	17.8
48	PPP	12.4	12.3	12.7	12.7	12.0	12.3	12.2	11.9	13.3	16.2	16.2
50	POS									2.9	3.7	4.4

Table 29 shows the TAG composition changes in the TS:FHCO 70:30 fat blend with 4% RM enzyme after the enzyme had been washed with acetone. The number of wash cycles achieved with acetone was six hence one batch of enzyme could be used to interesterify different batches of TS:FHCO blends seven times before slowly losing its interesterifying ability. The point when the enzyme was considered to have lost all its activity was when interesterified fat blends did not change in composition or melting point from the non-interesterified fats (Table 19, Table 28) Changes in specific TAGs included the reduction in loss of POP, PPP and POS TAGs as the enzyme lost activity and the reduced formation of LMP and PLO TAGs were observed after the seventh and the eighth washes. This result can be used to explain the change in melting point temperature indicated in Figure 38. When the TAG composition for the interesterified fats remained constant after the first 6 washes, similarly the melting point was also observed at 44 $\pm$ 1°C however once the enzyme began to lose its activity, ability to interesterify, there were less changes in the TAG composition which resulted in less of a reduction in the desired melting point.

6.3 Discussion for reusability trials

The objective for this stage of trials was to determine the maximum number of uses for the enzymes after washing one batch with organic solvents multiple times before the enzymes lost its interesterification ability in the batch processing technique. The two hard stocks retained from the previous selection process were the RM enzyme (added 4%) interesterified tallow stearin and fully hardened coconut oil at 70 to 30% and TL enzyme (added 4%) interesterified tallow stearin and fully hardened coconut oil at 70 to 30% and the lipase enzymes used for this reaction reacted differently with the solvents used.

The acetone washed enzymes differed from other solvents as the RM enzyme survived six washes and produced seven batches of hard stock while the TL enzyme was able to go through five washes and produce six batches of hard stocks before the melting point temperature became higher than 44°C. The change in the melting point temperature indicates changes in the quality of the hard stocks produced hence indicating that the enzymes were losing their ability to interesterify. However, it took four more washes for the RM enzyme to produce a hard stock with melting point temperature at 51°C (Figure 50), which was the melting point temperature of the non-interesterified hard stock when the enzymes has completely lost its ability to interesterify. This observation was supported by the changes in the TAG composition as indicated in Table 31 for hard stocks washed with acetone. The TAG composition for the acetone washed hard stock remained consistent within the first six washes as the percentage composition of the PPL and PLO TAGs dropped from 5.2 and 4.0% to 3.4 and 1.3%. The decrease of these medium molecular weight TAGs was associated with the increase in the high molecular weight TAGs.

The change in the results of melting point temperature and TAG composition suggests that as the number of reuses for the enzyme increases for the interesterification reaction, the quality of the hard stock decreases. Acetone was found to be the solvent that could deliver the greatest number of batches of hard stocks.

7.0 Final Discussion

The objective of this project has been met by the formulation of two enzymatically interesterified hard stocks. Rapid crystalizing fats can be formulated from tallow stearin and fully hardened coconut oil interesterified at 70:30% ratio by either enzyme Lipase RM TL (Novozyme) and enzyme Lipase TL IM (Novozyme) with 4% (w/w) of enzymes. Only simple equipments such as jacketed stirer tanks are required for the interesterification reaction when the raw materials are heated to 65 ± 1°C for 8 hours under constant low speed stirring. Palm steain and fully hardened coconut oil at 50:50% ratio can also achieve fast crystallization once interesterified by TL enzyme at 4% (w/w) but has lower melting point at 36°C instead of the required 44°C of the benchmark fat. Further studies were conducted in the second stage of trials on length of time required for the reaction and the amount of enzymes used in order to provide information for optimization during production. It was noted that the TAG composition and melting point for the interesterified hard stock has stopped after 8 hours of reaction with 4% (w/w) of enzymes but can be reduced to 6 hours with the increase of enzyme concentration to 8% (w/w). Reusability trials were conducted in the third stage of experiment in order to maximize the efficiency of the enzyme and to reduce costs for producing these hard stocks. The residual hard stocks can be separated from the enzymes by gentle washing using acetone followed by deionized water and drying. This allows each batch of enzyme to produce a maximum of seven batches of hard stocks before the physical properties of the hard stocks becomes inconsistent.

Rate of crystallization analysis was conducted during the cooling of interesterified hard stocks using low frequency pulsed NMR in order to determine the time required for the fats to crystallize. Differential scanning calorimetry was conducted to provide in depth analysis on the change of heat profile during crystallization of the interesterified hard stock and to also confirm and explain the results obtained from rate of crystallization analysis. Melting point and solid fat content analysis was conducted to monitor physical changes of the interesterified hard stock with different raw material and commercial enzymes. The changes occurred in melting point and solid fat content can be explained by the change in TAG composition using HPLC analysis. HPLC analysis monitors the changes the ratio of high and low molecular weight TAG composition caused by the rearrangement of fatty acids within and with other TAG molecule caused by the commercial lipase enzymes hence explaining the changes in physical characteristics of the hard stock.

7.1 Final Conclusions

The achievements accomplished during this project includes:

- The development of production method for two rapid crystalling hard stocks using tallow stearin and fully hardened coconut oil by enzymatic intereterification with similar physcial properties as the benchmark product

- The development of production method for an alternative hard stock using palm stearin and fully hardened coconut oil with different physical properties as the benchmark hard stock but also a fast crystallizer.

- Investigation of the impact on interesterification reaction with different concentration of enzymes were used and with different length of reaction time

- Determine processing method for reusability of commercial lipase enzymes in batch processing and determine the maximum number of washes possible with different solvents

- Fortifying TAG compositional analysis using HPLC for hard stock analysis and tallow stearin to help understand the physical changes that occurred to the fatty acids arrangement during the interesterification reaction.

7.2 Final Recommendations

Recommendations on future studies includes:

- Investigation of continuous interesterification process for large quantity production.

- Determine the maximum reusability of enzymes in continuous reaction and provide contrast with batch reaction.

- Formulation of more efficient commercial lipase enzyme so smaller quantities can be used during commercial batch production.

- Conduct analysis using x-ray diffraction to investigate the polymorphic changes of the hard stock during reaction.

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Appendix

Appendix A: Minitab output for experimental design

A1 Minitab command output for experimental design

Taguchi Design

```
Taguchi Orthogonal Array Design
L27(3**3)
Factors:  3
Runs:    27
Columns of L27(3**13) Array


1 2 3
```

A2 Key for Minitab result output for experimental design

Output	Enzyme type	Fat mix	Fat ratio
1	Novozyme 435	Tallow stearin/Fully hardened coconut oil	80/20
2	Lipase RM IM	Palm stearin/Fully hardened coconut oil	70/30
3	Lipase TL IM	Tallow stearin/ Palm stearin	50/50

A3 Minitab result output for experimental design

Enzyme type	Fat mix	Fat Ratio

1	1	1
1	1	1
1	1	1
1	2	2
1	2	2
1	2	2
1	3	3
1	3	3
1	3	3
2	1	2
2	1	2
2	1	2
2	2	3
2	2	3
2	2	3
2	3	1
2	3	1
2	3	1
3	1	3
3	1	3
3	1	3
3	2	1
3	2	1
3	2	1
3	3	2
3	3	2
3	3	2

Appendix B: Melting point analysis for fat blending trial

Raw data for all melting point temperature during fat blending trials (Including trial A and duplicate trial B)

B1 Melting point analysis for preliminary trail

Raw material and commercial fats						
Number of trials	Tallow Stearin	Palm Stearin	FHCO	IE 45	Olinera	IPL
1	55	54	36	50	41	44
2	55	54	36	50	41	44
3	55	54	36	50	41	44
4	55	54	37	49	40	44

B2 Melting point analysis for fat blending trial on tallow stearin and fully hardened coconut oil trial A

TS:FHCO(A)			
	80	70	50
Control	55	54	52
RM	47	45	41
TL	47	46	41
Novo	48	44	42

B3 Melting point analysis for fat blending trial on tallow stearin and fully hardened coconut oil trial B

TS:FHCO(B)			
	80	70	50
Control	55	54	52
RM	47	45	41
TL	47	46	41
Novo	48	44	42

B4 Melting point analysis for fat blending trial on tallow stearin and palm stearin trial A

TS:PS(A)			
	80	70	50
Control	54	53	51
RM	50	49	48
TL	51	49	48
Novo	52	51	50

B5 Melting point analysis for fat blending trial on tallow stearin and palm stearin trial B

TS:PS(B)			
	80	70	50
Control	54	53	51
RM	50	49	48
TL	51	49	48
Novo	52	51	50

B6 Melting point analysis for fat blending trial on palm stearin and fully hardened coconut oil trial A

PS:FHCO(A)			
	50	70	80
Control	43	51	52
RM	35	37	42
TL	36	40	42
Novo	35	41	42

B7 Melting point analysis for fat blending trial on palm stearin and fully hardened coconut oil trial B

PS:FHCO(B)			
	50	70	80
Control	43	51	52
RM	35	38	42
TL	36	40	42
Novo	35	40	42

Appendix C HPLC analysis on triglycerides composition (TAG) for fat blending trials

C1 TAG composition on for fat blending trial on tallow stearin and fully hardened coconut oil trial A

Trial A (TS: FHCO)													
ECN		Fat blend 8.2(TS:FHCO)	Fat blend 7.3(TS:FHCO)	Fat blend 5.5(TS:FHCO)	Novo8.2(TSFHCO)	Novo7.3(TSFHCO)	Novo5.5(TSFHCO)	RM8.2(TSFHCO)	RM7.3(TSFHCO)	RM5.5(TSFHCO)	TL8.2(TSFHCO)	TL7.3(TSFHCO)	TL5.5(TSFHCO)
24	CpCpCp				6.3019	4.8346	2.5562	4.6387	4.4937	2.0139	4.8927	4.8257	2.3659
	RT3.797				3.7921		3.6036	4.0427	2.92		4.5463	2.7032	
	RT4.830				3.2685	5.7086	4.9198	3.4967	3.7599		3.8393	2.2358	1.2587
	RT5.868				1.1629	2.5253	2.9322	1.73	2.2938	2.4018	1.7266	1.3967	2.3794
30	CCC	2.0438	2.6402	3.1739	2.5738	4.1668	4.2943	2.6903	3.7587	2.907	2.9131	2.7898	2.6137
	RT9.476							1.054					
32	CCL	6.4139	9.8472	11.3219	3.5472	9.4842	9.7892	3.6018	3.6393	4.7505	3.9531	3.8363	4.6321
	RT10.787							1.0834					5.0304
34	CLL	15.7161	12.7747	10.8147	5.2815	10.5163	9.9412	5.4744	5.6012	4.8175	4.0391	4.8909	4.4574
	RT14.085				2.0468			1.6721				1.1129	3.6209
	RT16.358				2.1168			1.1347	1.6882	4.3582	1.9792		3.7217
36	LLL	12.3126	16.3675	17.2139	9.7949	8.3564	13.933	8.3877	6.7895	5.3792	9.5129	6.2561	5.2081
	RT18.587	2.4169			2.1937			1.2713					1.3372
38	LLM					6.2944				6.3199	1.7823		6.2814
38	LLO	10.2936	14.6449	16.1184	5.3274	12.7498	12.9672	5.7544	6.0948	6.4816	5.876	7.0462	6.1927
38	RT24.425								1.6794	2.314		2.0319	1.3385

40	LMM	6.4832	8.5416	10.1853	5.3791	7.9399	9.2161	5.3173	8.0758	8.2194	5.8577	8.7426	8.9419
40	RT29.581				1.5472			1.3266	1.311		1.9643		
40	RT36.407				1.6328			1.7643	1.6575	1.2613			
40	LMO	4.3192	5.9227	6.7419	6.3327	7.2279	7.5814	6.2848	7.9408	9.0725	6.7414	8.0872	8.8205
	RT40.082									1.104	1.6662		
	RT49.172	2.9186			2.4918			2.6082	3.2954	1.8335	2.5136	2.5305	2.0156
42	LMP				8.7735			9.3265	12.5972	8.8762	8.6374	11.3215	8.9213
44	PLO				2.1647		4.9601	4.247	2.5345	4.3184	2.9876	1.0136	5.2103
44	PPL									2.204			
	RT76.099	2.7639	2.8195	2.7836								4.3694	
48	POO	17.2736	13.5186	10.9733	12.4151	10.1739	6.0026	11.1787	10.3851	11.2029	12.123	12.0229	6.4419
48	POP												
48	PPP	16.8174	12.4483	10.6219	11.6982	9.4905	7.1523	11.5317	9.4528	10.11341	11.7127	10.2977	8.3671
50	POS											2.1638	

C2 TAG composition on for fat blending trial on tallow stearin and fully hardened coconut oil trial B

Trial B (TS: FHCO)													
ECN		Fat blend 8.2(TS:FHCO)	Fat blend 7.3(TS:FHCO)	Fat blend 5.5(TS:FHCO)	Novo8.2(TSFHCO)	Novo7.3(TSFHCO)	Novo5.5(TSFHCO)	RM8.2(TSFHCO)	RM7.3(TSFHCO)	RM5.5(TSFHCO)	TL8.2(TSFHCO)	TL7.3(TSFHCO)	TL5.5(TSFHCO)
24	CpCpCp				6.8249	5.2647	4.3368	4.8849	4.2108	3.6793	5.1926	5.0136	4.6139
	RT3.797				3.9162		3.2153	4.3328		1.2149	4.6637	2.6405	2.7693
	RT4.830				3.2685	6.3627	5.4137	3.3671	4.0297	1.7649	4.2179	2.1679	1.2587
	RT5.868				1.8736	2.321	2.1236	1.3099	2.1339			2.3167	
30	CCC	2.3149	2.7694	3.3919	2.1948	4.1668	4.1084	3.1257	3.5783	3.1149	2.3364	3.1046	
	RT9.476							1.2194					
32	CCL	6.0814	9.2133	10.4919	3.4217	10.3326	7.9609	3.8211	4.6694	6.3824	3.5146	4.2439	6.3948
	RT10.787							1.3629		2.7768			3.2796
34	CLL	14.3629	11.8129	10.1538	5.0619	9.4593	8.7496	5.5481	5.6012	6.8175	4.7169	4.2194	3.7548
	RT14.085				2.1342			2.0367					2.6936
	RT16.358				2.3217			1.0699	2.3126	3.2483			
36	LLL	11.9694	15.4639	16.6423	9.4271	9.3327	7.0219	4.2138	6.6875	5.3211	7.7982	6.7499	6.3897
	RT18.587	2.5931			1.9176			1.5741			2.0874		
38	LLM					5.4647		2.9715		3.2146	2.3794	1.3678	5.0847
38	LLO	11.0934	14.2189	17.3162	5.1034	13.2694	10.2316	4.3988	7.2164	3.2201	5.4679	7.0498	11.3849
38	RT24.425								2.8936			1.2691	2.1316
40	LMM	7.2649	9.694	11.2631	5.0671	7.6437	7.3541	7.3628	9.3697	6.4532	6.1284	8.0303	10.3685
40	RT29.581				2.5425			1.2633					
40	RT36.407				2.5137			1.2637	1.0138	1.7626	2.093		

40	LMO	4.4938	6.5437	7.8316	6.8927	6.3692	9.8864	6.7864	8.4366	11.8469	7.3605	8.0872	8.1039
	RT40.082									2.0638			
	RT49.172	2.7619			3.7619			1.3697	3.3694		2.0637	2.4019	2.0051
42	LMP		2.2691		8.5138		5.3738	8.4493	10.0649	9.3372	8.8491	11.9638	8.7361
44	PLO						3.3849	4.3271	3.213	4.7784	5.2196	2.4527	
44	PPL						4.3219			3.3609			3.2138
	RT76.099	3.1842	2.3329	2.4428								2.3675	
48	POO	17.3196	12.9641	11.1932	11.9196	10.3627	8.2144	11.3917	10.3629	9.2167	12.9153	12.3941	9.1607
48	POP												
48	PPP	15.8149	12.3649	9.0814	10.7618	9.2637	7.3649	12.4166	10.6479	9.4198	12.5879	11.7639	8.2394

C3 TAG composition on for fat blending trial on tallow stearin and palm trial A

Trials A (TS:PS)													
		Fat blend8.2(TSPS)	Fat blend7.3(TSPS)	Fat blend5.5(TSPS)	Novo8.2(TSPS)	Novo7.3(TSPS)	Novo5.5(TSPS)	RM8.2(TSPS)	RM7.3(TSPS)	RM5.5(TSPS)	TL8.2(TSPS)	TL7.3(TSPS)	TL5.5(TSPS)
24	CpCpCp				7.0003	10.9136	10.9017	7.1659	10.9136	10.9017	8.2723	9.3739	10.2319
	RT4.642				4.2147	4.1619	4.0217	4.3161	4.1726	3.8216	4.5801	4.0103	3.8472
30	CCC	1.2369	1.1608	1.6963									
32	CCL	2.9827	4.0183	6.5954	3.4681	5.9216	7.2185	4.2129	5.3184	7.4319	2.7849	4.1508	6.8197
34	CLL	4.9146	6.2773	8.7247	5.2164	7.0132	9.1704	5.8316	7.7269	9.0178	5.4249	7.3908	9.2117
	RT13.591					3.019	3.6418		3.5127	3.8176		3.6825	3.5185
36	LLL	4.759	6.3191	9.7737	9.0642	9.1738	9.1614	9.1627	9.1624	9.0419	9.0622	9.0443	9.1627
38	LLM	3.3955	4.6884	7.1969	3.6182	3.3629	3.0174	3.6182	3.3629	3.8517	3.401	2.6629	2.4684
40	LLO				3.2085	3.4219	3.9137	3.4019	3.6185	3.8624	3.6698	3.9102	3.7739
40	LMM	4.5424	4.6326	5.5228									
42	LMO	1.7687	2.4224	3.9472									
42	LMP												
44	PLO	4.362	3.7059	2.6974									
44	PPL	4.8811	4.6057	3.1672	3.0819	3.1129	3.2681	3.2176	3.1074	3.3185	3.1718	3.0794	3.2185
	RT82.502	4.1679	5.5133	4.267									
48	POO	12.5613	9.5744	7.4757	8.6513	7.2351	5.2219	7.8219	5.1638	4.7928	7.402	5.2164	4.2176
48	POP	23.3649	22.1911	15.7107	10.3019	8.1362	7.0391	9.7638	7.9435	6.7429	9.5731	9.2392	7.2188
48	PPP	26.8206	24.1528	20.0245	12.9615	12.0151	11.6017	13.1614	11.2164	9.5637	12.7938	12.4142	10.7316
52	PSS			2.6203	28.9317	22.4619	21.4371	28.2477	24.7319	22.9739	29.7709	24.9134	24.7139

C4 TAG composition on for fat blending trial on tallow stearin and palm trial B

Trials B (TS:PS)													
		Fat blend8.2(TSPS)	Fat blend7.3(TSPS)	Fat blend5.5(TSPS)	Novo8.2(TSPS)	Novo7.3(TSPS)	Novo5.5(TSPS)	RM8.2(TSPS)	RM7.3(TSPS)	RM5.5(TSPS)	TL8.2(TSPS)	TL7.3(TSPS)	TL5.5(TSPS)
24	CpCpCp	Fat blend8.2(TSPS)	Fat blend7.3(TSPS)	Fat blend5.5(TSPS)	Novo8.2(TSPS)	Novo7.3(TSPS)	Novo5.5(TSPS)	RM8.2(TSPS)	RM7.3(TSPS)	RM5.5(TSPS)	TL8.2(TSPS)	TL7.3(TSPS)	TL5.5(TSPS)
	RT4.642				6.3168	8.0269	10.1735	7.3624	8.2649	9.3476	7.6219	8.8436	10.2168
30	CCC				4.1823	5.2196	4.6132	4.7916	4.9618	4.6193	4.6821	4.1024	4.1732
32	CCL	1.4296	1.3517	2.0184									
34	CLL	3.1526	3.2106	6.9136	3.1628	5.5196	6.3314	3.4928	5.9318	7.8931	3.5129	4.7219	7.2168
	RT13.591	4.6257	5.9184	7.9618	6.2184	7.316	8.9417	6.2719	7.4129	9.8316	6.0194	7.7419	9.3142
36	LLL					2.3194	2.4579		3.1528	3.3619		3.2148	3.4217
38	LLM	4.2618	6.4816	8.6617	9.1736	8.9276	9.0019	9.0816	9.0419	9.1429	9.1637	9.1438	9.0637
40	LLO	3.162	4.9216	6.3729	3.8216	3.0419	3.7615	3.4619	3.1629	3.3157	3.2069	3.0168	3.5164
40	LMM				3.0884	3.4168	3.7627	3.1029	3.4219	3.6127	3.5429	3.1836	3.7739
42	LMO	4.2213	4.8109	4.9316									
42	LMP	1.4206	2.1063	3.1409									
44	PLO												
44	PPL	3.2416	3.806	3.2617									
	RT82.502	4.1584	4.8019	3.3219	3.1625	3.0219	3.8216	3.0184	2.9618	3.0816	3.08216	3.1248	3.3281
48	POO	4.0629	5.3918	4.0162									
48	POP	13.9217	9.7613	7.0419	8.4158	7.8628	6.4173	8.2149	7.38149	5.0814	7.9264	7.1495	5.9416
48	PPP	24.1699	22.0819	19.3373	10.2618	8.9662	7.4038	10.2164	8.2136	7.6294	10.3814	8.9639	7.0138
52	PSS	27.4365	25.0613	22.1628	12.6718	11.4019	10.2617	12.3815	11.7184	10.6195	12.0637	11.6219	10.8416

C5 TAG composition on for fat blending trial on palm trial and fully hardened coconut oil trial A

Trials A (PS:FHCO)													
		Fat blend8.2(PSFHCO)	Fat blend7.3(PSFHCO)	Fat blend 5.5(PSFHCO)	Novo8.2(PSFHCO)	Novo7.3(PSFHCO)	Novo5.5(PSFHCO)	RM8.2(PSFHCO)	RM7.3(PSFHCO)	RM5.5(PSFHCO)	TL8.2(PSFHCO)	TL7.3(PSFHCO)	TL5.5(PSFHCO)
24	CpCpCp			3.8329	5.00375	4.368	3.3414	3.97795	4.3039	3.35235	3.92855	4.0425	3.29615
	RT5.295					1.2638				1.3073			1.68905
	RT6.314	1.1144	1.0723	2.3368	1.0131	1.4392	1.0638	1.2136	1.6728		1.2164	1.7749	2.12635
	RT6.593			1.75855	1.1367			1.4457		1.89365	1.585		
30	CCC	1.3681	1.2218	3.22755	2.7034	4.1432	3.10805	2.81905	2.70295	3.508	2.0352	2.8852	3.0867
	RT8.903												
32	CCL	3.56915	4.7046	8.6286	2.9518	2.917	3.2063	3.0811	3.4375	2.1639	3.1766	2.57	2.85545
	CLL	5.2897	6.2174	8.4665	4.17735	4.2789	4.27095	4.2724	4.53575	4.5763	3.9859	4.07605	4.73055
34	RT10.767				1.3187	3.6889	2.0687	1.27045	2.1681	2.1638	1.1279	1.2684	
	RT13.561				4.4076	1.2408	3.4034			2.7146			1.8871
	LLL	4.15525	5.544	8.7849	1.8417	3.6003	3.79965	1.9304	3.6131	3.89225	2.10455	2.77635	4.152
36	RT17.339	1.477	1.449	1.5709			1.89355			1.88785		1.2577	2.525
	LLM	3.1155	3.38265	4.4869	3.4433	3.093	5.165	3.54565	3.0539	3.7968	3.4693	2.58735	5.9197
38	RT24.987	1.55285	1.735	4.3602	1.1835	1.99625	3.9799	2.31245	2.0277	4.28595	2.4355	2.158	4.4502
38	LLO	3.1692	3.90185	4.72695	3.8718	4.134	7.80955	3.1155	3.7974	8.0017	3.90035	3.86115	7.3542
38	RT25.255			2.0614	2.5467	1.2889	1.66355	2.61105	1.5907	1.3694	2.6386		
40	RT30.674				2.23985	2.1167	1.7567	2.7451	2.23475	2.7669	1.8409	1.7523	1.8112

40	LMM	3.2047	4.29695	5.26745	3.3249	3.9224	6.5137	3.54925	3.5901	6.2208	3.61765	3.37725	6.6762
40	RT35.543			1.4653	2.11005			2.04535	1.29865		2.06755	2.37225	3.4165
40	RT41.139	1.0471	1.19845			1.2074		1.3464	3.1953		1.3193	2.51655	
	LMO	2.23115	2.5731	3.8104	6.6881	3.9244	5.30655	6.86295	3.8645	6.18545	6.5246	7.2428	5.2382
	LMP				6.4742	5.3413	8.4051	7.98455	7.25655	9.0715	8.34705	8.30275	8.11635
42	PLO	4.1766	3.63025	2.5819	4.8078	5.21195	3.4396	3.73015	6.8698	3.8792	5.6998	4.32915	2.6183
44	PPL	4.99285	4.3939	2.94835	6.9122	3.8397	6.0799	4.9651	3.8444	5.13025	6.5393	4.0247	4.6839
44	RT60.247	2.07475	2.65535	2.1307	2.7534	4.4463			6.2103			4.397	
	RT63.007		1.4163	2.9232	5.3729	4.7364		4.8173	5.8722		6.7916	4.29565	
48	POO	11.1719	10.12155	6.05655	6.33625	5.6717	3.79205	6.56255	6.13895	3.9484	6.353	5.54445	3.936
48	POP	24.5533	21.45705	15.6807	15.03825	11.5697	10.50875	16.5184	10.83435	11.2278	14.6384	11.9243	10.89115
48	PPP	22.04065	20.10625	14.84585	12.86235	10.8474	9.2775	12.3153	11.2367	9.00345	12.4809	10.9238	9.44245
50	POS												
52	PSS		3.0701	2.965		6.3527			6.3089	4.6455		5.8942	

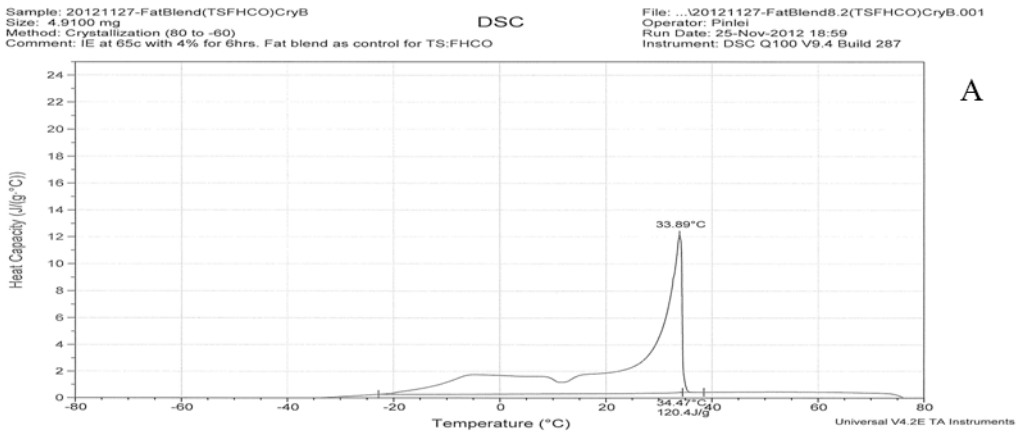
C6 TAG composition on for fat blending trial on palm trial and fully hardened coconut oil trial B

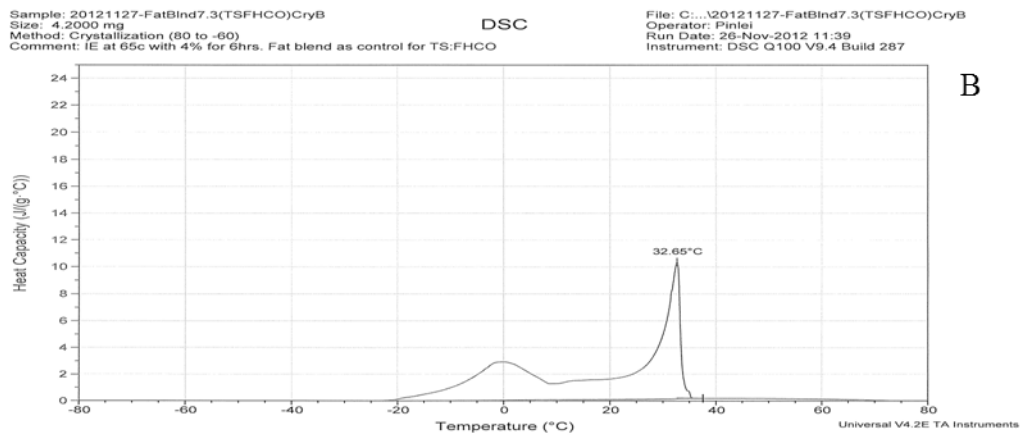
Trials B (PS:FHCO)													
		Fat blend8.2(PSFHCO)	Fat blend7.3(PSFHCO)	Fat blend 5.5(PSFHCO)	Novo8.2(PSFHCO)	Novo7.3(PSFHCO)	Novo5.5(PSFHCO)	RM8.2(PSFHCO)	RM7.3(PSFHCO)	RM5.5(PSFHCO)	TL8.2(PSFHCO)	TL7.3(PSFHCO)	TL5.5(PSFHCO)
24	CpCpCp			0	0.00995	0.4455	0.8751	0.81515	0.3134	0.59905	1.46765	0.7334	1.09555
	RT5.295					0				0			0.47475
	RT6.314	0.0313	0.0414	0	0	0	0	0	0		0	0	0.76095
	RT6.593			0.56115	0			0		0.82995	0.2171		
30	CCC	0.0745	0.2043	1.17945	0.0615	0	0.10165	0.05595	0.51465	0.4657	0.6067	0.713	0.4922
	RT8.903												
32	CCL	0.14125	0.5591	0.1591	0.5901	0.6988	0.083	0.1353	0.4454	0	0.6702	0.0981	0.50405
	CLL	0.2026	0.1035	0.5292	0.08455	2.0362	1.94265	0.0457	0.18425	1.2161	0.3057	0.53585	1.48685
34	RT10.767				0	0	0.8606	0.13235	0	0	0.1063	0	
	RT13.561				0	0	0.1855			0			0
	LLL	0.18265	0.8951	0.4319	0.39	1.2868	0.41395	0.0131	0.6588	0.50055	0.08485	0.49345	0.0136
36	RT17.339	0.0954	0	0			0.67765			0.17405		0.0277	0.4371
	LLM	0.154	0.86925	2.1252	0	0.272	0	0.58375	0.7385	0	0.2526	0.52945	0.7033
38	RT24.987	0.19005	0.5999	0	0	0.67265	1.2128	0.90425	0.2881	1.49325	0.9263	0.2457	0.5224
38	LLO	0	0.46375	0.68885	1.4299	0.2028	0.15855	0.8313	0.529	0.5929	0.81335	0.35275	0.9987
38	RT25.255			0	0	0.0183	0.36805	0.59775	0	0	0.2618		
40	RT30.674				0.02125	0.0518	0.5929	0	0.09535	0.609	0.6272	0.4168	0.5506

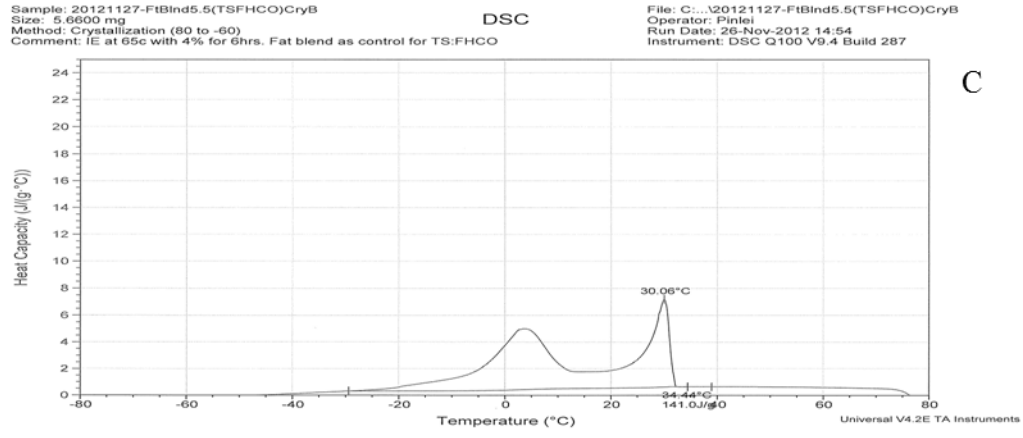
40	LMM	0.1232	1.03595	0.10515	0.2167	0.45	0.2818	0.37605	0.0444	0.7507	0.40095	0.38665	0.0974
40	RT35.543			0	0.47795			0.28155	0.02995		0.09975	1.23535	1.4996
40	RT41.139	0	0.09215			0.1255		0	1.0217		0	1.05085	
	LMO	0.04045	0	0	0.1444	0.6883	0.26275	0.75215	0.6886	0.39255	0.9278	0.5	0.2408
	LMP				0.8525	2.0497	0.351	0.76765	0.15835	0.1398	0.70335	1.08825	0.52235
42	PLO	0.1047	0.00235	0	0.4419	0.87505	0.2229	0.58745	0.534	0.2038	0.2367	1.33455	0.5461
44	PPL	0.17905	0.135	1.77335	0.119	0.5531	0.1126	1.7484	0.0265	0.15345	0	0	0.1134
44	RT60.247	0.06005	0.32025	0	0	1.1795			0			1.3668	
	RT63.007		0	0	0	0		0	0		0	1.03395	
48	POO	0.87	1.12955	0.38495	0.01945	0.6538	0.57465	0.00935	0.74465	0.0767	0.5319	0.22915	0.3803
48	POP	0.3885	0.06935	0.3188	0.82185	0.207	1.61085	1.4787	0.13305	1.2341	1.3608	0.1073	0.27675
48	PPP	0.12125	0.25515	0.01155	1.54065	0.5179	0.9596	1.5774	0	0.95385	1.3273	0.2419	0.87715
50	POS												
52	PSS		0	0		0			0	0		0	

Appendix D DSC crystallization curve

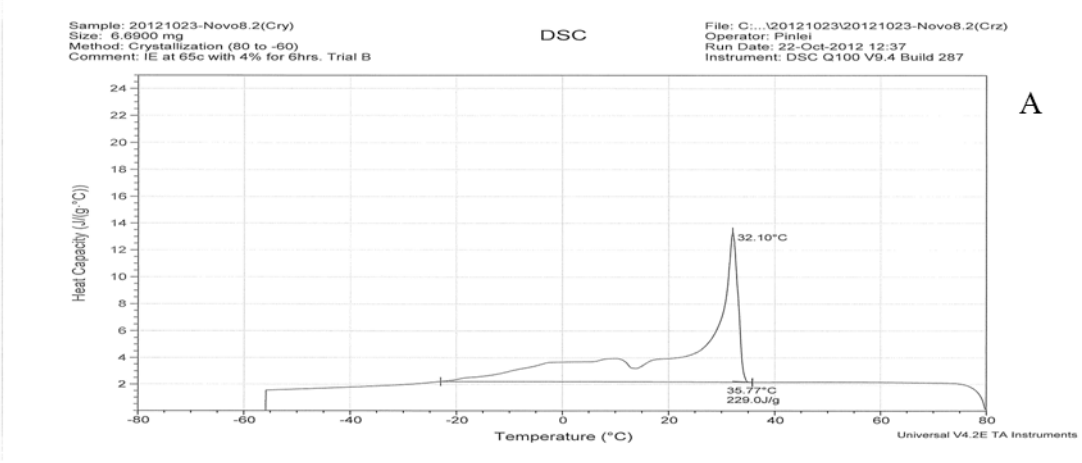
D1 DSC crystallization curve for fat blending trial on tallow stearin and fully hardened coconut oil on non-interesterified solid fat blends trial B

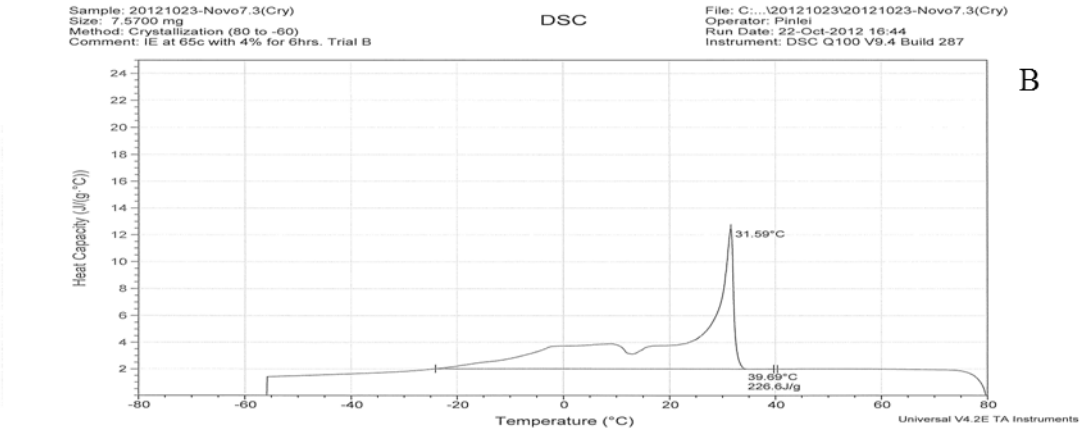


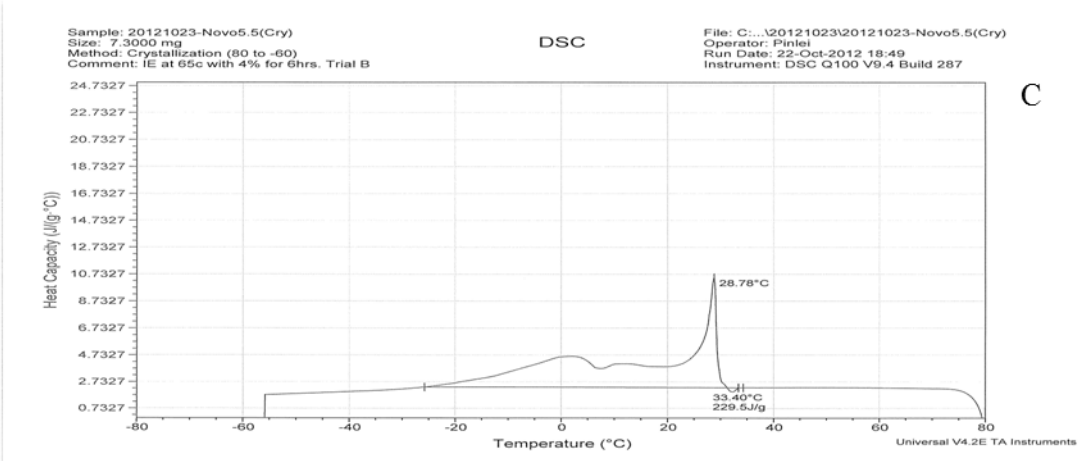




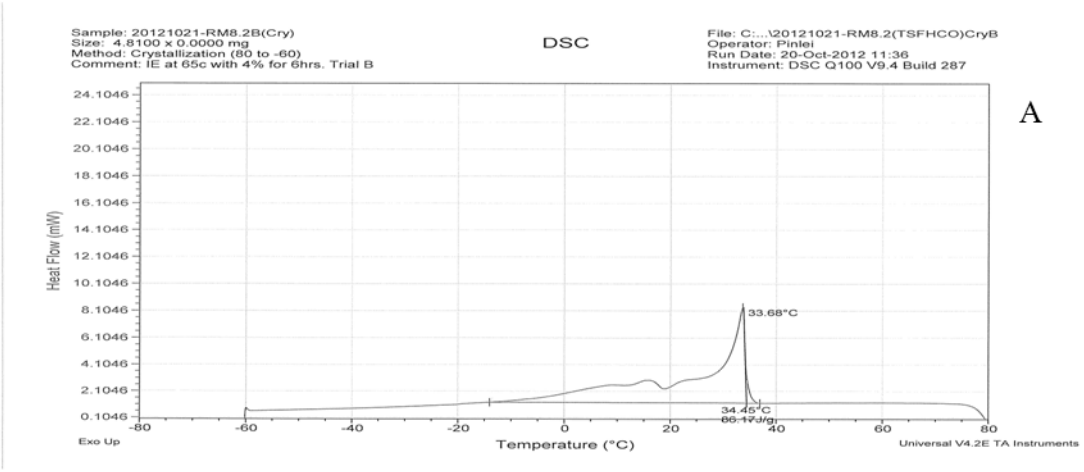
D2 DSC crystallization curve for fat blending trial on tallow stearin and fully hardened coconut oil on Novozyme 435 lipase enzyme interesterified fats trial B

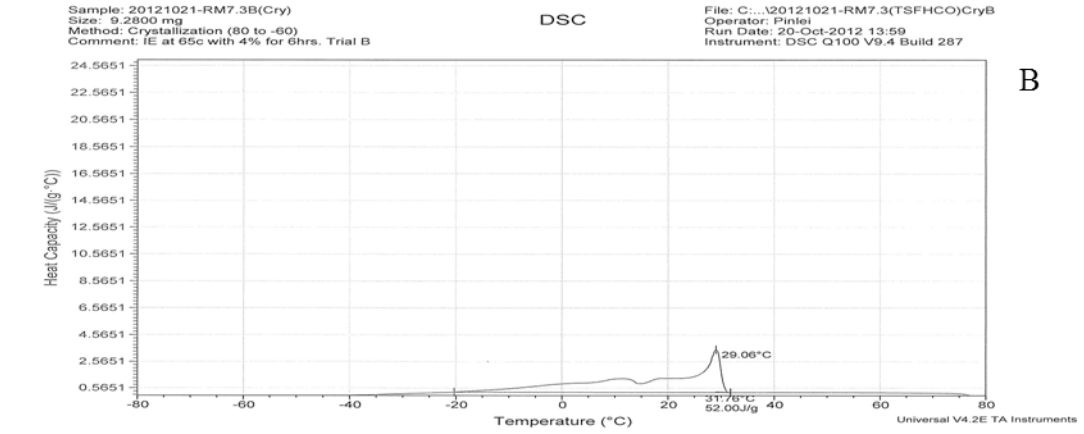


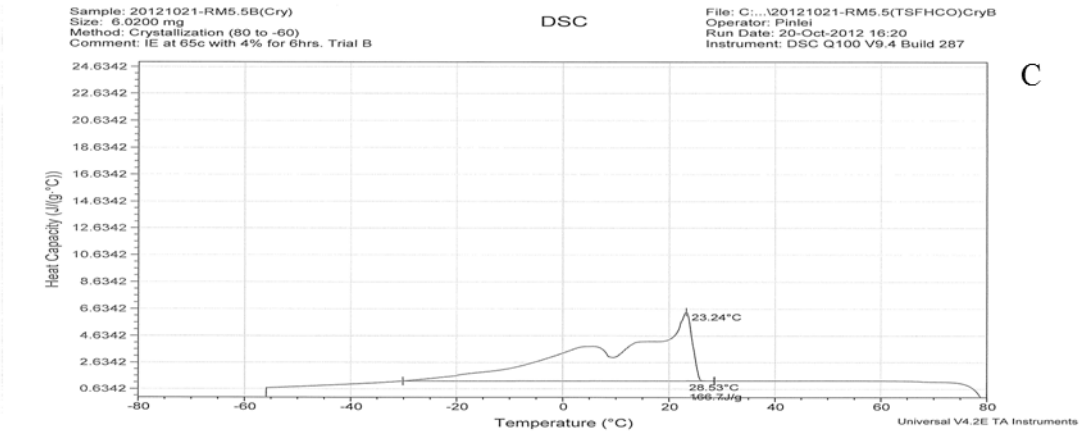




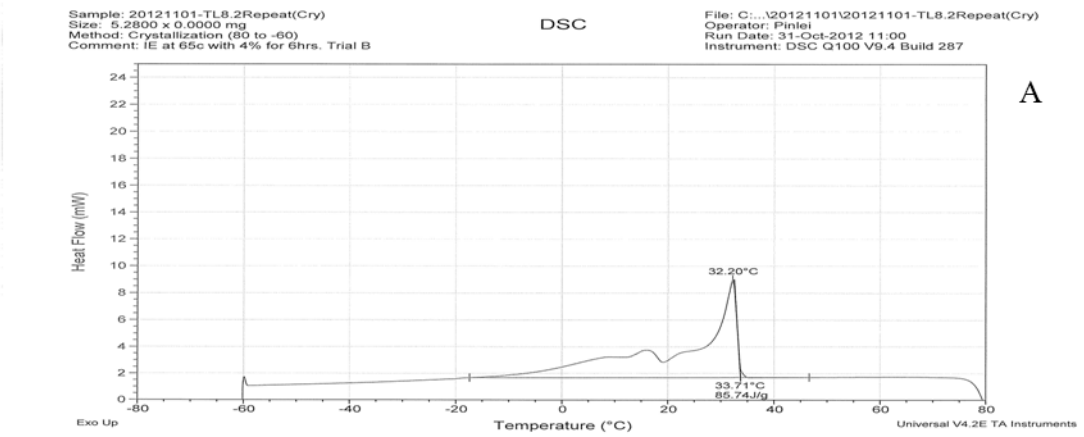
D3 DSC crystallization curve for fat blending trial on tallow stearin and fully hardened coconut oil on Lipozyme RM IM lipase enzyme interesterified fats trial B

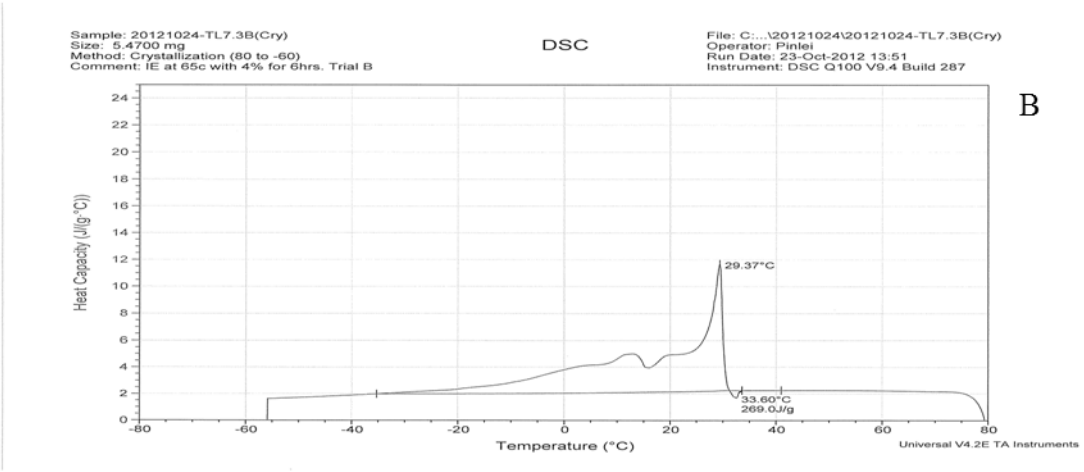


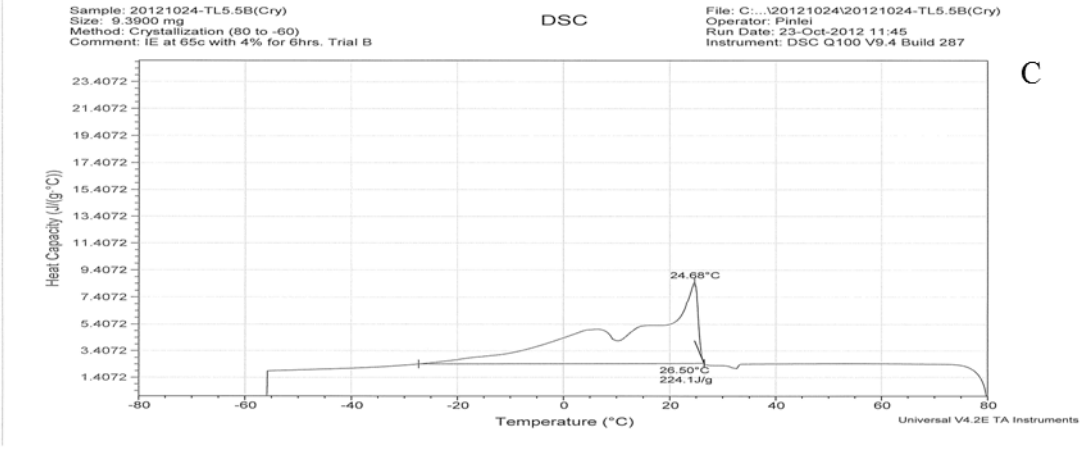




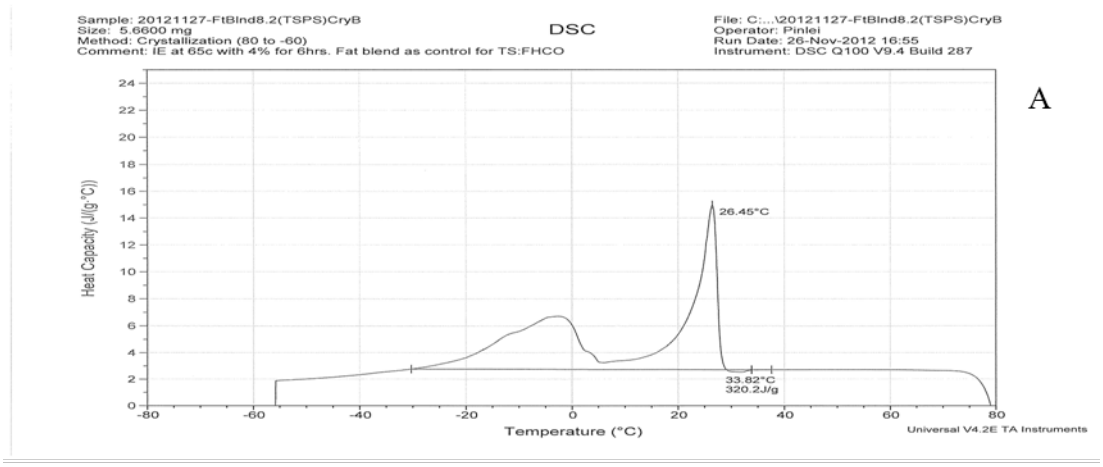
D4 DSC crystallization curve for fat blending trial on tallow stearin and fully hardened coconut oil on Lipozyme TL IM lipase enzyme interesterified fats trial B

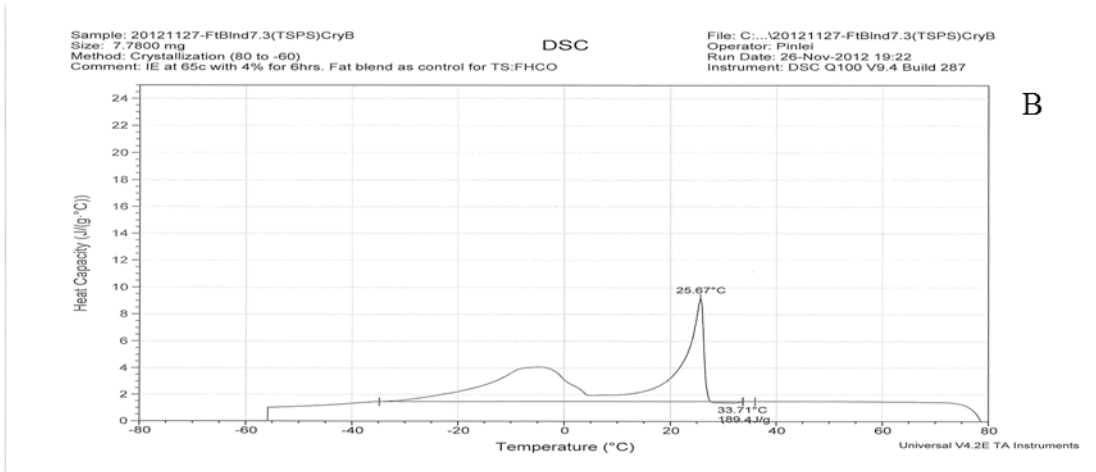


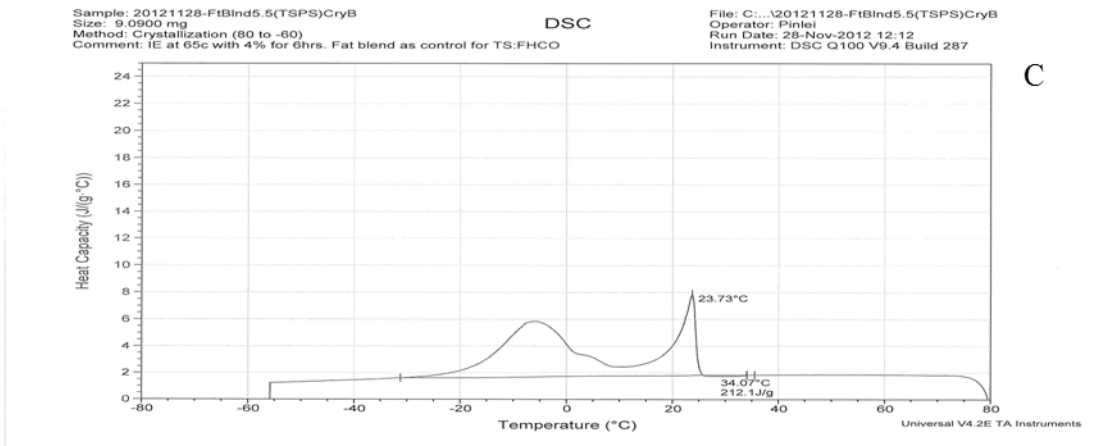




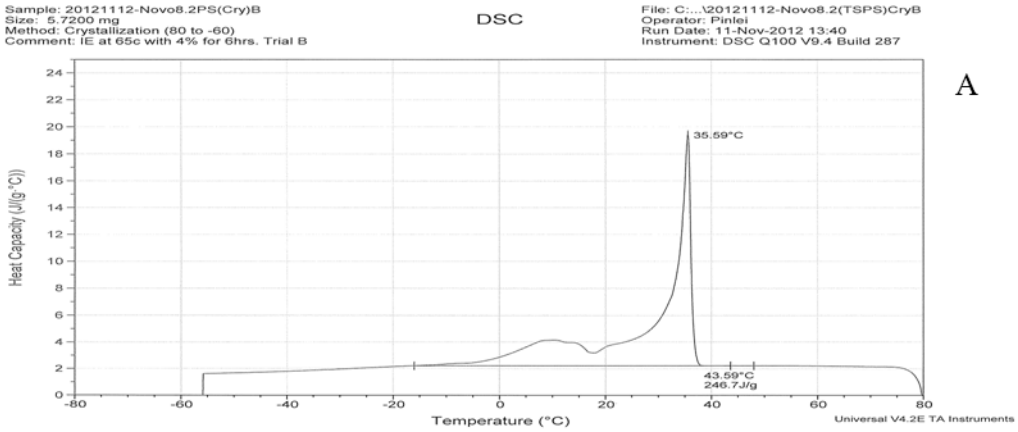
D4 DSC crystallization curve for fat blending trial on tallow stearin and palm stearin on non-interesterified solid fat blends trial B

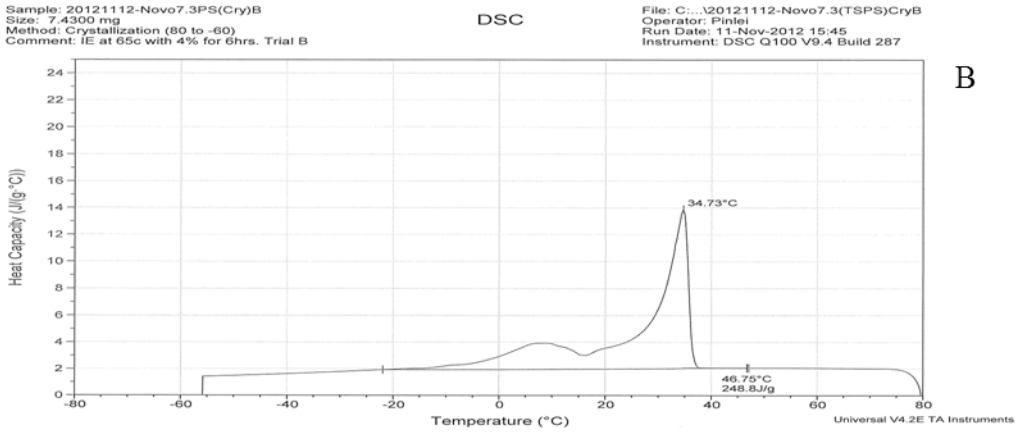


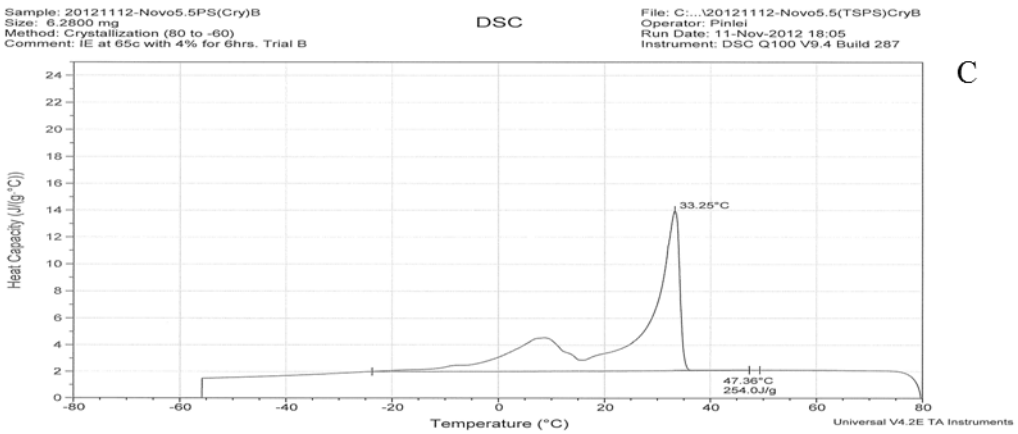




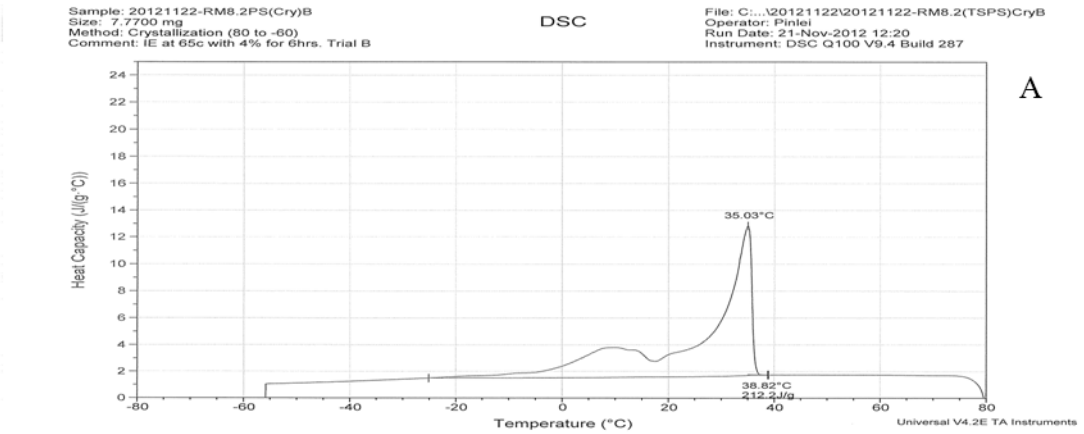
D5 DSC crystallization curve for fat blending trial on tallow stearin and palm stearin on Novozyme 435 lipase enzyme interesterified fats trial B

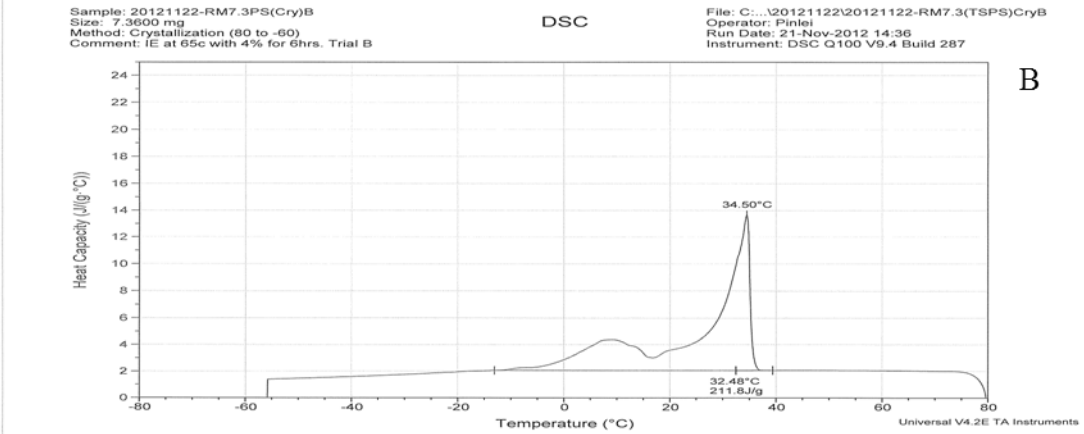


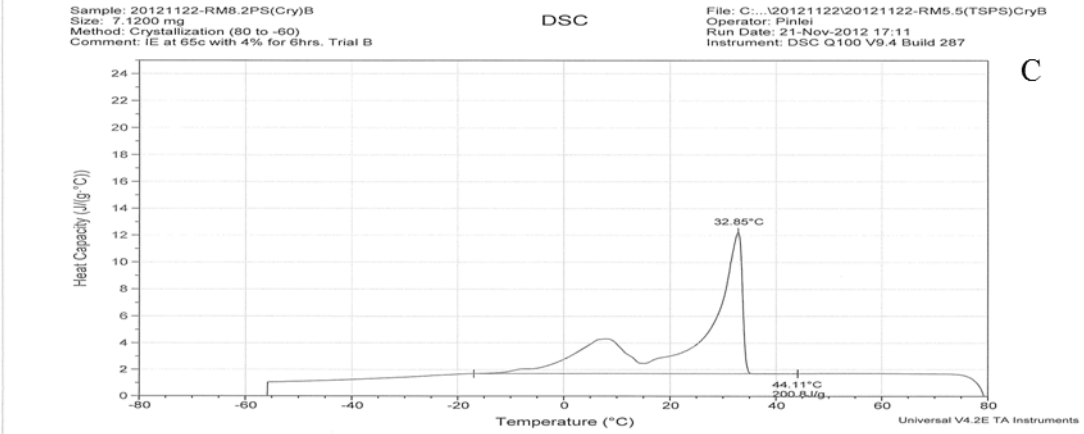




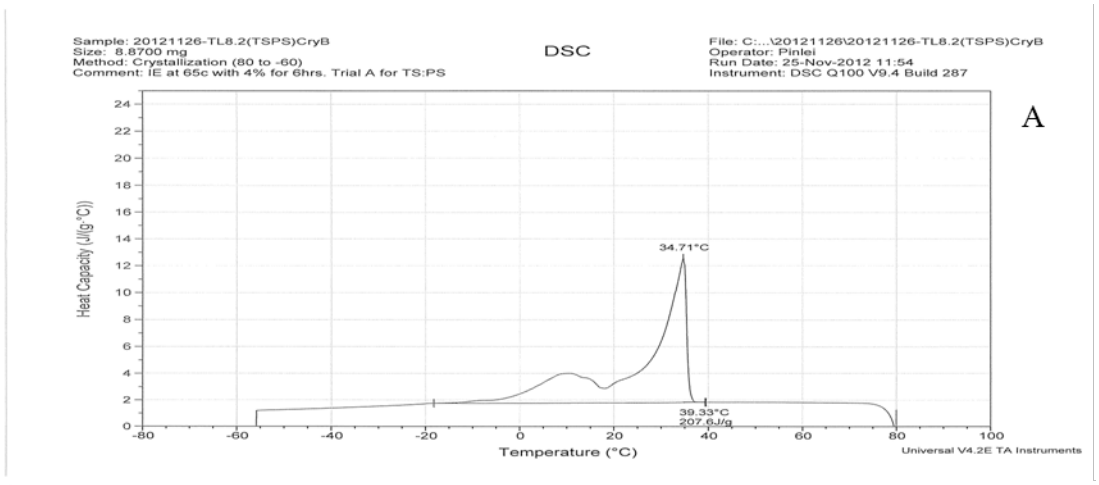
D6 DSC crystallization curve for fat blending trial on tallow stearin and palm stearin on Lipozyme RM IM lipase enzyme interesterified fats trial B

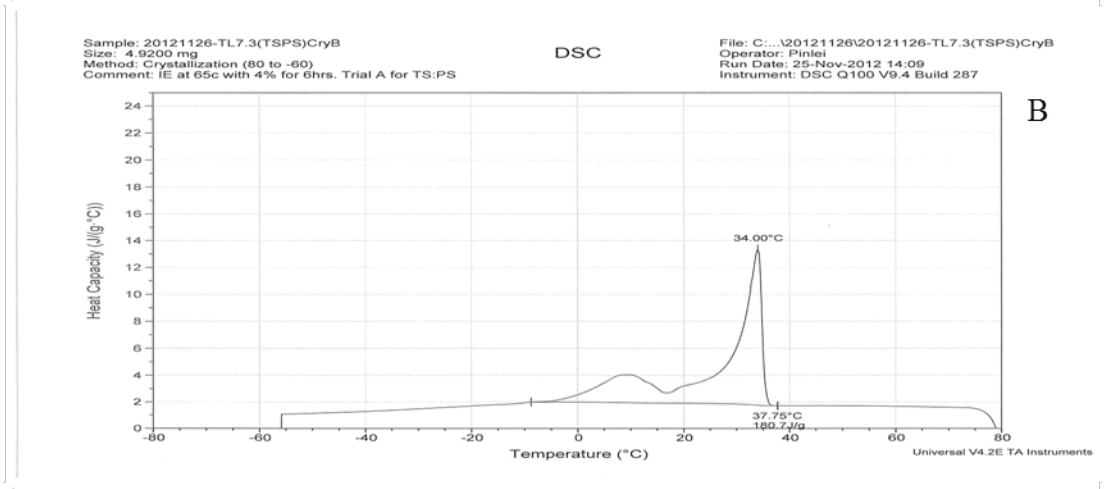


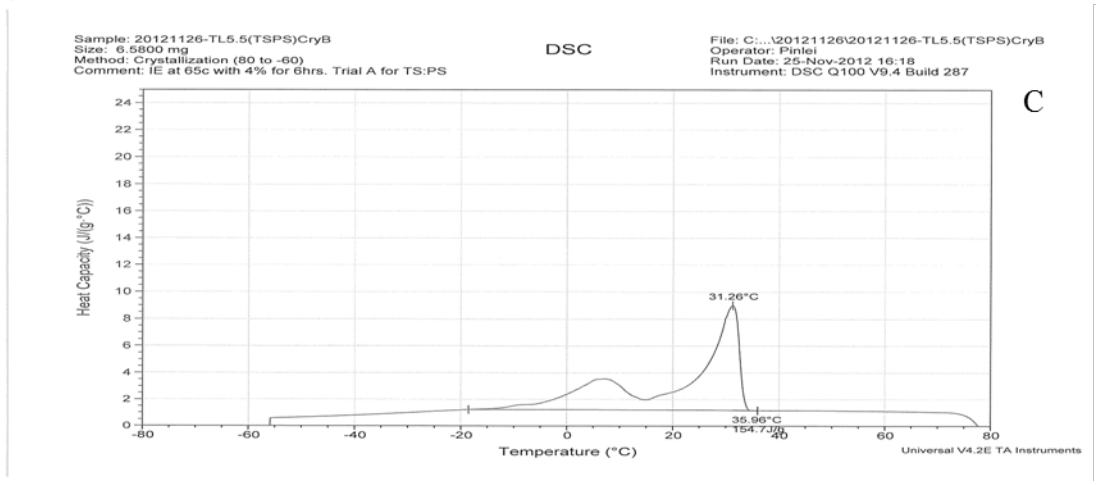




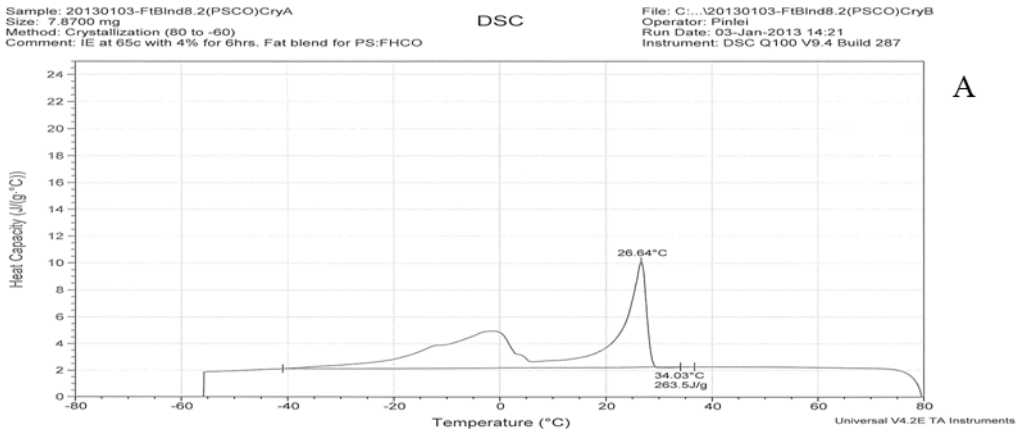
D7 DSC crystallization curve for fat blending trial on tallow stearin and palm stearin on Lipozyme TL IM lipase enzyme interesterified fats trial B

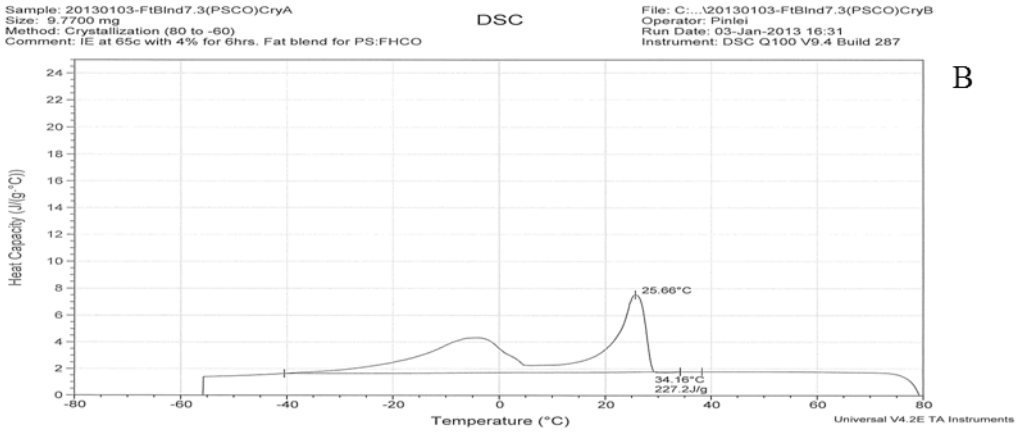


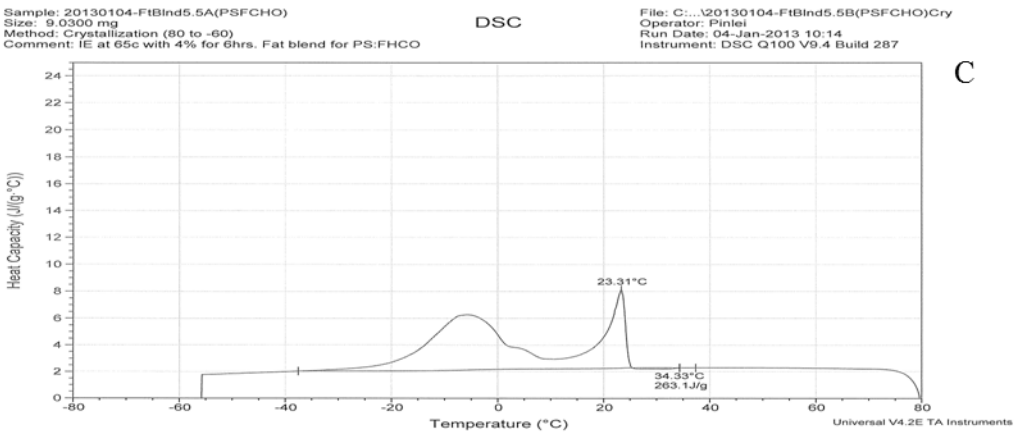




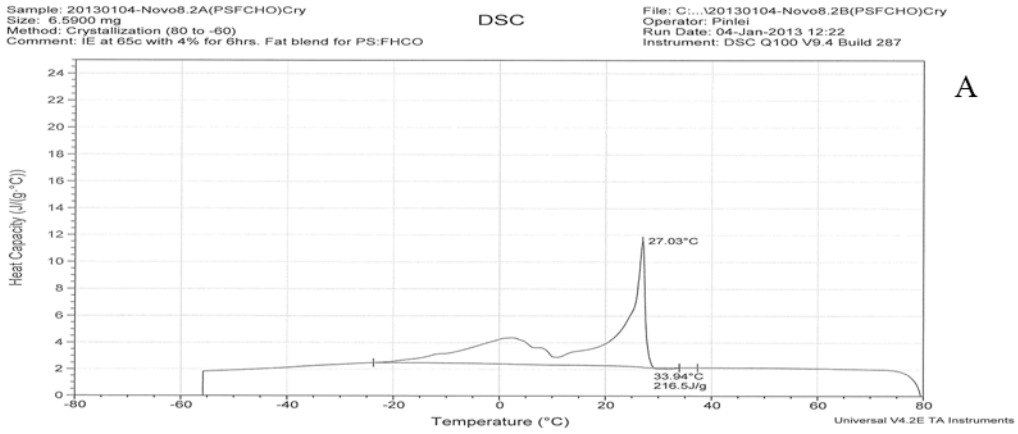
D8 DSC crystallization curve for fat blending trial on palm stearin and fully hardened coconut oil on non-interesterified fats trial B

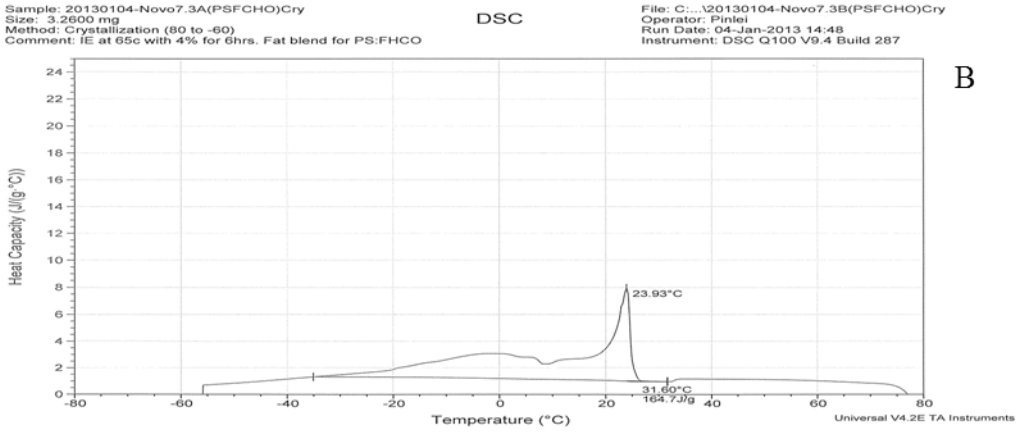


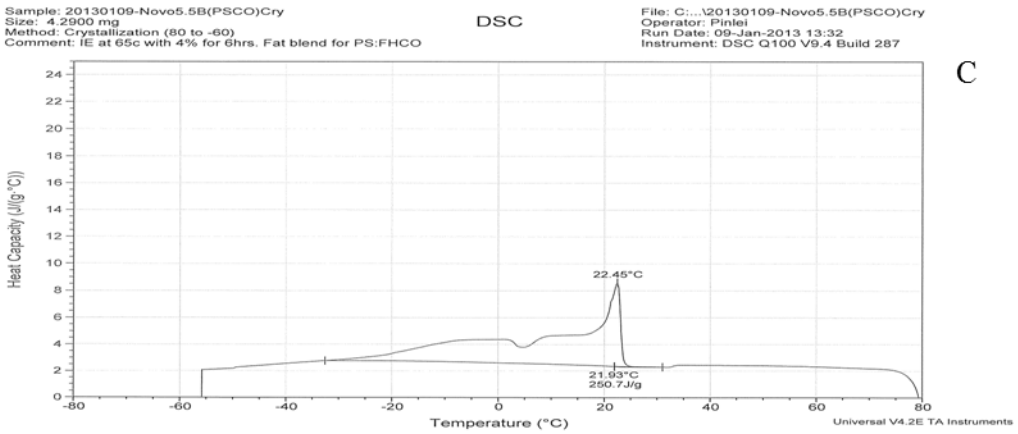




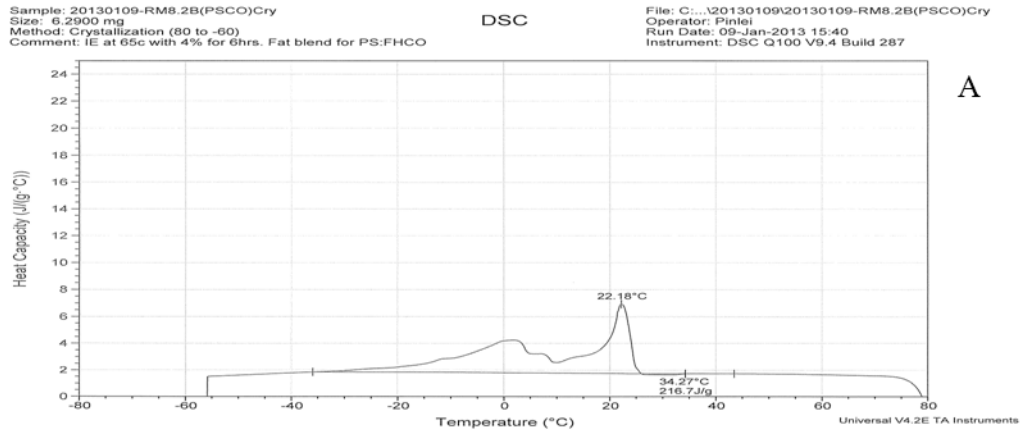
D9 DSC crystallization curve for fat blending trial palm stearin and fully hardened coconut oil on Novozyme 435 lipase enzyme interesterified fats trial B

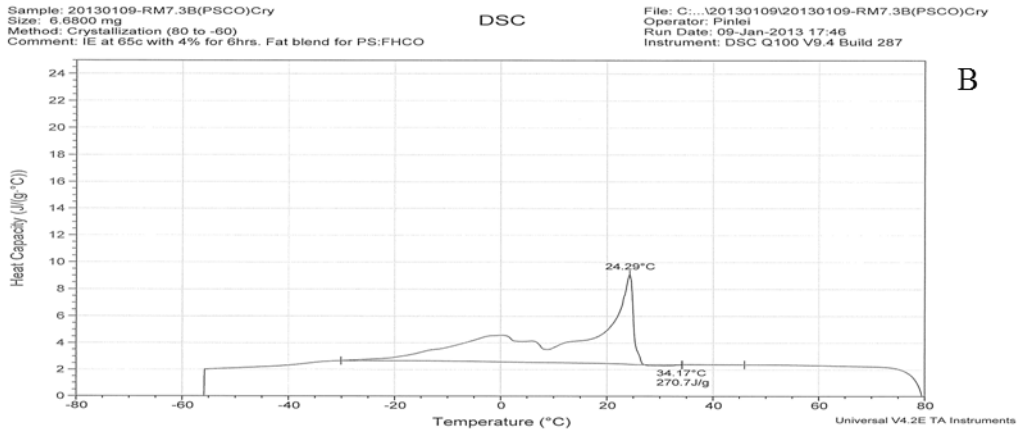


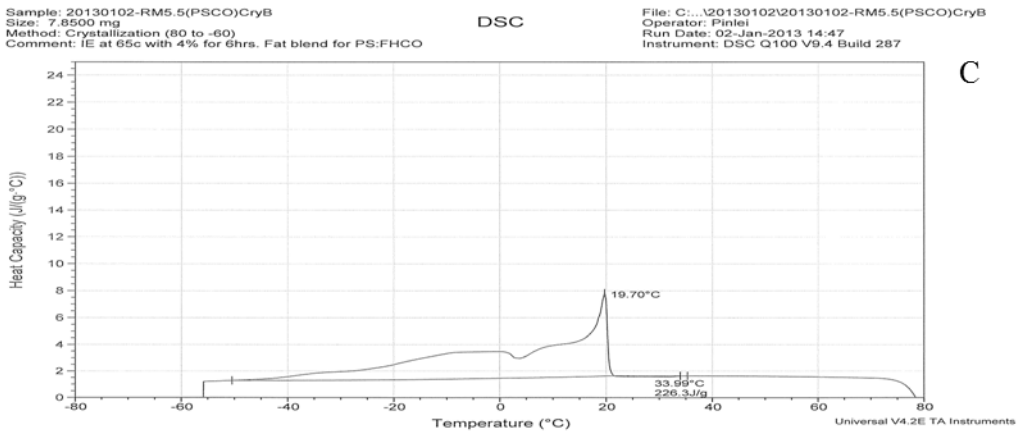




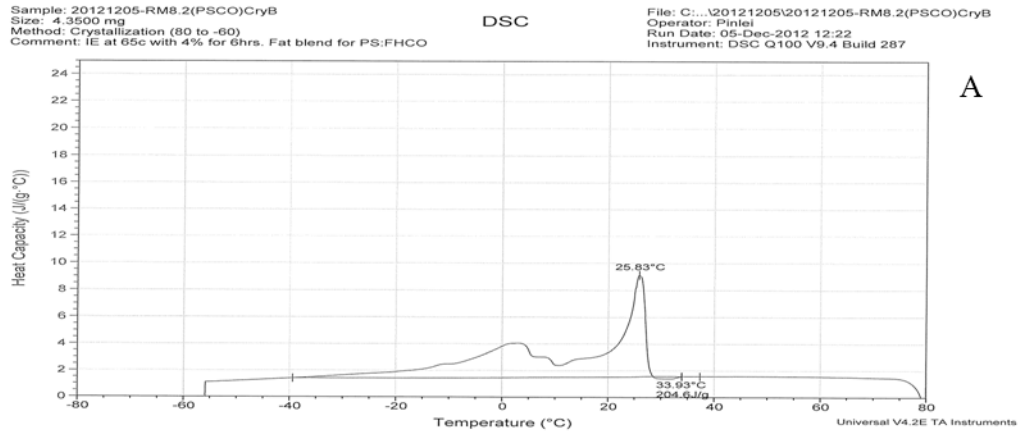
D10 DSC crystallization curve for fat blending trial palm stearin and fully hardened coconut oil on RM IM lipase enzyme interesterified fats trial B

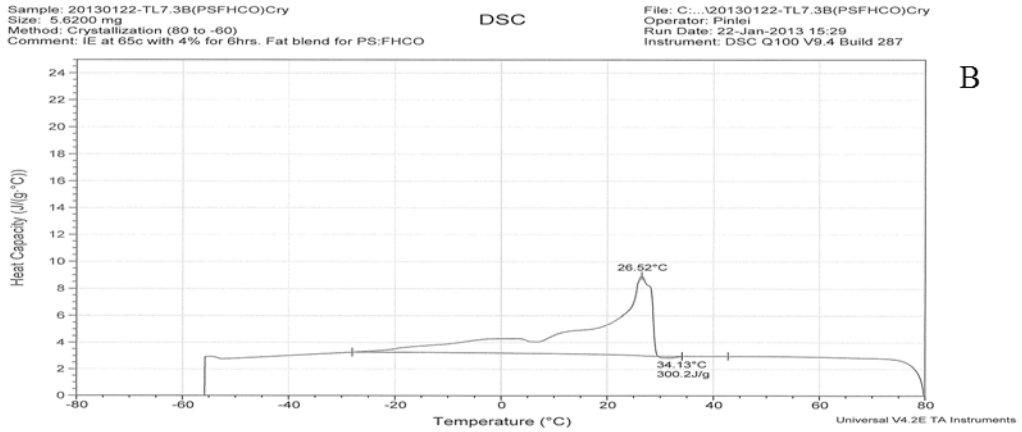


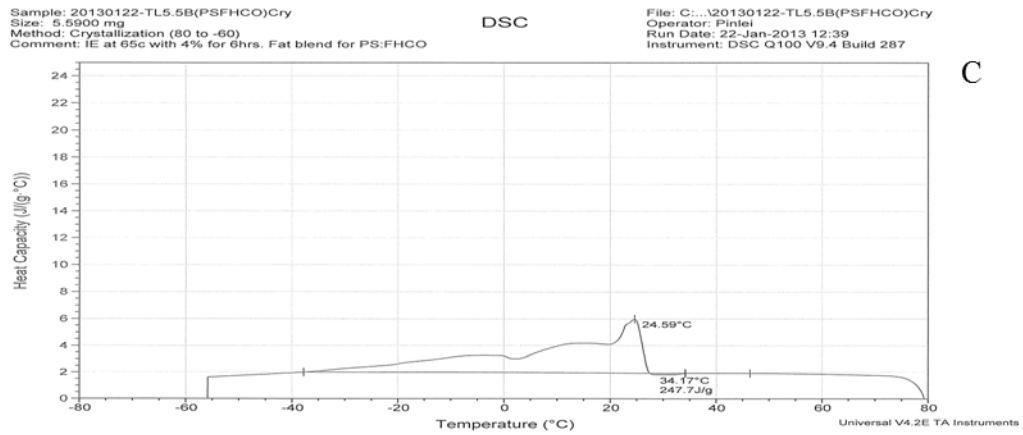




D11 DSC crystallization curve for fat blending trial palm stearin and fully hardened coconut oil on TL IM lipase enzyme interesterified fats trial B







Appendix E Solid fat content on fat blending trial

E1 Solid fat content on fat blending trial for tallow stearin and fully hardened coconut oil trial B

TS:FHCO(B)	0°C	10°C	20°C	30°C	40°C
Control8.2	93.13	90.73	80.94	63.75	42.41
Control7.3	93.25	91.36	75.75	55.81	34.31
Control5.5	92.76	89.94	67.54	40.15	21.75
Novo8.2	91.36	86.94	77.21	54.3	28.02
Novo7.3	91.56	87.72	74.42	48.35	20.97
Novo5.5	89.62	84.33	61.81	31.17	9.54
RM8.2	92.18	88.14	79.38	54.54	22.89
RM7.3	92.96	89.48	76.11	46.66	15.42
RM5.5	92.31	88.3	67.31	31.93	5.84
TL8.2	92.43	88.83	80.74	55.3	22.88
TL7.3	92.18	87.56		45.11	12.82
TL5.5	91.47	87.61	65.33	29.24	3.38

E2 Solid fat content on fat blending trial for tallow stearin and palm stearin trial B

TS:PS (B)	0°C	10°C	20°C	30°C	40°C
Control8.2	0.14	0.25	1.18	1.205	0.96
Control7.3	0.18	0.225	0.145	0.275	0.47
Control5.5	0.1	0.16	0.05	0.2	0.5
Novo8.2	0.57	1.05	0.925	0.505	0.135
Novo7.3	0.67	0.875	1.185	0.75	2.595
Novo5.5	1.45	0.36	1.01	0.225	0.1
RM8.2	0.46	0.79	0.835	1.145	0.515
RM7.3	0.605	1.225	1.38	2.255	0.44
RM5.5	0.08	0.405	0.13	0.06	0.175
TL8.2	0.745	0.725	3.205	4.925	2.83
TL7.3	0.38	0.615	0.625	0.91	0.4
TL5.5	0.14	0.65	1.62	0.955	0.14

E3 Solid fat content on fat blending trial for palm stearin and fully hardened coconut oil trial B

PS:FHCO(B)	0°C	10°C	20°C	30°C	40°C
Control8.2	86.16	78.69	59.06	38.42	19.49
Control7.3	85.92	78.94	52.6	31.8	14.28
Control5.5	88.54	81.8	46.7	23.21	8.32
Novo8.2	79.62	73.78	52.45	29.47	10.55
Novo7.3	79.09	71.59	46.67	24.02	5.27
Novo5.5	78.28	72.46	42.57	16.8	-0.14
RM8.2	82.64	76.3	51.11	26.09	6.05
RM7.3	81.41	73.18	45.78	21	1.39
RM5.5	82.58	74.39	41.39	13.61	-0.14
TL8.2	82.96	74.64	51.48	27.07	5.81
TL7.3	81.92	74.75	48.92	23.54	2.77
TL5.5	78.74	70.68	41.26	15.13	0.3

Appendix F Time for crystallization on fat blending trials

F1 Time for crystallization on fat blending trials with tallow stearin and fully hardened coconut oil trial B

Time for crystallization																			
TS:FH CO	Time (Minute)																		
	0	1	2	3	4	6	8	10	12	14	16	18	20	30	40	50	60	70	80
Control 8.2	0.1	66.2	66.6	67.2	67.6	83.1	85.7	87.4	88.2	88.4	87.4	88.9	89.1	91.5	92	92.2	92.4	92.4	92.4
Control 7.3	0.1	61.6	63.2	68.4	79.6	85.7	87.3	88.9	88.9	89.1	89.34	89.3	89.7	91.9	92.2	92.5	92.6	92.5	92.5
Control 5.5	0.3	52.1	55.9	71.4	85.6	85.4	87.6	88.3	88.1	88.9	88.85	88.6	88.8	91.3	91.5	91.8	91.9	91.6	91.6
NOVO 8.2	0.1	63.8	65.9	77.2	77.9	79.7	82.4	84.9	86.1	89.7	90.37	90.9	90.2	90.4	90.8	90.8	90.8	90.9	90.8
NOVO 7.3	0.2	61.2	62.1	75.1	75.4	80.9	82.9	83.9	84.4	84.5	85.73	87.4	88.0	89.2	90.1	90.1	90.1	90.1	90.1
NOVO 5.5	0.1	52.7	54.7	74.1	74.7	76.3	79.3	83.2	85.1	86.9	89.21	90.2	90.8	91.5	91.2	91.2	91.3	91.2	91.3
RM8.2	0.0	68.8	79.6	81.4	83.3	83.8	83.7	84.7	86.3	89.4	91.3	91.5	91.5	91.5	91.4	91.0	91.5	91.4	91.4
RM7.3	0.1	60.3	77.7	77.9	78.3	83.1	83.3	88.5	89.6	90.7	90.7	90.7	90.7	90.7	90.7	90.6	90.7	90.7	90.7
RM5.5	0.2	54.3	66.2	74.1	76.4	80.2	83.2	84.3	87.3	89.3	89.26	89.4	89.4	89.4	89.2	89.3	89.3	89.3	89.3
TL8.2	0.1	69.4	80.7	82.8	83.0	83.9	85.0	85.9	86.4	87.7	88.14	88.4	88.4	88.4	88.4	88.4	88.4	88.4	88.2
TL7.3	0.2	65.0	75.9	81.4	82.6	84.9	86.4	88.4	89.2	90.3	91.03	91.0	91.1	91.0	90.8	91.1	91.0	91.0	91.0
TL5.5	0.4	51.8	67.1	75.8	76.5	82.1	84.6	85.3	85.9	86.9	86.74	85.3	86.1	86.1	85.3	85.9	86.0	86.2	86.1

F2 Time for crystallization on fat blending trials with tallow stearin and palm stearin trial B

Time for crystallization																		
TS:PS	Time (Minute)																	
	0	1	2	3	4	6	8	10	12	14	16	18	20	30	40	50	60	70	80
Control 8.2	0.2	33.2	38.4	46.2	49.9	64.3	69.4	70.3	71.8	71.6	74.3	76.9	78.5	82.25	83.2	85.4	85.7	86.1	86.3
Control 7.3	0.1	34.3	40.2	49.9	60.5	68.4	70.2	72.3	73.7	73.9	74.0	74.3	74.3	78.8	82.0	82.1	84.4	84.5	85.0
Control 5.5	0.0	35.6	44.2	51.3	67.9	75.4	78.2	81.1	81.5	81.8	82.1	82.5	82.8	83.5	84.1	85.2	86.5	87.3	87.4
NOVO 8.2	0.1	62.2	76.5	79.8	81.4	82.2	83.8	84.1	84.3	85.3	85.4	87.5	87.4	87.5	87.8	87.7	87.9	87.4	88.0
NOVO 7.3	0.0	62.0	75.2	79.2	80.1	81.1	80.4	80.5	80.7	81.5	82.4	83.2	84.7	85.0	85.7	85.9	86.3	86.5	86.5
NOVO 5.5	-0.1	70.8	72.2	73.2	73.7	74.2	75.2	76.2	77.4	77.8	78.3	79.1	80.8	83.2	84.3	84.5	84.6	84.6	84.6
RM7.3	0.1	67.9	75.1	77.4	78.8	79.6	80.0	80.1	80.3	80.5	80.6	80.8	81.0	82.5	83.0	83.2	84	84.3	84.3
RM5.5	0.0	69.3	70.3	78.4	78.6	79.1	79.2	79.3	79.5	81.1	81.5	82.4	83.2	83.2	84.0	79.9	84.2	84.2	84.2
TL8.2	0.1	65.4	77.6	78.3	78.9	79.9	80.3	81.1	81.8	82.2	82.9	83.2	83.7	83.9	84.4	84.3	85.1	85.4	85.5
TL7.3	0.1	61.6	76.7	78.9	79.2	80.3	80.9	81.6	82.2	82.3	82.4	82.4	82.7	82.7	83.3	83.7	84.7	84.7	84.6
TL5.5	0.0	64.2	78.2	79.1	80.7	81.4	81.9	82.1	82.6	82.8	83.0	83.3	84.1	84.4	84.5	84.6	84.7	84.7	84.6

F3 Time for crystallization on fat blending trials with palm stearin and fully hardened coconut oil trial B

Time for crystallization																			
PS:FHCO	Time (Minute)																		
	0	1	2	3	4	6	8	10	12	14	16	18	20	30	40	50	60	70	80
Control 8.2	0.2	42.5	44.6	49.9	59.3	62.1	63.2	72.4	73.6	73.8	74.2	79.4	80.1	80.6	81.4	82.1	82.5	82.9	82.7
Control 7.3	0.1	41.4	42.4	49.3	52.3	61.4	73.2	74.3	75.5	77.2	77.7	78.3	79.6	81.8	82.2	82.7	83.2	83.8	83.6
Control 5.5	0.0	40.1	40.9	50.0	51.1	63.2	75.2	75.9	76.2	76.4	77.1	77.7	78.5	86.3	86.5	86.9	86.9	87.3	87.3
NOVO 8.2	0.2	42.3	49.4	56.2	58.0	58.3	64.4	65.1	65.3	74.8	75.2	75.3	76.4	77.9	78.4	79.6	79.5	79.6	79.4
NOVO 7.3	0.1	44.9	51.8	57.3	60.1	60.2	65.2	66.6	67.4	75.1	76.3	77.0	77.3	77.6	77.9	78.4	79.0	78.5	78.8
NOVO 5.5	0.1	49.8	50.2	53.3	54.8	55.3	56.7	58.9	59.3	76.4	74.1	74.2	74.3	74.4	75.4	76.8	76.2	76.4	74.4
RM8.2	0.2	41.9	48.4	53.2	54.3	60.9	61.2	61.5	62.2	62.4	76.3	78.3	79.4	80.3	81.4	81.4	81.4	81.4	81.4
RM7.3	0.2	43.9	49.8	54.8	56.7	61.8	63.2	65.2	65.3	74.9	75.3	80.4	80.3	80.2	80.4	80.2	80.4	80.4	80.4
RM5.5	0.1	45.3	50.2	55.0	57.5	61.5	63.8	65.9	76.3	78.8	80.6	80.4	80.3	80.3	80.4	80.4	80.5	80.4	80.5
TL8.2	0.1	40.8	42.9	49.4	51.8	56.5	58.3	60.4	73.7	74.0	75.7	75.8	75.9	76.5	81.9	81.8	81.8	81.9	81.9
TL7.3	0.1	42.4	44.2	52.2	60.4	61.8	63.1	63.2	76.4	78.4	80.9	80.9	80.9	80.9	80.9	80.4	80.8	80.9	80.9
TL5.5	0.0	43.8	46.8	54.3	63.2	64.2	65.2	64.5	77.2	78.4	80.4	80.2	80.4	80.4	80.4	80.4	80.4	80.4	80.4

Appendix G Melting point analysis for reaction time and concentration trials

G1 Melting point analysis for reaction time and concentration trial on TL enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow Stearin :FHCO (70:30) with 0-8% TL enzyme over 8 hours					
	0 hours	2 hours	4 hours	6 hours	8 hours
TL7.3(TS:FHCO)0%	52	52	52	52	52
TL7.3(TS:FHCO)2%	52	51	48	46	45
TL7.3(TS:FHCO)4%	52	49	47	45	44
TL7.3(TS:FHCO)8%	52	47	45	45	44

G2 Melting point analysis for reaction time and concentration trial on RM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow Stearin :FHCO (70:30) with 0-8% RM enzyme over 8 hours					
	0 hours	2 hours	4 hours	6 hours	8 hours
RM7.3(TS:FHCO)0%	52	52	52	52	52
RM7.3(TS:FHCO)2%	52	51	48	45	45
RM7.3(TS:FHCO)4%	52	49	47	45	44
RM7.3(TS:FHCO)8%	52	47	45	45	44

G3 Melting point analysis for reaction time and concentration trial on TL IM enzyme interesterified palm stearin and fully hardened coconut oil

Palm Stearin :FHCO (50:50) with 0-8% TL enzyme over 8 hours					
	0 hours	2 hours	4 hours	6 hours	8 hours
TL5.5(PS:FHCO)0%	43	43	43	43	43
TL5.5(PS:FHCO)2%	43	43	42	40	39
TL5.5(PS:FHCO)4%	43	40	38	37	36
TL5.5(PS:FHCO)8%	43	38	37	36	36

Appendix H HPLC analysis on triglycerides composition (TAG) for reaction time and concentration trials

H1 TAG composition for time and concentration trial with 2% TL IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by TL enzyme at 2%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL7.3(TSFHCO)2%0hr	TL7.3(TSFHCO)2%2hr	TL7.3(TSFHCO)2%4hr	TL7.3(TSFHCO)2%6hr	TL7.3(TSFHCO)2%8hr
CpCpCp	4.6	4.2	3.9	3.7	3.6
RT5.6	1.8	1.5	1.3	1.1	
CCC	4.6	4.4	3.9	3.8	3.7
RT7.5					
CCL	5.9	5.2	5.4	5.0	4.9
RT9.2	1.2	1.1	1.2		
CLL	6.4	5.9	5.7	4.4	4.0
RT13.0					
RT15.2					
LLL	8.2	7.4	7.1	7.1	6.9
RT16.2		4.4	4.2	4.1	3.9
RT22.7		2.0	1.1		
LLM	7.5	7.4	7.3	7.1	6.8
LLO	8.3	7.9	7.5	7.8	6.9
LMM	8.9	7.9	7.7	7.2	6.9
RT41.9					
RT51.2	3.0			1.9	2.8
LMP		2.7	2.9	3.6	3.9
RT71.8	1.6		1.2	1.9	2.3
PLO		1.5	1.9	3.0	3.3
PPL			2.1	2.8	3.5
POP	16.8	15.9	15.7	15.9	15.0
PPP	16.6	15.9	15.1	14.9	14.2
POS	4.5	4.1	4.1	3.8	3.6
PPS					2.1

H2 TAG composition for time and concentration trial with 4% TL IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by TL enzyme at 4%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL7.3(TSFHCO)4%0hr	TL7.3(TSFHCO)4%2hr	TL7.3(TSFHCO)4%4hr	TL7.3(TSFHCO)4%6hr	TL7.3(TSFHCO)4%8hr
CpCpCp	4.4	4.2	4.0	4.1	3.3
RT5.71	2.0	1.6	1.5	1.2	
CCC	4.7	4.3	4.2	3.8	3.1
RT7.51		1.2			1.4
CCL	5.5	5.2	5.0	3.6	2.9
RT9.8	1.4	1.1		1.5	2.9
CLL	6.6	6.1	5.0	3.8	5.0
RT13.0					1.5
RT15.2		1.0	2.7	2.1	1.4
LLL	8.3	8.1	7.7	6.7	7.2
RT17.6				1.5	2.0
RT23.6			3.4	2.4	6.4
LLM	7.4	7.1	6.7	6.2	
LLO	8.1	7.8	7.5	7.2	5.3
LMM	8.7	8.1	7.7	7.2	6.9
RT41.9				1.2	1.9
RT52.6	2.8	1.5		1.4	2.6
LMP		2.0	2.8	3.2	7.5
RT70.3	1.5	1.4	2.1	2.5	2.2
PLO		1.8	2.9	3.3	4.3
PPL		2.5	3.1	3.3	4.9
POP	16.2	15.1	14.2	13.9	12.2
PPP	16.9	15.3	14.6	13.7	12.7
POS	4.3	4.1	4.0	3.7	2.2

H3 TAG composition for time and concentration trial with 8% TL IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by TL enzyme at 8%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL7.3(TSFHCO)8%0hr	TL7.3(TSFHCO)8%2hr	TL7.3(TSFHCO)8%4hr	TL7.3(TSFHCO)8%6hr	TL7.3(TSFHCO)8%8hr
CpCpCp	4.4	4.0	3.8	3.3	3.3
RT6.5	1.5	1.4	1.1		
CCC	4.6	3.9	3.5	3.4	3.4
RT9.0		1.5	2.2	1.7	1.2
RT10.4					2.8
CCL	5.3	3.7	3.7	3.7	3.4
RT11.8	1.6	1.8	1.4	1.3	1.1
CLL	6.5	5.1	5.5	5.3	5.6
RT15.7		1.2	1.8	1.7	1.6
LLL	7.6	6.9	6.5	6.6	6.9
R9.237		1.6	1.3	1.1	1.4
RT23.5			2.1	1.7	
LLM	7.7	6.3	6.1	6.3	6.3
RT26.2		2.1			
RT28.6		1.4			
LLO	8.7	7.5	6.3	6.2	6.2
RT36.6		1.6	1.1	1.3	1.2
RT39.3		1.8			
RT41.5		1.2		2.0	2.0
RT45.7		1.9			
LMM	9.3	7.6	6.1	6.3	6.3
RT52.4	4.2		1.9	2.9	2.7
LMP		3.1	7.3	7.3	7.4
RT69.81		2.7	2.5	2.7	2.5
PLO		2.9	4.3	4.4	4.2
PPL		2.1	5.6	5.7	5.3
POP	17.1	12.9	12.5	12.6	12.2
PPP	16.6	12.8	12.4	12.0	12.8
POS	4.4				

H4 TAG composition for time and concentration trial with 2% RM IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by RM enzyme at 2%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	RM7.3(TSFHCO)2%0hr	RM7.3(TSFHCO)2%2hr	RM7.3(TSFHCO)2%4hr	RM7.3(TSFHCO)2%6hr	RM7.3(TSFHCO)2%8hr
CpCpCp	4.5	4.3	4.1	3.9	3.5
RT5.5	1.6	1.5	1.8		
CCC	4.5	4.3	4.4	4.6	3.9
CCL	5.5	5.2	5.0	4.3	4.6
RT12.6	1.8				
CLL	7.0	6.8	6.4	5.6	5.2
LLL	7.4	7.1	7.0	5.7	5.6
RT15.6		4.0	4.0	4.3	4.3
LLM	7.1	7.4	5.8	5.8	5.4
RT20.6				1.8	1.9
RT21.1		1.7		3.2	2.2
RT22.0			2.0	1.4	
LLO	8.6	8.2	8.2	8.6	7.9
LMM	9.5	7.0	10.1	10.7	8.9
LMO	4.5				
LMP		3.0	2.9	3.4	3.7
PLO		1.4	1.7	2.5	3.0
PPL				1.6	3.8
RT76.0					3.2
POO				1.3	2.6
POP	16.8	16.2	15.9	14.3	13.5
PPP	16.5	15.6	15.2	12.7	12.3
POS	4.4	5.5	4.9	3.9	3.7

H5 TAG composition for time and concentration trial with 4% RM IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by RM enzyme at 4%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	RM7.3(TSFHCO)4%0hr	RM7.3(TSFHCO)4%2hr	RM7.3(TSFHCO)4%4hr	RM7.3(TSFHCO)4%6hr	RM7.3(TSFHCO)4%8hr
CpCpCp	4.6	4.5	4.2	3.9	3.9
RT4.3	1.8	1.4	1.1		
CCC	4.6	4.3	4.1	3.9	3.6
CCL	5.1	4.3	4.1	3.8	3.6
RT9.585	1.3			1.9	1.8
RT11.1					2.0
CLL	7.1	6.3	6.0	5.4	5.2
RT12.7			1.2		2.7
RT13.5			2.2	2.0	
LLL	7.5	7.2	6.9	6.5	6.5
RT17.0				1.8	2.3
LLM	6.8	6.6	6.3	6.0	6.1
RT26.6		1.3			2.5
LLO	8.3	7.8	7.4	7.0	6.3
LMM	9.2	8.6	8.2	8.4	6.3
LMO	4.3	3.2	2.6		
RT50.1		1.3	1.1	1.9	1.8
LMP		2.4	4.5	6.4	6.5
PLO		1.9	2.1	3.2	4.6
RT65.2		1.4	1.3	3.7	
PPL		1.2	1.8	1.9	5.2
RT69.2		1.2	1.7	1.4	2.7
POO					
POP	16.9	15.4	14.3	13.2	12.9
PPP	16.8	15.4	14.8	13.3	12.6
PSS	4.6	4.3	4.2	4.0	

H6 TAG composition for time and concentration trial with 8% RM IM enzyme interesterified tallow stearin and fully hardened coconut oil

Tallow stearin and fully hardened coconut oil interesterified by RM enzyme at 8%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	RM7.3(TSFHCO)8%0hr	RM7.3(TSFHCO)8%2hr	RM7.3(TSFHCO)8%4hr	RM7.3(TSFHCO)8%6hr	RM7.3(TSFHCO)8%8hr
CpCpCp	4.3	4.1	4.0	4.0	3.9
RT5.6	1.2	1.1	1.1	1.1	
CCC	4.5	3.8	3.7	3.6	3.6
RT7.4			1.7		2.7
CCL	5.3	4.3	3.9	3.6	3.7
RT9.2	1.7	1.4		1.6	1.8
CLL	6.7	5.3	5.2	5.3	5.3
RT11.9					1.9
LLL	8.0	6.4	6.0	5.9	6.4
LLM	7.4	6.7	6.7	6.1	6.0
RT21.0				3.7	1.7
LLO	8.6	6.8	6.8	6.5	6.5
RT31.9			1.2	1.8	1.0
LMM	9.2	6.1	6.1	6.1	6.1
LMO	4.2		2.0	1.1	
RT50.8		3.7	3.5	3.3	1.7
LMP		6.8	7.8	7.8	7.7
RT69.8		2.4	2.5	2.5	2.7
PLO		5.0	4.9	4.9	4.9
PPL		5.7	5.9	5.1	5.1
POP	17.1	13.0	13.6	12.8	12.8
PPP	16.8	12.9	12.8	12.4	12.4
PSS	4.2	4.3			
RT141.4					1.2

H7 TAG composition for time and concentration trial with no enzyme interesterified palm stearin and fully hardened coconut oil

Palm stearin and fully hardened coconut oil interesterified by no enzyme 0%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL5.5(PSFHCO)0hr0%	TL5.5(PSFHCO)2hr0%	TL5.5(PSFHCO)4hr0%	TL5.5(PSFHCO)6hr0%	TL5.5(PSFHCO)8hr0%
CpCpCp	4.8	4.6	4.2	3.5	5.1
RT6.8	1.4	1.4	1.4	1.3	1.3
CCC	5.2	5.0	5.1	5.0	5.0
CCL	9.0	8.8	8.8	8.9	8.8
CLL	7.8	7.4	7.4	7.6	7.5
LLL	8.3	8.3	8.4	8.4	8.5
LLM	7.8	7.1	7.2	7.3	7.1
LLO	3.1	3.8	3.9	3.9	3.9
LMM	5.8	5.0	5.1	5.1	5.0
LMO	3.8	3.8	3.9	3.9	3.8
LMP					
PLO	1.2	1.2	1.2	1.2	1.2
PPL					
RT64.6	2.2	2.5	2.5	2.5	2.4
POO	6.4	6.2	6.7	6.4	6.6
POP	15.6	15.9	15.3	15.6	15.5
PPP	14.7	15.4	15.3	15.6	15.1
PSS	2.9	2.9	2.9	2.9	2.9

H8 TAG composition for time and concentration trial with 2% TL IM enzyme interesterified palm stearin and fully hardened coconut oil

Palm stearin and fully hardened coconut oil interesterified by TL enzyme at 2%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL5.5(PSFHCO)0hr2%	TL5.5(PSFHCO)2hr2%	TL5.5(PSFHCO)4hr2%	TL5.5(PSFHCO)6hr2%	TL5.5(PSFHCO)8hr2%
CpCpCp	4.8	4.6	4.2	3.5	5.1
RT4.4					
RT5.4					
RT6.9	1.4	1.4	1.4	1.3	
RT7.1					1.3
CCC	5.2	5.0	5.1	5.0	5.0
RT11.5					
CCL	9.0	8.8	8.8	8.9	8.8
RT15.4					
CLL	7.8	7.4	7.4	7.6	7.5
LLL	8.3	8.3	8.4	8.4	8.5
RT20.5					
LLM	7.8	7.1	7.2	7.3	7.1
RT22.3					
LLO	3.1	3.8	3.9	3.9	3.9
RT28.4					
RT29.2					
LMM	5.8	5.0	5.1	5.1	5.0
RT38.5					
LMO	3.8	3.8	3.9	3.9	3.8
LMP					
RT50.3					
PLO	1.2	1.2	1.2	1.2	1.2
PPL					
RT88.0	2.2	2.5	2.5	2.5	2.4
POO	6.4	6.2	6.7	6.4	6.6
POP	15.6	15.9	15.3	15.6	15.5
PPP	14.7	15.4	15.3	15.6	15.1
PSS	2.9	2.9	2.9	2.9	2.9

H9 TAG composition for time and concentration trial with 4% TL IM enzyme interesterified palm stearin and fully hardened coconut oil

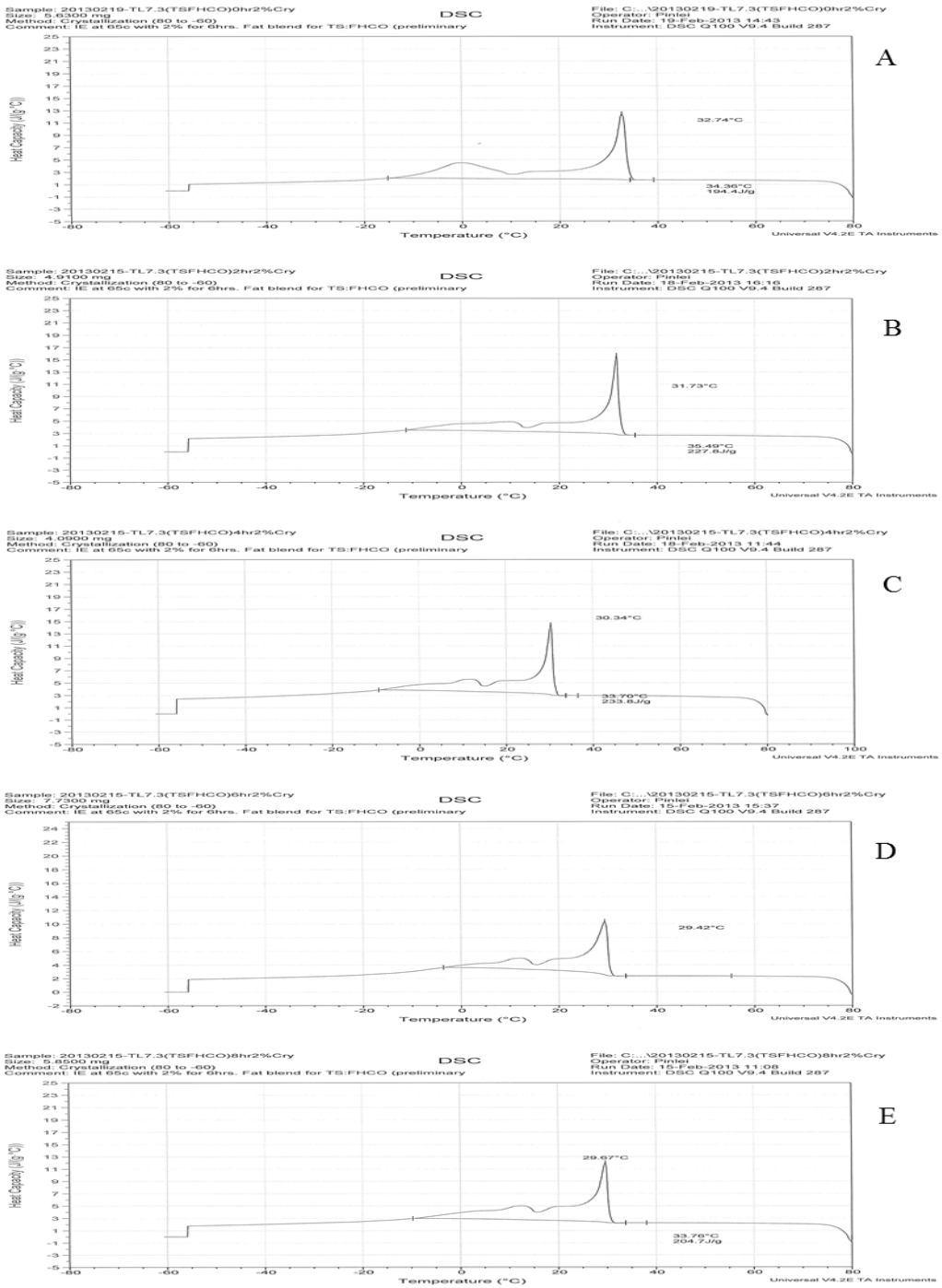
Palm stearin and fully hardened coconut oil interesterified by TL enzyme at 4%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL5.5(PSFHCO)0hr4%	TL5.5(PSFHCO)2hr4%	TL5.5(PSFHCO)4hr4%	TL5.5(PSFHCO)6hr4%	TL5.5(PSFHCO)8hr4%
CpCpCp	4.6	4.0	3.7	3.4	3.5
RT4.4		1.2	1.7	1.4	1.9
RT5.2		1.1	1.5	1.6	1.9
RT6.6	1.3	1.6	2.1	1.9	2.6
RT7.0				2.5	4.0
RT7.8					
CCC	5.3	4.7	4.3	3.7	3.4
RT12.4				1.4	1.4
RT10.5		1.1	1.1	1.3	2.1
CCL	8.8	6.9	5.8	5.6	5.2
RT14.1				1.4	1.7
RT14.6			2.8		1.8
CLL	7.7	7.2	6.8	6.4	2.5
LLL	8.4	7.8	6.9	5.8	5.0
RT22.0					
LLM	7.7	6.4	5.5	5.4	5.0
LLO	3.1	3.3	3.6	3.0	2.9
RT26.2					
LMM	5.8	5.7	5.4	5.3	5.2
RT33.8					1.5
LMO	3.9	4.3	4.8	5.0	5.2
RT46.3		1.1		1.7	1.3
LMP		4.7	5.4	5.5	5.6
PLO	1.4	2.6	3.2	4.4	4.5
PPL		3.2	3.5	5.7	5.7
RT92.2	2.5				
POO	6.7	5.2	5.0	3.9	3.8
POP	15.7	13.0	12.0	10.6	10.1
PPP	14.4	12.2	11.5	10.1	9.7
PSS	2.3	2.6	2.7	2.3	2.5

H10 TAG composition for time and concentration trial with 8% TL IM enzyme interesterified palm stearin and fully hardened coconut oil

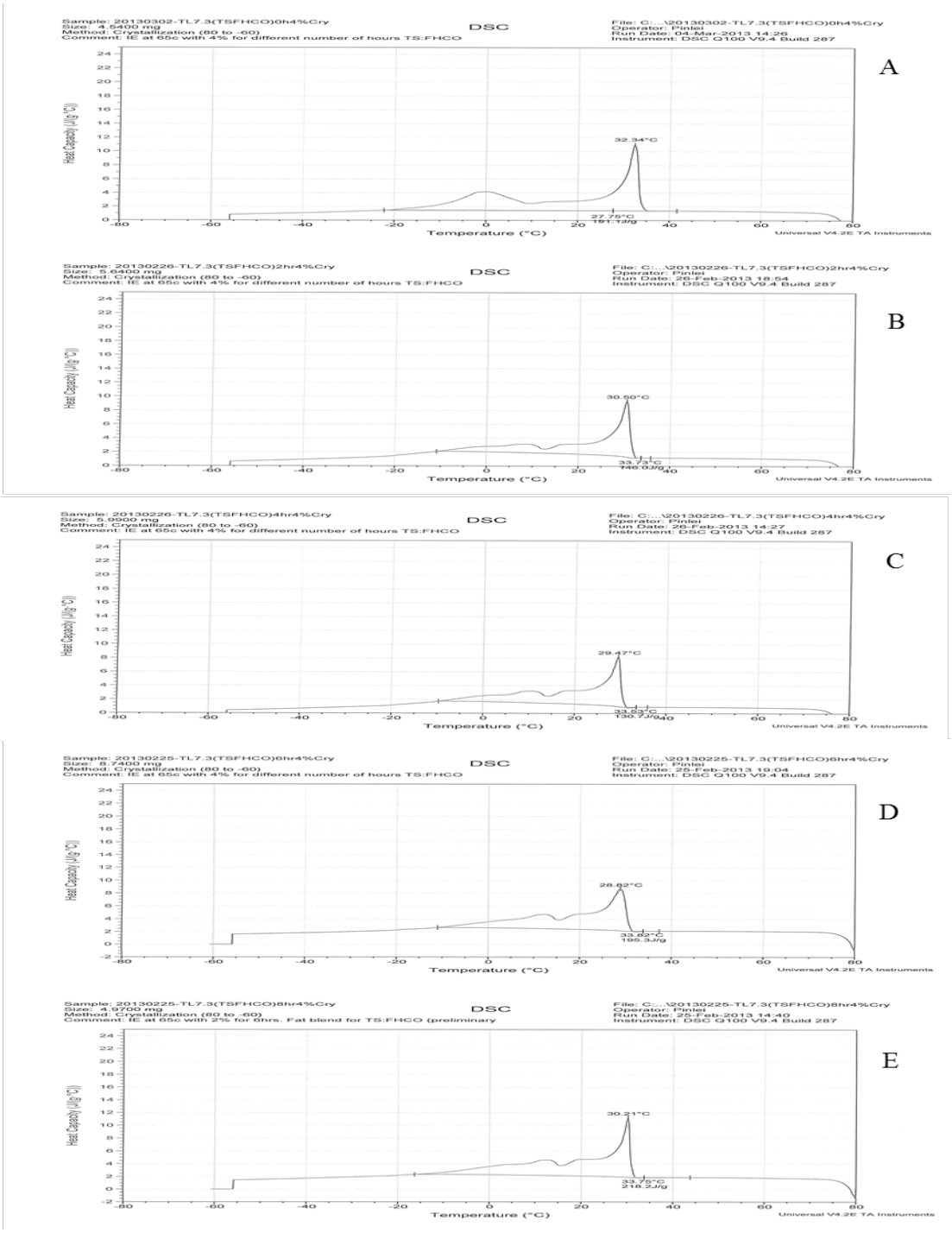
Palm stearin and fully hardened coconut oil interesterified by TL enzyme at 4%					
	0 hour	2 hour	4 hour	6 hour	8 hour
Name	TL5.5(PSFHCO)0hr8%	TL5.5(PSFHCO)2hr8%	TL5.5(PSFHCO)4hr8%	TL5.5(PSFHCO)6hr8%	TL5.5(PSFHCO)8hr8%
CpCpCp	4.7	3.9	3.6	3.1	3.1
RT3.7		1.3	2.2	2.3	2.4
RT4.5			1.6	2.2	2.0
RT6.6	1.4	1.4	1.1	1.6	1.5
RT7.4		3.0	3.3	3.5	3.5
RT7.8				1.3	1.3
CCC	5.3	4.7	4.0	3.3	3.2
RT12.1		1.1	1.8	2.0	1.8
CCL	8.9	7.3	5.7	5.0	4.7
RT16.4		1.3			2.1
CLL	7.6	6.6	4.3	4.0	2.1
LLL	8.4	6.1	5.7	4.3	4.6
RT22.0		1.5			
LLM	7.5	4.7	4.8	4.8	4.5
LLO	3.8	2.6	2.4	2.3	4.2
RT26.2				1.2	2.0
LMM	5.9	4.9	5.6	5.6	5.6
RT28.8			1.4	1.2	
LMO	3.9	4.9	5.0	4.9	5.2
LMP		5.4	6.6	7.7	7.8
PLO	1.2	2.0	4.6	5.8	5.7
PPL		4.1	5.9	6.3	6.2
RT88.0	2.7	2.8	2.8	2.5	2.0
POO	6.5	5.8	5.4	5.0	4.7
POP	15.2	11.1	9.7	9.2	9.1
PPP	14.3	10.4	9.4	8.8	8.5
PSS	2.3	2.1	2.2	2.0	2.0

Appendix I DSC crystallization curve for time and concentration trials

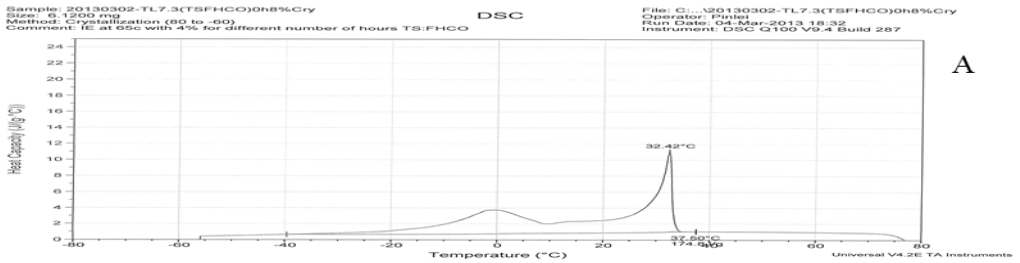
I1 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 2% TL enzyme

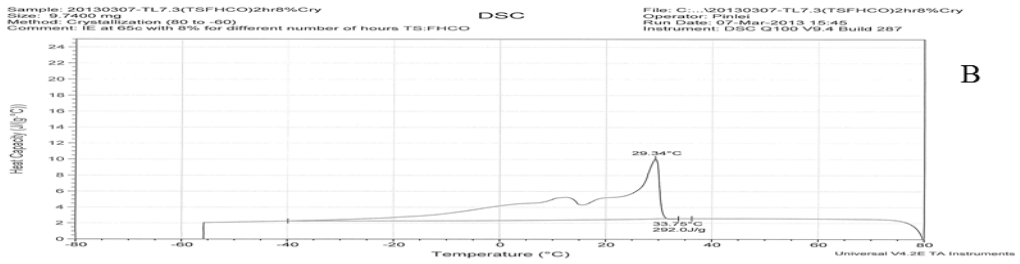


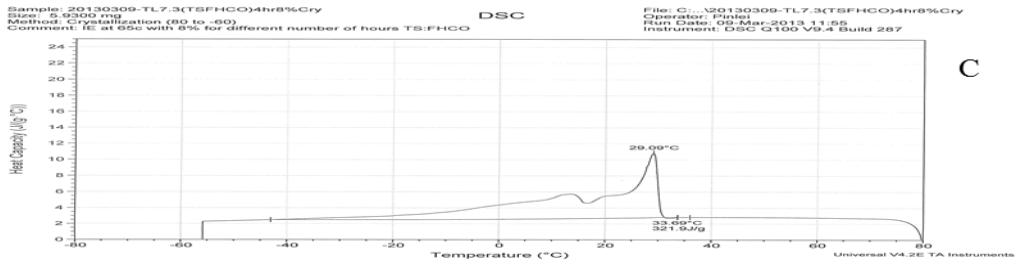
I2 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 4% TL enzyme

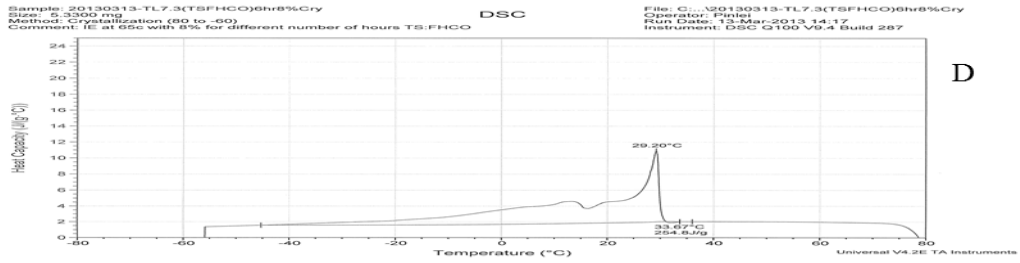


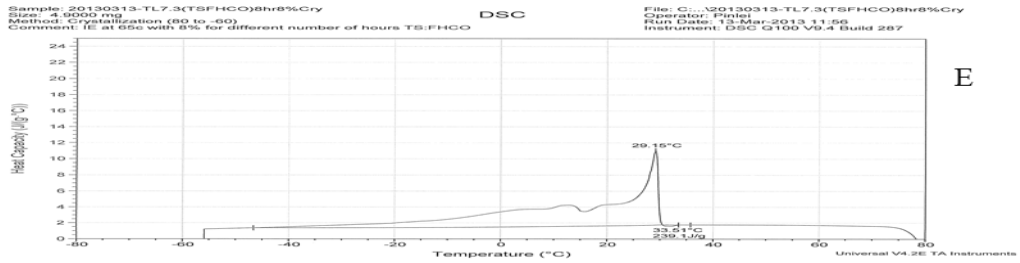
I3 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 8% TL enzyme



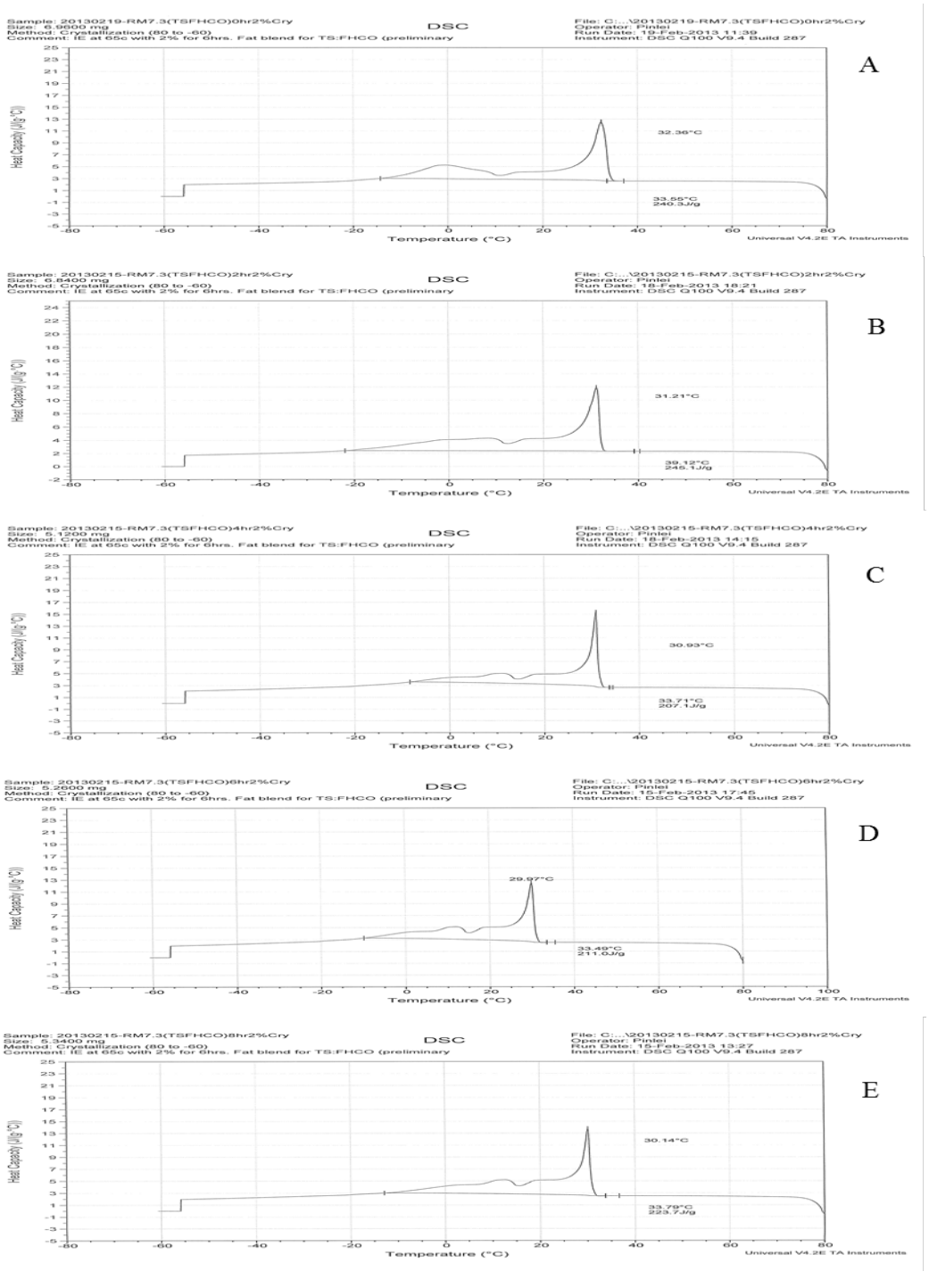




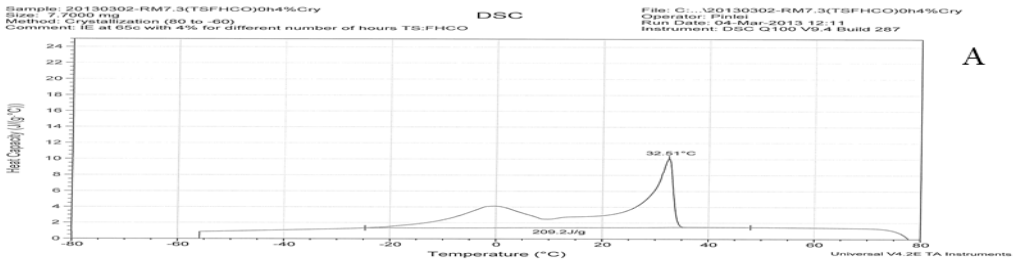


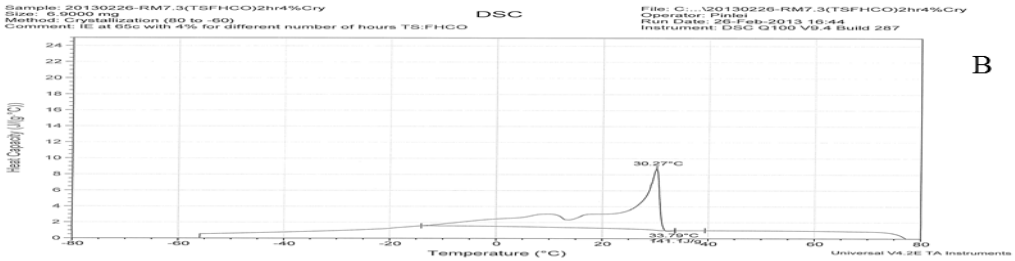


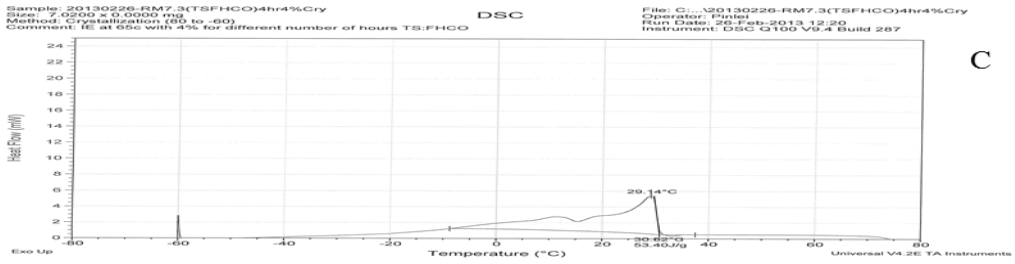
I4 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 2% RM enzyme

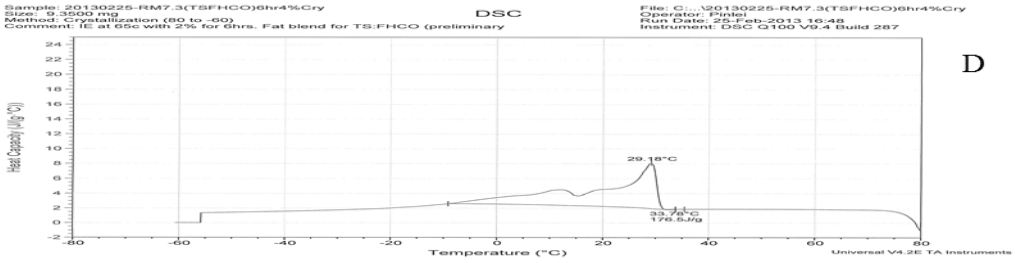


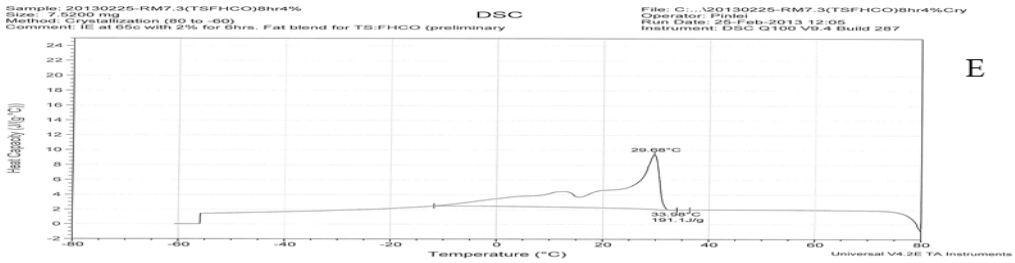
I5 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 4% RM enzyme



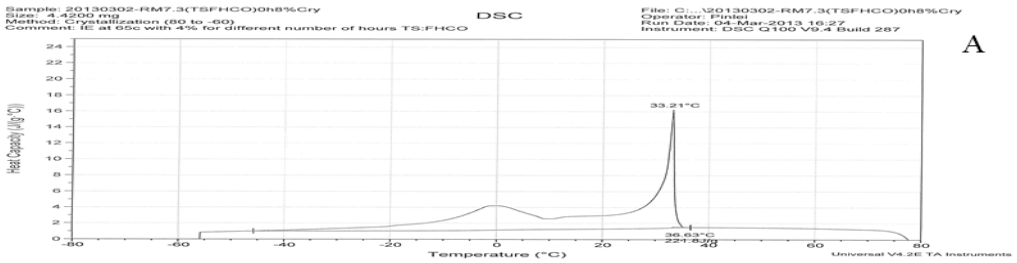


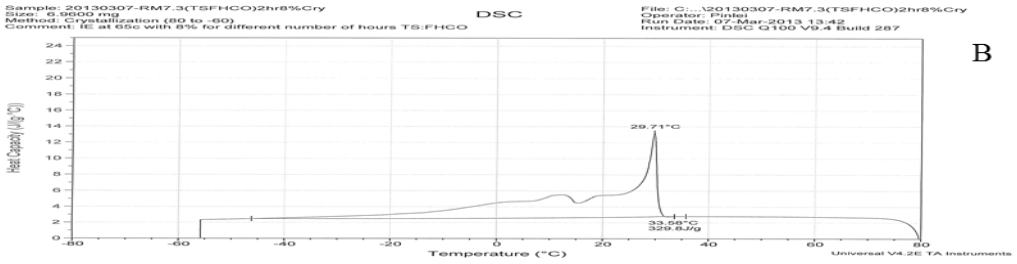


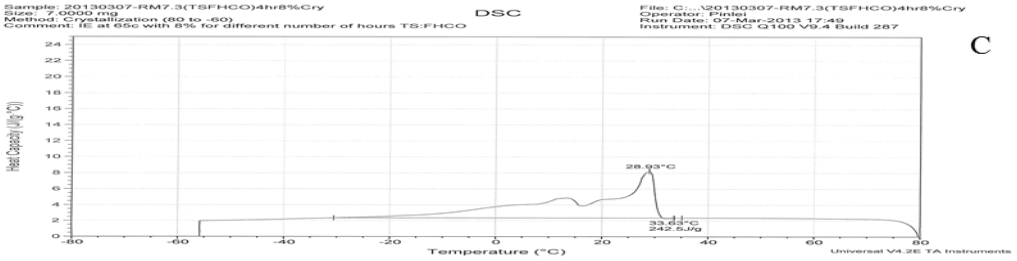


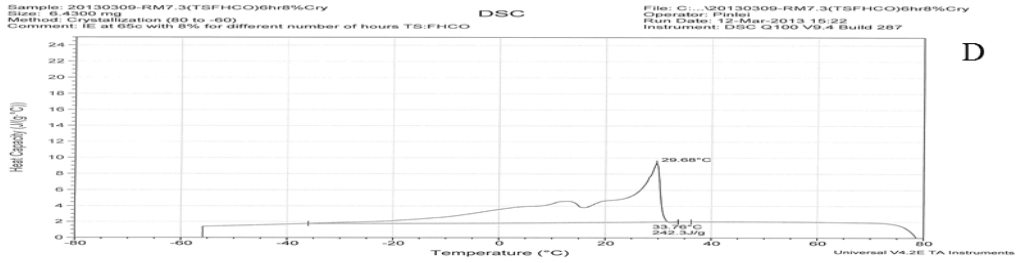


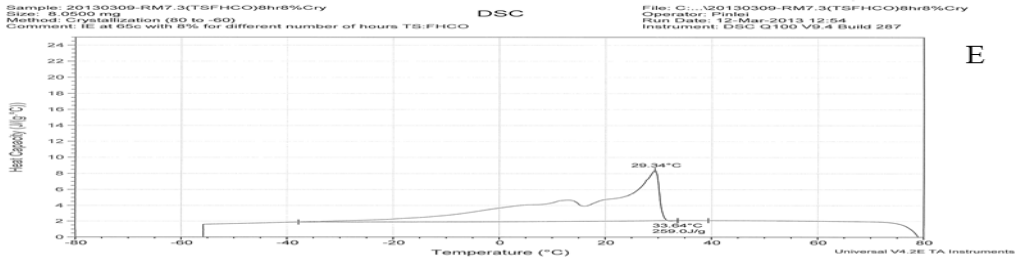
I6 DSC crystallization curve for time and concentration trial on tallow stearin and fully hardened coconut oil with 8% RM enzyme



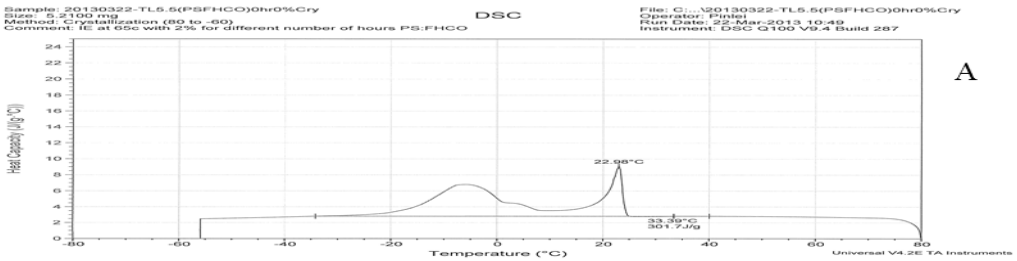


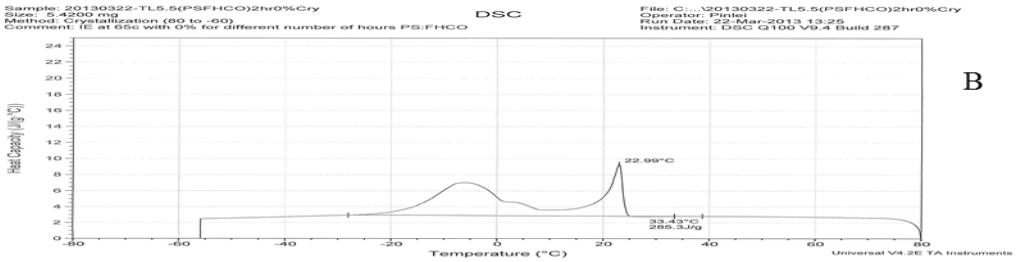


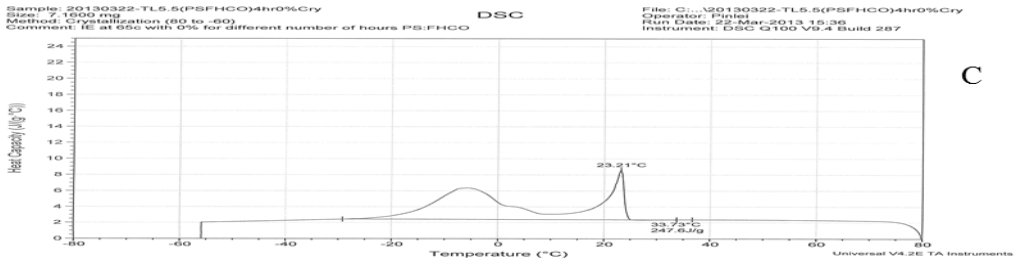


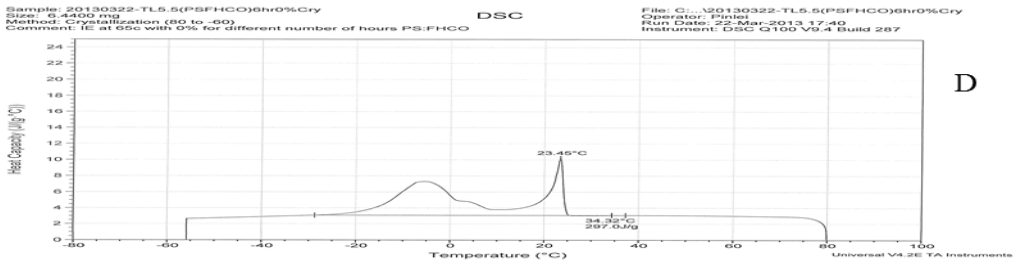


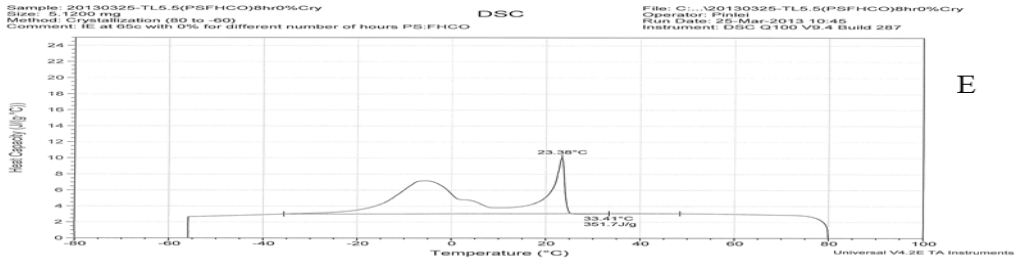
I7 DSC crystallization curve for time and concentration trial on palm stearin and fully hardened coconut oil with no TL enzyme



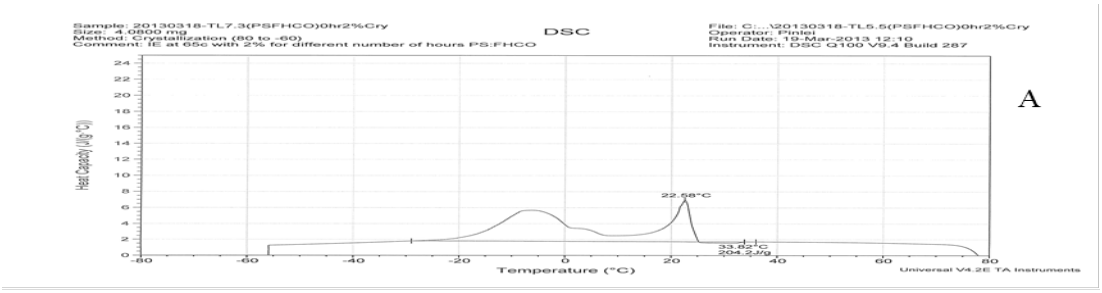


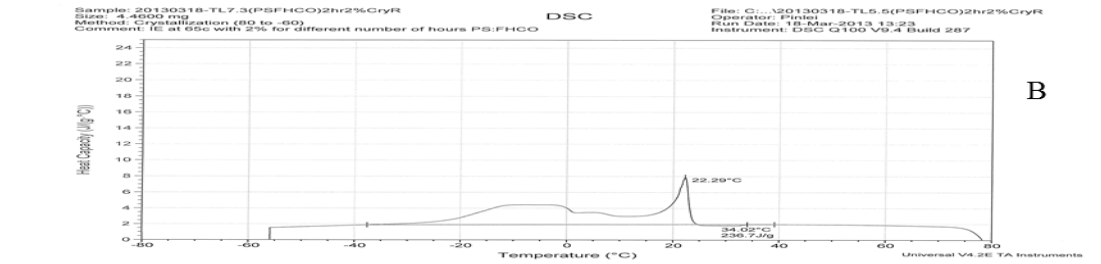


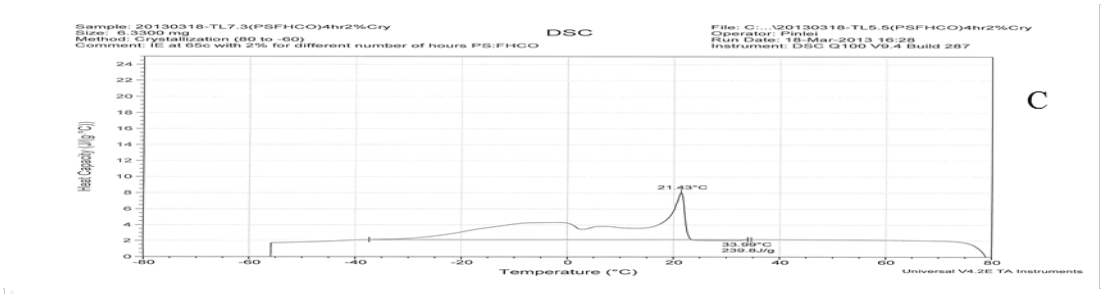


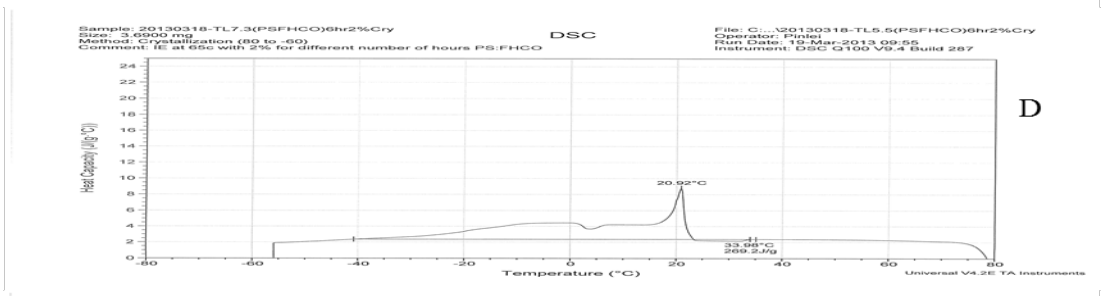


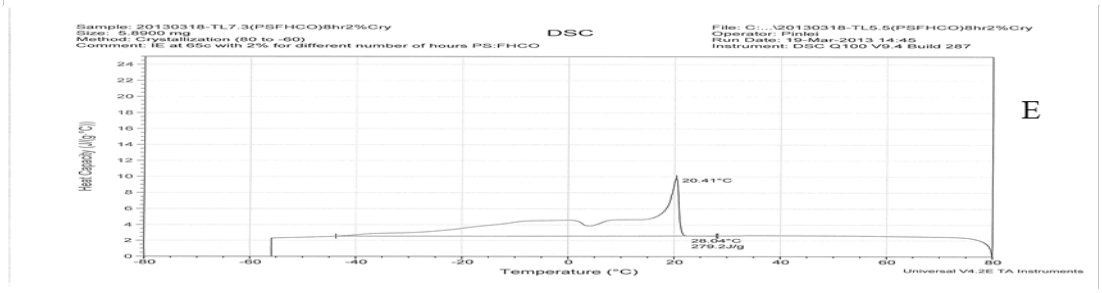
I8 DSC crystallization curve for time and concentration trial on palm stearin and fully hardened coconut oil with 2% TL enzyme



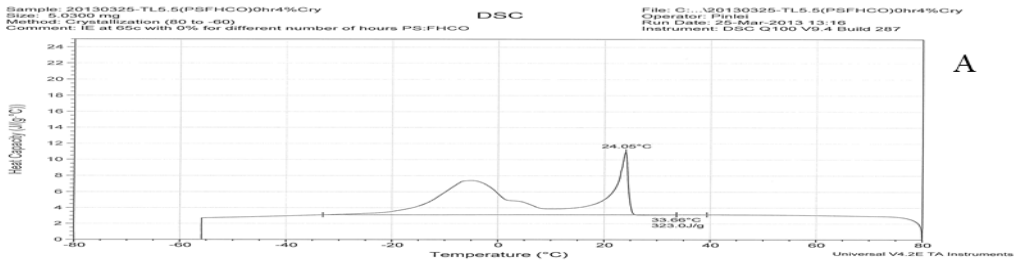


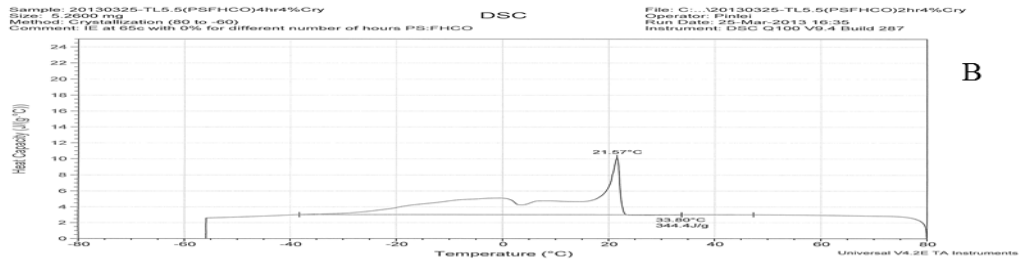


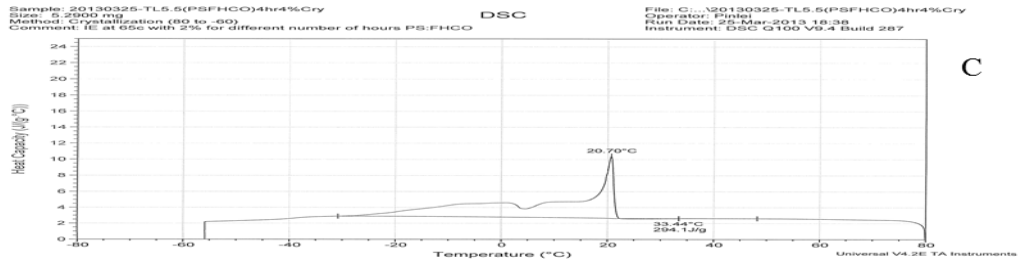


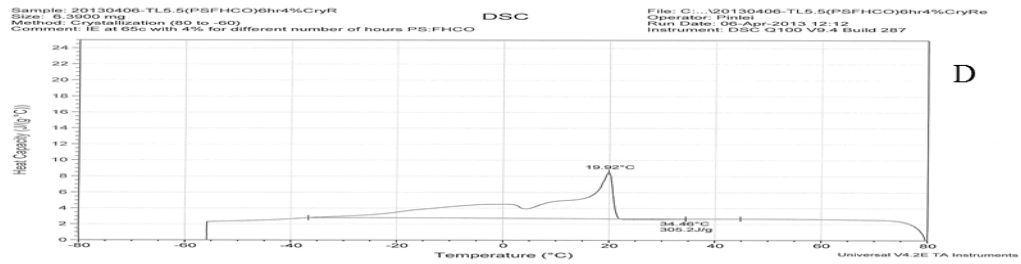


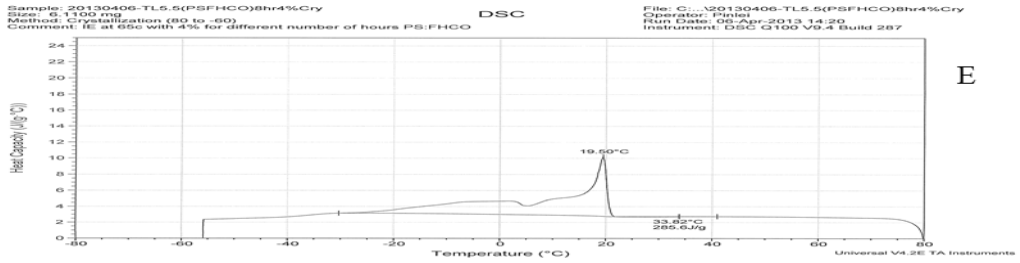
I9 DSC crystallization curve for time and concentration trial on palm stearin and fully hardened coconut oil with 4% TL enzyme



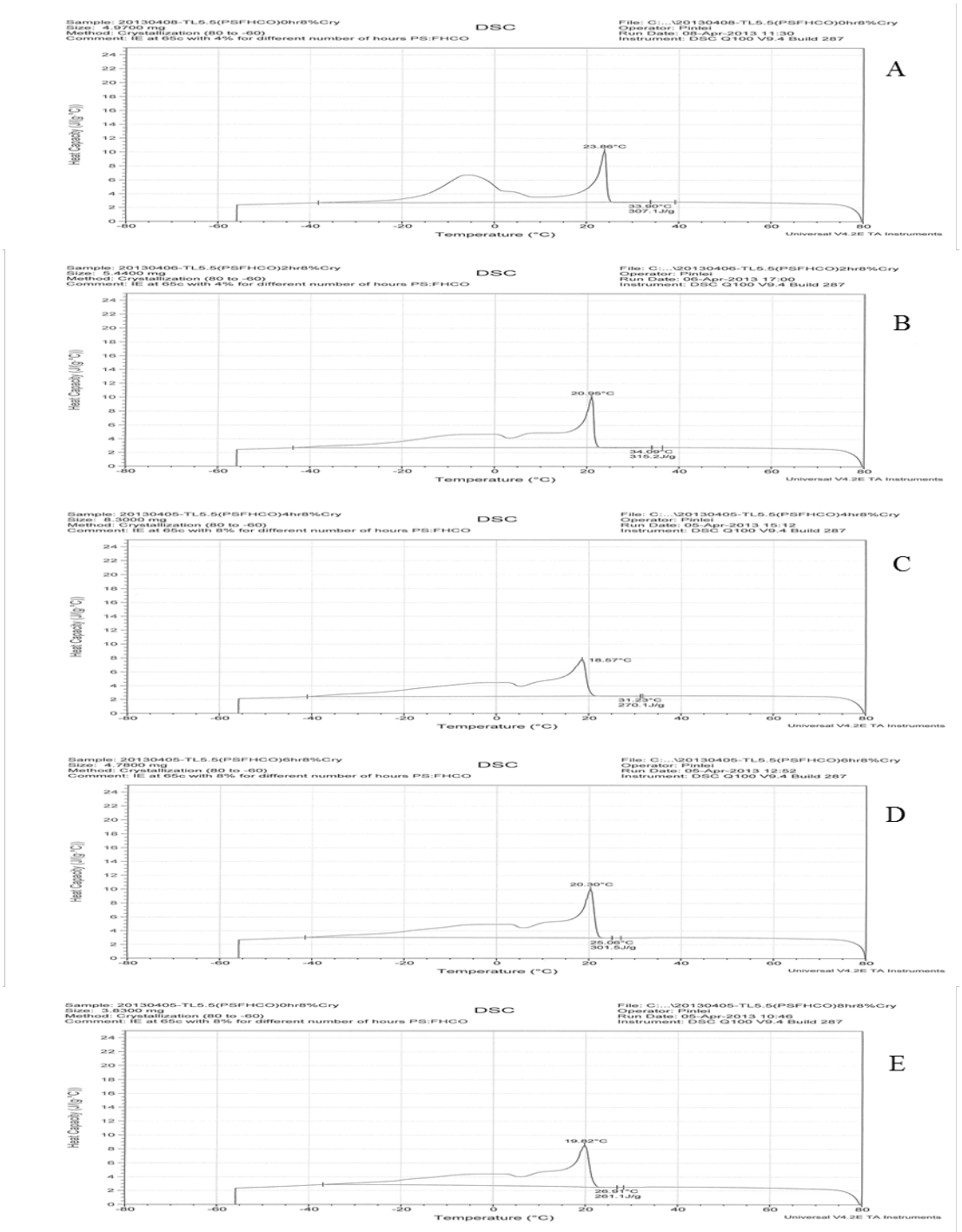








I10 DSC crystallization curve for time and concentration trial on palm stearin and fully hardened coconut oil with 8% TL enzyme



Appendix J Solid fat content for time and concentration trials

J1 Solid fat content for time and concentration trial on TL enzyme interesterified tallow stearin and fully hardened coconut oil

TL7.3(TSFHCO)					
	0°C	10°C	20°C	30°C	40°C
0% 0 hour	93.3	90.9	70.0	45.4	26.9
0% 2 hour	89.3	91.0	69.8	45.4	27.1
0% 4 hour	93.5	91.0	69.7	45.3	26.9
0% 6 hour	92.9	90.7	70.3	45.8	27.4
0% 8 hour	93.1	90.6	70.0	45.7	27.6
2% 0 hour	93.3	90.9	74.8	54.6	34.7
2% 2 hour	93.4	90.6	74.6	51.3	27.4
2% 4 hour	92.9	89.5	75.6	48.4	22.0
2% 6 hour	91.6	88.7	76.4	47.2	17.8
2% 8 hour	91.6	88.6	76.2	45.5	16.6
4% 0 hour	93.0	91.0	75.5	55.1	35.3
4% 2 hour	93.0	90.0	74.9	49.4	25.6
4% 4 hour	93.2	89.9	76.6	46.9	19.8
4% 6 hour	90.4	87.2	76.8	47.8	19.2
4% 8 hour	91.3	87.4	74.8	42.7	14.2
8% 0 hour	93.0	89.9	69.4	45.0	26.8
8% 2 hour	92.3	89.1	76.4	46.2	17.3
8% 4 hour	91.5	87.8	75.7	45.5	15.4
8% 6 hour	90.0	86.9	75.6	44.1	15.0
8% 8 hour	90.9	86.9	74.7	44.5	15.1

J2 Solid fat content for time and concentration trial on RM enzyme interesterified tallow stearin and fully hardened coconut oil

RM7.3(TSFHCO)					
	0°C	10°C	20°C	30°C	40°C
0% 0 hour	91.6	90.1	72.0	45.4	26.9
0% 2 hour	91.1	90.4	72.0	45.4	27.1
0% 4 hour	91.3	91.0	70.1	45.7	26.9
0% 6 hour	91.3	90.8	71.0	45.5	27.3
0% 8 hour	91.3	90.9	69.0	45.8	27.2
2% 0 hour	91.1	89.4	72.2	52.7	32.5
2% 2 hour	91.3	88.5	73.2	47.9	26.4
2% 4 hour	91.4	88.8	74.9	48.0	21.8
2% 6 hour	91.3	88.5	75.5	46.1	18.5
2% 8 hour	91.2	87.8	75.6	45.0	16.7
4% 0 hour	92.2	90.0	74.3	53.8	34.1
4% 2 hour	90.7	87.4	74.1	47.5	21.6
4% 4 hour	90.3	87.5	75.6	46.8	17.3
4% 6 hour	90.4	87.6	75.3	43.7	14.4
4% 8 hour	88.7	85.7	74.8	45.4	15.3
8% 0 hour	92.0	90.0	73.7	53.0	33.6
8% 2 hour	92.3	89.4	75.6	46.6	18.3
8% 4 hour	91.7	88.0	76.0	44.7	15.4
8% 6 hour	90.5	87.0	75.1	43.4	12.0
8% 8 hour	89.6	86.6	74.4	42.9	13.8

J3 Solid fat content for time and concentration trial on TL enzyme interesterified palm stearin and fully hardened coconut oil

TL5.5(TSFHCO)					
	0°C	10°C	20°C	30°C	40°C
0% 0 hour	87.3	81.3	44.9	21.2	8.4
0% 2 hour	87.6	81.1	44.8	21.5	8.7
0% 4 hour	87.3	81.3	44.7	21.6	8.1
0% 6 hour	87.5	81.1	44.7	21.3	8.4
0% 8 hour	87.7	81.3	44.8	21.5	8.5
2% 0 hour	87.9	82.6	45.0	21.9	8.6
2% 2 hour	85.9	87.7	42.5	20.4	5.7
2% 4 hour	84.4	80.8	42.8	19.0	3.3
2% 6 hour	82.1	74.4	42.7	17.3	1.3
2% 8 hour	81.6	74.6	42.9	16.6	0.0
4% 0 hour	87.3	81.5	45.2	21.3	8.5
4% 2 hour	85.3	76.2	43.3	17.6	3.1
4% 4 hour	82.7	74.3	43.3	15.4	-0.2
4% 6 hour	80.0	73.5	43.0	14.9	-0.1
4% 8 hour	79.8	73.2	41.5	13.8	0.2
8% 0 hour	87.6	81.4	45.3	22.7	8.9
8% 2 hour	79.9	71.2	39.9	16.0	-0.4
8% 4 hour	80.2	71.5	41.6	14.9	0.0
8% 6 hour	79.2	72.8	42.3	15.6	-0.5
8% 8 hour	79.2	71.1	42.0	15.6	-0.3

Appendix K Melting point analysis for reusability trial

K1 melting point analysis on reusability for interesterified tallow stearin and fully hardened coconut oil washed by ethanol

Number of wash	0	1	2	3	4	5	6
RM7.3(TSFHCO)4%8hrEthanol	44	44	44	44	47	48	52
TL7.3(TSFHCO)4%8hrEthanol	44	44	45	46	47	49	52

K2 melting point analysis on reusability for interesterified tallow stearin and fully hardened coconut oil washed by chloroform

Number of wash	0	1	2	3	4	5	6
RM7.3(TSFHCO)4%8hrChloroform	44	44	44	46	48	48	51
TL7.3(TSFHCO)4%8hrChloroform	44	44	44	47	48	48	50

K3 melting point analysis on reusability for interesterified tallow stearin and fully hardened coconut oil washed by iso-octane

Number of wash	0	1	2	3	4	5	6	7
RM7.3(TSFHCO)4%8hrIso-octane	44	44	44	45	47	48	49	51
TL7.3(TSFHCO)4%8hrIso-octane	44	44	44	45	48	48	48	51

K3 melting point analysis on reusability for interesterified tallow stearin and fully hardened coconut oil washed by Acetone

Number of wash	0	1	2	3	4	5	6	7	8	9	10
RM7.3(TSFHCO)4%8hrAcetone	44	44	44	44	44	44	44	45	45	48	51
TL7.3(TSFHCO)4%8hrAcetone	44	44	44	44	44	44	45	45	47	48	51

Appendix L HPLC analysis on triglycerides (TAG) composition on reusability trial

L1 TAG composition reusability trials on interesterified tallow stearin and fully hardened coconut oil washed by ethanol

ECN	NAME	Zero wash	First wash	Second wash	Third wash
24	CpCpCp	3.4	4.8	4.1	4.3
	RT7.1		1.2		1.3
30	CCC	3.4	4.3	4.2	4.7
	RT10.1	1.2	1.2	1.6	1.2
	RT11.2		2.3	1.4	
32	CCL	3.2	3.7	5.1	5.4
32	RT10.9	1.9	1.2	1.1	1.1
34	CLL	5.1	5.6	6.2	6.1
	RT13.9	1.4			
36	LLL	6.9	6.3	6.9	7.6
	RT20.1			1.0	
38	LLM	6.5	7.0	7.4	7.5
38	LLO	6.5	5.4	8.1	8.1
	RT37.2	3.6	3.9	1.1	
42	LMO	1.1	1.6		
40	LMM	6.3	7.1	9.3	9.0
42	RT50.8	2.9	3.9	4.2	4.3
42	LMP	7.1	6.2	2.7	
	RT74.2	2.3	1.2		
44	PLO	4.7	2.1		
44	PPL	5.3	2.1		
48	POO				
48	POP	12.6	14.6	16.2	17.3
48	PPP	12.7	13.3	15.3	16.2
50	POS			3.2	4.5

L2 TAG composition reusability trials on interesterified tallow stearin and fully hardened coconut oil washed by chloroform

ECN	NAME	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash
24	CpCpCp	3.2	3.2	3.2	3.3	3.0	3.9	4.2	4.3
	RT6.8								1.5
30	CCC	3.4	3.4	3.4	3.6	4.2	4.2	4.3	4.4
	RT9.4	1.4	1.5	1.6	1.1	1.9	2.2	1.8	1.1
	RT10.2	2.2	2.2	2.2	1.3				
32	CCL	3.4	3.4	3.4003	3.3	3.7	3.8	4.6	5.2
32	RT12.1	1.4	1.4	1.5	4.7	1.1	1.9	1.0	1.1
34	CLL	5.2	5.1	5.2	4.7	5.3	5.7	6.2	6.3
	RT16.5	1.4	1.3						
36	LLL	7.1	7.2	7.2	6.1	6.3	6.3	7.2	7.4
	RT20.0				1.8				
38	LLM	6.2	6.2	6.2	6.5	6.9	7.0	7.2	7.4
38	LLO	6.0	6.1	6.2	6.5	7.5	7.9	7.9	8.7
	LMM	3.2	3.2	3.2	2.8	2.3	1.5	1.0	
42	RT40.9	1.6	1.5	1.4	1.3				
42	LMO	1.1	1.1	1.2	1.2	1.9	3.7		
40	RT41.6							2.4	
42	LMM	6.1	6.1	6.2	6.7	7.4	8.2	8.7	9.2
42	RT51.2	2.7	2.7	2.6	3.4	3.6	4.0	4.2	4.5
44	LMP	7.8	7.7	7.6	6.2	5.1	3.3	1.6	
44	RT75.7	2.2	2.2	2.3	1.2	4.5	1.1		
44	PLO	4.2	4.3	4.1	3.9	3.2	2.4	1.1	
48	PPL	5.1	5.2	5.2	2.1	1.2			
48	POO								
48	POP	12.3	12.3	12.2	13.9	15.8	16.8	17.0	18.0
48	PPP	12.2	12.2	12.1	13.7	14.9	15.8	16.6	16.2
50	POS							2.2	4.2

L3 TAG composition reusability trials on interesterified tallow stearin and fully hardened coconut oil washed by iso-octane

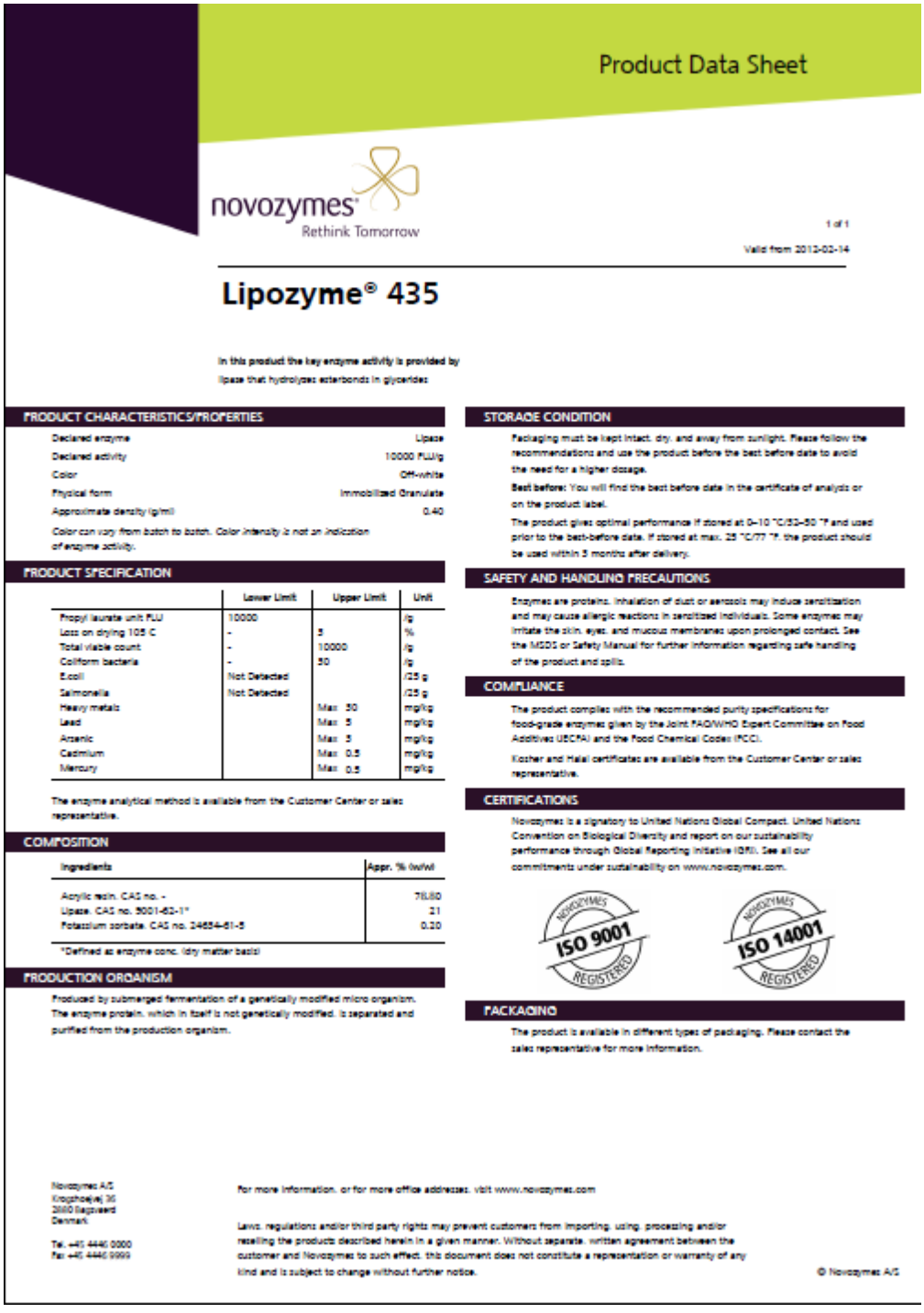
ECN	NAME	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash
24	CpCpCp	3.1	3.2	3.3	3.4	3.1	2.9	5.3	4.3
	RT6.1							1.3	1.0
30	CCC	3.0	3.1	3.3	3.5	3.5	4.7	4.9	4.9
	RT9.9	1.0	1.1	1.1		1.8	1.9	1.0	1.8
	RT10.5	2.2	2.1	2.3	4.4	4.3	3.2	1.0	
32	CCL	3.7	3.5	3.1	4.1	4.7	4.9	5.1	5.1
32	RT13.1	1.6	1.3	1.1	1.0	1.0			1.5
34	CLL	5.2	5.1	5.6	5.5	1.5	1.4	5.2	5.9
	RT15.3	1.3	1.3	1.3	2.6	1.9			
36	LLL	7.4	7.4	7.6	7.1	7.2	6.8	6.8	7.5
	RT22.2	1.3	1.2	1.3		3.5			
38	LLM	6.7	6.8	6.9	7.1	7.1	7.6	7.6	7.5
38	LLO	6.7	6.7	6.8	7.3	7.7	8.1	8.0	8.0
	RT35.9	3.6	3.3	3.0	2.6	2.0	1.1		
42									
42	LMO	1.2	1.3	1.3	1.1				
40	LMM	6.2	6.1	6.0	6.5	7.2	8.6	9.2	9.2
42	RT51.1	2.5	2.6	2.6	3.0	3.8	4.3	4.2	4.1
42	LMP	7.2	7.1	7.2	6.5	4.7	3.9		
44	RT73.4	1.1	1.5	1.9					
44	PLO	4.1	4.2	4.4	2.9	2.4	1.0		
44	PPL	5.2	5.3	5.3					
48	POO								
48	POP	12.4	12.6	12.1	15.2	16.4	17.5	17.9	17.6
48	PPP	12.0	12.0	11.2	14.9	15.1	16.18	16.2	16.7
50	POS						3.1	3.9	4.2

L4 TAG composition reusability trials on interesterified tallow stearin and fully hardened coconut oil washed by acetone

ECN	Name	Zero wash	First wash	Second wash	Third wash	Fourth wash	Fifth wash	Sixth wash	Seventh wash	Eighth wash	Ninth wash	Tenth wash
24	CpCpCp	3.2	3.2	3.4	3.3	3.3	3.4	3.3	3.4	3.7	4.1	4.4
	RT6.6											1.5
30	CCC	3.2	3.4	3.6	3.6	3.5	3.5	3.3	3.8	4.1	4.1	4.4
	RT9.4	1.3	1.3							1.0	1.2	1.2
	RT10.5	2.4	2.3	2.5	2.3	2.3	2.5	2.5	2.6	1.8	1.2	
32	CCL	3.5	3.6	3.6	3.5	3.7	3.4	3.6	3.3	4.7	5.1	5.3
32	RT11.2	1.2	1.3	1.3	1.9	2.0	2.2	2.4	2.0	1.5	1.1	1.0
34	CLL	5.3	5.3	5.1	5.2	5.3	5.1	5.2	5.2	4.5	5.5	6.3
36	RT15.7	1.2	1.2	1.2	1.3	1.3	1.2	1.2	1.3			
36	LLL	7.1	7.0	6.9	6.4	6.8	6.7	6.7	6.5	6.8	7.3	7.6
	RT19.6									1.1	1.2	
38	LLM	6.2	6.2	6.1	6.2	6.2	6.2	6.2	6.8	6.9	7.2	7.7
38	LLO	6.4	6.1	6.3	6.6	6.3	6.2	6.3	6.5	7.3	8.1	8.4
	RT36.2	3.4	3.3	1.4	1.6	1.7	1.7	1.7	1.6	1.3	1.1	
42	RT40.5	1.9	1.9	1.8	1.9	1.9	2.0	1.9	2.2	1.5		
42	LMO	1.1	1.1	1.1	1.1	1.0	1.0	1.1	1.1	1.1		
40	RT43.4			1.6	1.8	1.7	1.7	1.7	1.7	1.1		
42	LMM	6.1	6.1	6.4	6.4	6.4	6.5	6.5	6.4	6.9	8.1	9.3
42	RT50.6	2.7	2.8	2.7	2.7	2.5	2.4	2.4	2.2	2.6	3.5	4.4
44	LMP	7.3	7.5	7.6	7.2	7.7	7.5	7.6	7.1	3.9	2.0	
44	RT75.9	2.2	2.2	2.2	2.2	2.4	2.2	2.3	2.2	1.6	1.1	
44	PLO	4.2	4.2	4.1	4.4	4.5	4.2	4.2	4.0	1.3		
48	PPL	5.3	5.1	5.3	5.1	5.3	5.1	5.4	5.2	3.4	1.9	
48	POO									2.5		

Appendix M Enzyme specification sheet

M1Enzyme specification sheet for Novozyme 435



Product Data Sheet

novozymes
Rethink Tomorrow

1 of 1
Valid from 2013-02-14

Lipozyme® 435

In this product the key enzyme activity is provided by lipase that hydrolyzes esterbonds in glycerides

PRODUCT CHARACTERISTICS/PROPERTIES

Declared enzyme	Lipase
Declared activity	10000 FLU/g
Color	Off-white
Physical form	Immobilized Granulate
Approximate density (g/ml)	0.40

Color can vary from batch to batch. Color intensity is not an indication of enzyme activity.

PRODUCT SPECIFICATION

	Lower Limit	Upper Limit	Unit
Propyl laurate unit FLU	10000		/g
Loss on drying 105 C	-	5	%
Total viable count	-	10000	/g
Coliform bacteria	-	50	/g
E.coli	Not Detected		/25 g
Salmonella	Not Detected		/25 g
Heavy metals		Max 30	mg/kg
Lead		Max 5	mg/kg
Arsenic		Max 3	mg/kg
Cadmium		Max 0.5	mg/kg
Mercury		Max 0.5	mg/kg

The enzyme analytical method is available from the Customer Center or sales representative.

COMPOSITION

Ingredients	Appr. % (w/w)
Acrylic resin. CAS no. -	78.80
Lipase. CAS no. 9001-62-1*	21
Potassium sorbate. CAS no. 24634-61-5	0.20

*Defined as enzyme conc. (dry matter basis)

PRODUCTION ORGANISM

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.

STORAGE CONDITION

Packaging must be kept intact, dry, and away from sunlight. Please follow the recommendations and use the product before the best before date to avoid the need for a higher dosage.

Best before: You will find the best before date in the certificate of analysis or on the product label.

The product gives optimal performance if stored at 0–10 °C/32–50 °F and used prior to the best-before date. If stored at max. 25 °C/77 °F, the product should be used within 3 months after delivery.

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. See the MSDS or Safety Manual for further information regarding safe handling of the product and spills.

COMPLIANCE

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).

Kosher and Halal certificates are available from the Customer Center or sales representative.

CERTIFICATIONS

Novozymes is a signatory to United Nations Global Compact. United Nations Convention on Biological Diversity and report on our sustainability performance through Global Reporting Initiative (GRI). See all our commitments under sustainability on www.novozymes.com.

NOVOZYMES ISO 9001 REGISTERED

NOVOZYMES ISO 14001 REGISTERED

PACKAGING

The product is available in different types of packaging. Please contact the sales representative for more information.

Novozymes A/S
Krogshoejvej 36
2880 Bagsvaerd
Denmark

Tel. +45 4446 0000
Fax +45 4446 9999

For more information, or for more office addresses, visit www.novozymes.com

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M2Enzyme specification sheet for Lipozyme RM IM

Lipozyme® RM IM

Valid from 2011-09-30

Product Characteristics:

Declared enzyme	Lipase
Declared activity	275 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	275		/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.

M3 Enzyme specification sheet for Lipozyme TL IM

Lipozyme® TL IM

Valid from **2011-06-10**

Product Characteristics:

Declared enzyme	Lipase
Declared activity	250 IUN/g
Colour	Off-white Colour can vary from batch to batch. Colour intensity is not an indication of enzyme activity.
Physical form	Immobilized Granulate
Approximate density (g/ml)	0.40
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	250		/g
Loss on Drying 105 C	-	8	%
Laser Diffraction > 1180 micro	-	15	%
Laser Diffraction < 250 micron	-	10	%
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.