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Partitioning of Triacylglycerols in the Fractional Crystallisation of Palm Oil

Loughborough University

by

Elina Hishamuddin

A Doctoral Thesis submitted in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy of Loughborough University

May 2009

©E. Hishamuddin (2009)
To my parents
ABSTRACT

Palm oil is industrially fractionated on a large scale to yield a liquid olein (OL) product composed primarily of low melting triacylglycerols (TAGs) and a solid stearin (ST) product primarily of high melting TAGs. The physical and chemical properties of these fractionated products differ greatly from the original oil, and have added value. The aim of the work presented in this thesis is to study the partitioning of TAGs during the fractional crystallisation of palm oil and how this relates to their theoretical thermodynamic driving forces for crystallisation. Palm oil was studied under isothermal, non-isothermal and post-crystallisation stepwise remelting conditions. Filtered OL and ST products from the experiments were analysed for their TAG compositions by High Performance Liquid Chromatography (HPLC). Raw composition results showed fully saturated TAGs partitioning significantly to the ST phase, but little difference was observed in the compositions of the more unsaturated TAGs between the OL and ST (it would be expected that these would naturally concentrate in the OL). These observations are attributed to high levels of entrained liquid in the filter retentate, which has also been previously reported in the literature. A correction method based on the assumption that no triunsaturated TAGs should be able to crystallise to any significant extent was proposed to recalculate “true” ST compositions. These calculations indicated very high levels of entrainment (with the retentate possessing more liquid than solid), with typically only about 10% of palm oil TAGs crystallising despite forming a thick slurry. Although this assumption has not been directly verified, the corrected compositions showed behaviour that was very consistent with that which would be expected from thermodynamic driving force considerations. In the isothermal and non-isothermal studies conducted, the corrected ST composition revealed that PPP and other saturated TAGs showed the fastest transformation into the ST phase, followed by POP and other monounsaturated TAGs which predominated only once the saturated TAGs had been depleted from the OL phase. Slightly higher concentrations of PPP were achieved at higher isothermal temperatures (in isothermal studies) and lower cooling rates (in non-isothermal studies). Remelting studies on palm oil revealed that the melting process was largely dominated by trisaturated TAGs. This work has also demonstrated that the Focused Beam Reflectance Measurement (FBRM) technique was capable of detecting particle size and population numbers within the crystallising palm oil system and is a useful probe for detecting multiple events occurring in the crystalliser such as nucleation, melting, agglomeration and deagglomeration.
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NOMENCLATURE

$A$  Global kinetic coefficient / Surface area of a crystal / Peak area
$A'$  Global kinetic coefficient in melts
$A_{12}/A_{21}$ Binary interaction parameters
$B$  Nucleation rate
$C$  Concentration of solute
c*  Concentration of solute at saturation point
$C_s$  Chord
$C_p$  Molar heat capacity at constant pressure
$D$  Interfacial transport coefficient
$F$  Surface step density / Fraction
$f_w$  Experimental refractive index unit
$G$  Exponential term denoting order of overall growth process
$g'$  Exponential term denoting order of overall growth process in melts
$g_{E}$  Molar excess Gibbs energy
$\bar{g}_E$  Partial molar excess Gibbs energy
$g_i$  Crystal growth rate of species $i$
$G$  Gibbs free energy / Overall linear growth rate
$G'$  Activation energy of molecule between crystal-melt interface
$G^*_{crit}$  Critical excess free energy for heterogeneous nucleation
$G_{crit}$  Critical excess free energy
$G^e$  Excess Gibbs energy
$G^{ideal}$  Gibbs free energy in an ideal system
$G_s$  Surface excess free energy
$G_v$  Volume excess free energy
$h$  Planck's constant
$H$  Molar enthalpy
$H_f$  Heat of fusion
$H_m$  Melting enthalpy
$J$  Nucleation frequency
$k$  Boltzmann's constant / Upper channel number
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_b$</td>
<td>Constant related to secondary nucleation</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Coefficient of mass transfer by diffusion</td>
</tr>
<tr>
<td>$k_i$</td>
<td>Crystallisation rate coefficient</td>
</tr>
<tr>
<td>$k_r$</td>
<td>Rate constant for the surface reaction process</td>
</tr>
<tr>
<td>$K_G$</td>
<td>Overall crystal growth coefficient</td>
</tr>
<tr>
<td>$K'_G$</td>
<td>Overall growth mass transfer coefficient</td>
</tr>
<tr>
<td>$L$</td>
<td>A characteristic size of the crystal / Liquid phase</td>
</tr>
<tr>
<td>$m$</td>
<td>Mass of solid deposited</td>
</tr>
<tr>
<td>$m_E$</td>
<td>Mass fraction of entrained liquid</td>
</tr>
<tr>
<td>$m_{OL}$</td>
<td>Mass fraction of olein</td>
</tr>
<tr>
<td>$m_{PO}$</td>
<td>Total mass fraction of triacylglycerol in palm oil</td>
</tr>
<tr>
<td>$m_{ST}$</td>
<td>Mass fraction of stearin</td>
</tr>
<tr>
<td>$m_T$</td>
<td>Concentration of crystals in suspension</td>
</tr>
<tr>
<td>$M$</td>
<td>Midpoint of an individual channel</td>
</tr>
<tr>
<td>$M_i$</td>
<td>Initial mass</td>
</tr>
<tr>
<td>$M_f$</td>
<td>Final mass</td>
</tr>
<tr>
<td>$M_{OL}$</td>
<td>Mass of olein</td>
</tr>
<tr>
<td>$M_{ST}$</td>
<td>Mass of stearin</td>
</tr>
<tr>
<td>$n$</td>
<td>Number of moles of a component / Number of counts in channel / Filtration number</td>
</tr>
<tr>
<td>$N$</td>
<td>Measure of fluid mechanics interaction in secondary nucleation rate / Number of individual crystals / Net crystallisation flux / Time range</td>
</tr>
<tr>
<td>$N_A$</td>
<td>Avogadro number</td>
</tr>
<tr>
<td>$N_d$</td>
<td>Number of time steps</td>
</tr>
<tr>
<td>$N_R$</td>
<td>Number of Gaussians</td>
</tr>
<tr>
<td>$P$</td>
<td>Phase / Pressure</td>
</tr>
<tr>
<td>$P_s$</td>
<td>System pressure</td>
</tr>
<tr>
<td>$r$</td>
<td>Radius</td>
</tr>
<tr>
<td>$r_c$</td>
<td>Critical radius</td>
</tr>
<tr>
<td>$R$</td>
<td>Universal gas constant</td>
</tr>
<tr>
<td>$R_G$</td>
<td>Mass deposition rate</td>
</tr>
<tr>
<td>$s$</td>
<td>Chord length / Length square weight</td>
</tr>
</tbody>
</table>
List of Tables

\begin{itemize}
\item $S$: Supersaturation ratio / Molar entropy of pure component
\item $t$: Time
\item $t_f$: Filtration time
\item $T$: Equilibrium temperature
\item $T_i$: Isothermal temperature
\item $T^*$: Melting point of a crystal
\item $T_j$: Jacket temperature
\item $T_m$: Melting temperature
\item $T_{\text{off}}$: Offset temperature
\item $T_{\text{oil}}$: Oil temperature
\item $T_{\text{on}}$: Onset temperature
\item $T_{\text{sp}}$: Setpoint temperature
\item $\bar{v}$: Mean linear velocity
\item $v_b$: Velocity of scanning beam
\item $V$: Molar volume of a pure component
\item $w$: Weight of Gaussian / Composition of triacylglycerol in palm oil
\item $x$: Composition of a component / Composition of triacylglycerol in olein
\item $x_{\text{min}}$: Lower boundary
\item $x_{\text{max}}$: Upper boundary
\item $y$: Composition of triacylglycerol in stearin
\item $Y$: Percentage per channel
\item $z$: Composition of triacylglycerol in filter cake
\end{itemize}
Greek Symbols

\( \alpha \)  
Polymorph with the lowest stability and melting point / Volume shape factor

\( \beta' \)  
Polymorph with intermediate stability and melting point

\( \beta \)  
Polymorph with the highest stability and melting point / Surface shape factor

\( \gamma \)  
Interfacial energy / Activity coefficient of a component

\( \lambda \)  
Interfacial distance

\( \mu \)  
Mean of Gaussian

\( \mu^l \)  
Chemical potential of a component in liquid phase

\( \mu^s \)  
Chemical potential of a component in solid phase

\( \rho \)  
Density

\( \rho_c \)  
Crystal density

\( \rho^s \)  
Molar density

\( \sigma \)  
Relative supersaturation / Standard deviation of Gaussian

\( \phi \)  
Factor relating overall free energy change for heterogeneous nucleation with homogeneous nucleation

\( \theta \)  
Wetting angle

Abbreviations

DAG  
Diacylglycerol

DSC  
Differential scanning calorimetry

FBRM  
Focused beam reflectance measurement

HPLC  
High performance liquid chromatography

MAG  
Monoacylglycerol

TAG  
Triacylglycerol

SSS  
Trisaturated

SUS  
Disaturated

SUU  
Monosaturated

UUU  
Triunsaturated
1 INTRODUCTION

1.1 BACKGROUND TO THIS WORK

Palm oil is undoubtedly the largest produced vegetable oil in the world, accounting for about 60% of world vegetable oil exports (Carter et al., 2007). The predominance of this commodity over other vegetable oils has resulted from the exponential increase in its global output over the past three decades, with current production figures hovering well in excess of 40 million metric tonnes. The functionality of palm oil can be enhanced by various modification processes, the most widely practiced of which is through the fractionation of the constituent triacylglycerols (TAG). To date, palm oil in its modified form has been extensively utilised as raw material in the production of food and non-food products alike. This encompasses domestic consumables ranging from cooking and salad oils, margarines and shortenings to cosmetics, detergents and most recently in the development of biodiesel as fuel.

The fractionation process has become an increasingly important fat modification technique in the world of oils and fats. It principally involves the partial crystallisation of TAG components followed by physical separation of the crystallised portion of higher-melting TAGs from the lower melting liquid TAGs in the original oil (Deffense, 1985). The most widely practised fractionation technique is via the dry route. The advantages of dry fractionation over other techniques lie in its operation where it is considered an economical and environmentally-friendly process, in that it does not require the use of any chemicals or additives and post-treatment. In addition, dry fractionation is fully reversible, thus making it the preferred oil modification method most widely practiced around the world (Kellens et al., 2007).

The fundamental process that forms the core of fractionation is crystallisation. Melt crystallisation is driven by the differences between the melting points of the individual TAG components and the system temperature. The TAGs in palm oil span...
a wide range of (pure component) melting points both above and below the typical temperatures used in a fractionation vessel, leading to the preferential crystallisation of the higher melting TAGs. In fractionation, the partitioning of individual TAG components between the liquid (olein) and solid (stearin) phases during the crystallisation stage plays a major role in determining the quality and characteristics of the final products. In the last 30 years, various authors have reported findings on studies pertaining to the crystallisation behaviour and fractionation of palm oil (Jacobsberg & Oh, 1976; Kawamura, 1979 & 1980; Deffense, 1985; Ng & Oh, 1994; Zaliha et al., 2004; Kellens et al., 2007). Although these studies have provided a substantial amount of information on this topic, the majority are, however, focused on palm oil crystallisation in static conditions for very small quantities of palm oil. Indeed most crystallisation studies of oils and fats in general have been performed on static systems using various techniques such as differential scanning calorimetry (DSC), polarised light microscopy (PLM), laser particle counters, synchroton X-ray diffraction (XRD) and scanning electron microscopy (SEM). Such systems allow well controlled and precise measurements to be made but are a far cry from imitating the highly agitated processes carried out in the industry. There is, however, a lack of systematic studies on the crystallisation and partitioning behaviour of TAGs within palm oil under stirring conditions, particularly looking at the effect of temperature and cooling conditions. The main difficulty in studying the crystallisation of oils and fats in stirred systems is due to the limited number of instrumentation capable of providing sound on-line information on crystal behaviour.

The Focused Beam Reflectance Measurement (FBRM) technique has been widely utilised as a tool for the real time and in situ monitoring of particulate characteristics in various industrial processes such as crystallisation/precipitation, flocculation, grinding and has seen vast application in the pharmaceutical and petroleum industries (Heath et al., 2002). What is unique about FBRM is that there are not many devices which can provide continuous in situ monitoring of particles concentration, size and behaviour in real time. The advantage of FBRM over more conventional particle analysis techniques lies in its ability to characterize particles during the crystallisation process and no sampling or dilution is required as these are often not representative of the actual conditions during the process and may alter particle characteristics. Despite

Partitioning of triacylglycerols in the fractional crystallisation of palm oil
these facts, the application of FBRM in the crystallisation of oils and fats from the melt in general, and in palm oil systems has yet to be explored.

Thermodynamic theories in solid-liquid separation systems and in particular, fat crystallisation have shown that the transformation of liquid to solid is principally governed by the differences in the chemical potential driving forces of the TAG components between these two phases (Himawan et al., 2006). However, whilst this fundamental concept has long been established, there is a lack of published studies on the crystallisation driving forces of components with respect to the crystallisation of oils and fats and its application in palm oil crystallisation in stirred systems is unprecedented.

Hence, in order to fill the gaps within this area, the research study presented in this thesis is aimed at investigating the effects of crystallisation temperature and cooling rate conditions on the partitioning behaviour of individual TAG components in palm oil and how this relates to the chemical potential driving force for crystallisation. The identification of the partitioning tendency of the TAG components in fractional crystallisation will facilitate better understanding of the crystallisation behaviour of TAGs and thus allowing better control of the desired properties and quality of the final oil. This will further equip us with knowledge on determining the process conditions required during the fractional crystallisation process which is crucial for producing products for its intended end use. It is hoped that results from this study will benefit the palm oil industry in extending current knowledge on the thermodynamic fundamentals of the fractionation of palm TAGs and be utilised to design products with tailor-made quality and specifications.
1.2 OBJECTIVES OF PRESENT STUDY

The main objectives of conducting this research are, but not limited to, the following:

i. To study the effects of crystallisation temperature on the partitioning of individual lipids in the fractional crystallisation process of RBD palm oil, investigate the fractionation behaviour of triacylglycerols (TAGs) under isothermal, non-isothermal and post-crystallisation stepwise remelting conditions and the factors responsible,

ii. To quantify the driving force for crystallisation of palm oil TAGs using selected thermodynamic expressions based upon chemical potential and how this relates to the partitioning behaviour of TAGs,

iii. To investigate the capability of Focused Beam Reflectance Measurement (FBRM) method as a tool in monitoring the evolution of palm oil crystallisation and in the detection of crystallisation events, whether useful information can be obtained via this method.

This research study was carried out on a lab-scale crystallisation system using a stirred vessel with palm oil as the material under study. Palm oil fractionation experiments at isothermal, non-isothermal and post-crystallisation stepwise remelting conditions were conducted within this system and an FBRM probe was used as the main particulate characterisation and monitoring tool. The crystallising slurry was sampled at different time intervals and filtered to separate the liquid olein (OL) from the solid stearin (ST). The OL and ST products were then analysed for their TAG composition by High Performance Liquid Chromatography (HPLC). The partitioning behaviour of TAGs and their approximate chemical potential driving forces for crystallisation were investigated and quantified based on the compositional data provided by HPLC.
1.3 ORGANISATION OF THE DISSERTATION

The thesis comprises seven chapters, the contents of which are presented in the following order:

Chapter 1  contains the introduction to the thesis, explaining the background to this work and the main objectives of the study with a detailed outline of the thesis.

Chapter 2  reviews the relevant literature beginning with an introduction on palm oil followed by the fractionation process, with an elaborate study on literature comprising fundamental theories and thermodynamic aspects of fat crystallisation.

Chapter 3  provides a detailed description of the experimental and analytical tools and techniques utilised in this work, which mainly comprise the High-Performance Liquid Chromatography (HPLC) and Focused Beam Reflectance Measurement (FBRM) techniques. Methods for the treatment of experimental data shall also be discussed.

Chapter 4  discusses the results from the study of the influence of temperature on the isothermal crystallisation on palm oil under shear. The chapter begins with results from FBRM response followed by the measured HPLC composition of TAGs. Subsequently, further analysis on the experimental data to quantify the crystallisation driving forces of TAGs and their growth rate behaviour shall be discussed and the effect of entrainment of liquid in the solid fraction that is obtained from filtration on these shall be assessed.

Chapter 5  presents the results obtained from the study on the influence of cooling rate on the non-isothermal crystallisation behaviour of palm oil under shear. The FBRM response, measure HPLC composition of TAGs and the quantification of crystallisation driving force of TAGs based on
experimental data shall be elaborated upon, together with the effect of liquid entrainment.

Chapter 6 introduces a new study on the post-crystallisation stepwise remelting of palm oil and presents results from the study of the effects of successive temperature increases on the TAGs composition and behaviour and the FBRM response associated with it.

Chapter 7 provides a general conclusion on the whole research study contained within this thesis and provides recommendations for future work.
2 LITERATURE REVIEW

2.1 PALM OIL

2.1.1 Introduction

Palm oil originates from the mesocarp of the African oil palm fruit, scientifically known as *Elaeis Guineensis* Jacq. This fruit bears two distinct types of commodity oils, crude palm oil from the fruit mesocarp and crude palm kernel oil from the kernel nut (see Figure 2-1). Oil palm fruits grow in bunches and each fruit bunch may contain about 200 to 2000 individual fruitlets with bunch weights varying from 4 to 20 kg (Gunstone & Harwood, 2007). Oil palm trees are grown on plantations in tropical countries such as Malaysia, Indonesia, Nigeria, Ivory Coast and Colombia. Malaysia is currently the largest producer and exporter of palm oil in the world. The main type of oil palm species grown in Malaysia is of the *Tenera* species, which is a hybrid of the *Dura* and *Pisifera* types. This species is preferred due to its thinner inner shell and thicker mesocarp compared to its parent species, thus producing more oil. The oil palm is a perennial crop and a tree can produce the two oil crops for up to 25 years.

Figure 2-1  An oil palm tree and oil palm fruit, showing the central kernel surrounded by the mesocarp

Partitioning of triacylglycerols in the fractional crystallisation of palm oil

7
2.1.2 Oil palm milling

Once planted, the trees take about 3 to 4 years to mature and bear fruit. Oil palm trees are harvested once the fruit bunches ripen. These fruit bunches are immediately transported to the mill by lorries or rail trucks for further processing. A schematic flow diagram of the processing of the oil palm fruit until the final refined, bleached and deodorised (RBD) product is outlined in Figure 2-2.

![Flow diagram for processing the oil palm fruit](image)

**Figure 2-2  Flow diagram for processing the oil palm fruit**

The first stage of processing involves steam sterilization of the fresh fruit bunches for about an hour at temperatures between 130 and 145 °C. This process inactivates fungal lipases that would otherwise rapidly hydrolyze the oil (Johnson, 2000). It also acts to loosen the fruits from the bunch stalks for ease of subsequent digestion. The
sterilised fruits are subsequently stripped from the bunch stalks using drum-type strippers. The loose fruits are then transported to a digestion unit where the fruits are reheated to a temperature of 95 to 100 °C for 20 to 30 minutes in order to loosen the pericarp from the nut and break the oil cells.

The digested pulp is conveyed to a continuous screw press where extraction of the oil is carried out. The product of the screw-press, known as the liquor, contains approximately 66% oil, 10% solids and 24% moisture (Basiron, 1996). These are fed through a vibrating screen and into settling tanks where a clarification process takes place, facilitated by the addition of water. After a residence time of about 2 hours, the top oil layer is skimmed off by centrifugation and dried under vacuum to a moisture content of between 0.10 to 0.15%. The remaining sludge is also centrifuged to recover any trapped oil and the recovered oil is recycled back into the clarifying tanks. The clarified oil is pumped to storage tanks to await transportation to ports or local manufacturers. Table 2-1 tabulates the characteristics of a typical batch of crude palm oil from a palm oil mill (Yusof, 2000).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids (%)</td>
<td>3.79</td>
</tr>
<tr>
<td>Peroxide value (PV)</td>
<td>2.80</td>
</tr>
<tr>
<td>Anisidine value</td>
<td>3.30</td>
</tr>
<tr>
<td>Carotenoids (ppm)</td>
<td>&gt;600</td>
</tr>
<tr>
<td>Deterioration of Bleachability Index (DOBI)</td>
<td>2.70</td>
</tr>
<tr>
<td>Iodine value</td>
<td>52.5</td>
</tr>
<tr>
<td>Saponification value</td>
<td>197</td>
</tr>
<tr>
<td>Moisture and impurities (%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Unsaponifiable matter (%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>36.50</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>0.12</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>4.20</td>
</tr>
<tr>
<td>Phosphatides (ppm)</td>
<td>694</td>
</tr>
</tbody>
</table>
The press cake from the screw press, which contains a mixture of fiber and nuts, are sent to a kernel processing plant where the nuts and fiber are separated. The fiber is usually used as boiler feed for steam generation in the palm oil mill while the nuts are sent to a kernel treatment plant for kernel recovery.

### 2.1.3 Crude palm oil refining and processing

Palm oil in its crude form bears a natural deep red colour due to a high carotenoid content of about 600 to 700 ppm. It contains small amounts of minor components such as free fatty acids, chlorophylls, phosphatides, metals, waxes, pigments and other ‘undesirable’ materials. These minor components, if not removed, give rise to oil quality deterioration, particularly in lowering the oxidative stability of the oil. It is important that these undesirable components are removed or inactivated in order to maintain the stability of the oil and to produce a clean, attractive looking and palatable oil (Yusof, 2000). Therefore, crude palm oil undergoes a further refining process in a dedicated refinery to make it edible and consumable.

There are two main methods of crude palm oil refining, namely physical/steam refining and chemical/alkali refining, each differing in the way the free fatty acids are removed. The former employs steam at high temperatures and under vacuum conditions to remove free fatty acids while the latter uses alkali to neutralize them. The main method of crude palm oil refining most widely practiced in Malaysia is physical/steam refining. This method does not involve effluent handling and has low operation costs when compared to its chemical counterpart (Kheiri, 1985).

The physical refining process can be divided into three stages; degumming, bleaching and deodorization. Degumming is a pre-treatment stage in which gums present as phosphatides and other polar lipids are agglomerated and precipitated with the introduction of a concentrated acidic solution. The crude oil is fed into a stirred conditioning tank where the oil is pre-heated to a temperature of between 90 to 110 °C before a 0.05% to 0.20% acid solution containing about 85% food grade phosphoric acid or citric acid is added to react with the oil for half an hour.
The second stage involves a bleaching process with the primary purpose of removing colour pigments and other undesirable impurities such as trace metals, chlorophylls, sulphur compounds and peroxides. It also extracts the gums precipitated from the preceding stage. This process takes place in a stirred bleaching tank where 0.2 to 2.0% of acid-activated bleaching earth is added to the oil. A temperature of approximately 95 °C is usually employed under vacuum conditions of 20 to 25 mmHg for 30 minutes (Yusoff, 2000). The bleached oil is usually filtered after being cooled to 60 to 70 °C and the remaining spent earth retained on the filter chamber is blown with nitrogen to expel entrained oil.

The final stage in crude palm oil refining is the deodorization step. This step serves as a deacidification process as free fatty acids are mainly removed together with mono- and diglycerides, oxidation products and pigment decomposition products that would otherwise impart undesired odour and flavour to the oil. Deodorization of the pretreated oil mainly incorporates the application of distillation at a high temperature i.e. between 200 to 260 °C and a low vacuum. Either high pressure steam or a thermal heating fluid is used to achieve these high temperatures (Allen, 1997). The oil is then cooled and sent for storage. The final oil product coming from this stage is termed as refined, bleached and deodorized (RBD) palm oil and is ready for shipment and used for conversion to edible or non-edible food products. Table 2-2 outlines a typical specification for RBD palm oil in terms of export quality.

Table 2-2 Typical specifications for RBD palm oil

<table>
<thead>
<tr>
<th>Quality Parameters</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Fatty Acids (FFA as palmitic)</td>
<td>0.1% max.</td>
</tr>
<tr>
<td>Moisture &amp; Impurities (M&amp;I)</td>
<td>0.1% max.</td>
</tr>
<tr>
<td>Iodine Value (Wijs)</td>
<td>50 – 55</td>
</tr>
<tr>
<td>Melting Point (°C) (AOCS Cc 3-25)</td>
<td>33 – 39</td>
</tr>
<tr>
<td>Colour (5 ¼” Lovibond cell)</td>
<td>3 Red max.</td>
</tr>
</tbody>
</table>
2.1.4 Palm oil production

Although the first palm oil refinery was only established in the early 1970’s, it is now a high volume product. In the year 2008, the world production of palm oil reached 42.8 million metric tonnes, making it the most produced vegetable oil in the world (USDA, 2008). This accounted for more than 32% of the world production of major vegetable oils in 2008, as illustrated in Figure 2-3. Palm oil can be utilised to produce a variety of food applications, ranging from cooking and salad oils to margarines and shortenings and also a number of non-food applications such as providing raw materials for the oleochemical, cosmetics and biodiesel industry.

![Figure 2-3 World production of major vegetable oils in 2008 (USDA, 2008)](image)

The remarkable increase in palm oil production signifies its importance as one of the world’s two main commodity crops, along with soybean oil. Various modification processes (including fractionation and interesterification) allows palm oil to be applied in many diverse areas with both edible and non-edible usages. Hence, there is a lot of motivation to research and explore the vast possibilities that palm oil modification processes can offer. However, before venturing into the topic on palm oil modification, with particular emphasis on the crystallisation and fractionation process, it is first worth examining the major components that make up the composition of this highly versatile oil.
2.2 CHEMICAL COMPOSITIONS OF PALM OIL

2.2.1 Introduction

Oils and fats are made up of a number of different lipid components. Triacylglycerols (TAGs) form a major part of the total composition of an oil or fat, accounting for about 94% of its chemical composition while the rest include minor components such as partial acylglycerols i.e. mono- and diacylglycerols (MAG & DAG), carotenes, sterols and phospholipids. The chemical composition of an oil or fat is the main determinant of its physical behaviour. Hence, it is important to understand the fundamental properties of the main components of oils and fats in order to determine the physical and chemical behaviour of oils and fats. This section shall present the chemical constituents of palm oil, starting with an introduction on fatty acids followed by the main TAG components of palm oil.

2.2.2 Fatty acids

Fatty acids are the starting points in lipid structure (O’Keefe, 2002). They are the main components forming TAGs, MAGs and DAGs in oils and fats. Their structure consists of a carbon chain of variable length with a carboxylic acid group attached at the end. Stearic acid and oleic acid both have 18 carbon atoms as illustrated in Figure 4, however the latter bears one double bond while the former doesn’t.

![Chemical Structures](image)

(a) Stearic acid

(b) Oleic acid

Figure 2-4 Chemical structures of (a) stearic acid and (b) oleic acid

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Fatty acids can be divided into two main classes which is of relevance to this work; namely saturated (which has no double bonds) and unsaturated which has one or more double bonds. A fatty acid bearing only one double bond is termed monounsaturated while the presence of two or more double bonds in a fatty acid makes it a polyunsaturated fatty acid. Table 2-3 lists some common fatty acids found in oils and fats and their abbreviations, along with the length of and number of double bonds in the carbon chain.

Table 2-3  Common fatty acids found in oils and fats

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Carbon number : Number of double bonds</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric</td>
<td>12:0</td>
<td>La</td>
</tr>
<tr>
<td>Myristic</td>
<td>14:0</td>
<td>My</td>
</tr>
<tr>
<td>Palmitic</td>
<td>16:0</td>
<td>P</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1</td>
<td>PO</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:0</td>
<td>S</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:1</td>
<td>O</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:2</td>
<td>L</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:3</td>
<td>Ln</td>
</tr>
<tr>
<td>Arachidic</td>
<td>20:0</td>
<td>A</td>
</tr>
<tr>
<td>Erucic</td>
<td>22:0</td>
<td>E</td>
</tr>
</tbody>
</table>

There are nine major fatty acids present in palm oil. Palmitic acid and oleic acid account for about 44% and 40% of the total fatty acid content of palm oil respectively. Other fatty acid constituents of palm oil include myristic, stearic and linoleic acids with trace amounts of lauric, palmitoleic, linolenic and arachidic acids. Table 2-4 tabulates the range of common fatty acids found in palm oil (Gunstone & Harwood, 2007).

RBD palm oil possesses approximately equal amounts of saturated and unsaturated fatty acids. This gives the oil a naturally semi-solid form at room temperature (Deffense, 1985). This inherent property is the reason why the base oil has few uses
as such. Hence, further processing of palm oil is required to render it suitable for use in the food industry by splitting it into liquid and solid fractions. It is also the only vegetable oil to contain a significant amount (12%) of saturated acids in the 2-position of the TAG.

**Table 2-4 Fatty acids in palm oil (Gunstone & Harwood, 2007)**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Carbon number :</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of double bonds</td>
</tr>
<tr>
<td>Lauric</td>
<td>12:0</td>
</tr>
<tr>
<td>Myristic</td>
<td>14:0</td>
</tr>
<tr>
<td>Palmitic</td>
<td>16:0</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:0</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:1</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:2</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:3</td>
</tr>
<tr>
<td>Arachidic</td>
<td>20:0</td>
</tr>
</tbody>
</table>

*ND : not detected, TR : trace levels

**2.2.3 Triacylglycerols (TAGs)**

Triacylglycerols (TAGs) consist of three fatty acid molecules esterified with a glycerol molecule. Glycerol molecules may also combine with less than three fatty acids to form what are termed as partial glycerols, namely monoacylglycerols (MAG) and diacylglycerols (DAG). The nature of the constituent fatty acids of a specific TAG determines its physical and biological properties (Mann *et al.*, 2001). Figure 2-5 illustrates the formation of a TAG.

In this work, TAGs will be identified by a 3-letter character abbreviating the 3 types of fatty acids esterified to the glycerol backbone. For example, a TAG with palmitic, oleic and stearic acids at the 1, 2 and 3 positions at the glycerol molecule shall be
represented by POS. This annotation, termed regiospecific, does not distinguish between positions 1 and 3 of the glycerol molecule.

![Chemical structure of glycerol and fatty acids forming triacylglycerol](image)

**Figure 2-5  Formation of a triacylglycerol molecule (Mann et al., 2001)**

TAGs can be classified according to their degree of saturation: trisaturated (SSS), disaturated (SSU or SUS), monosaturated (SUU) and triunsaturated (UUU) TAGs. Trisaturated TAGs exist when all the fatty acids on the glycerol molecule are from the saturated species. Some examples of SSS TAGs are tripalmitin (PPP), tristearin (StStSt) and trilaurin (LLL). Disaturated TAGs contain two saturated fatty acids constituents whereas monosaturated TAGs only have one saturated fatty acid on the glycerol backbone. When all the fatty acids attached to the glycerol backbone consist of only unsaturated fatty acids, these form a UUU TAG. In general, the greater the degree of unsaturation, the lower the melting point of the TAG. The fatty acids that make up a single TAG molecule may be distributed in any position and TAGs may contain different mixtures of saturated and unsaturated fatty acids.

Palm oil can be considered to consist of three main types of triacylglycerols: trisaturated (mainly PPP), disaturated (mainly POP but significant amounts of PLinP and PPO) and monosaturated (mainly POO but some OPO and PLinO) (Timms, 1984). Table 2-5 tabulates the main TAGs found in palm oil (Smith, 2001). It can be seen that the two dominant TAGs present in palm oil are POP (24%) and POO (20%). This is followed by PLP (7%), POS (6%), PLO (6%), PPP (5%), PPO (4%), OOO (4%) and POL (4%). The remaining TAGs are present at less than 3%.
Table 2-5 Triacylglycerol Composition of Palm Oil (Smith, 2001)*

<table>
<thead>
<tr>
<th>TAG</th>
<th>wt%</th>
<th>TAG</th>
<th>wt%</th>
<th>TAG</th>
<th>wt%</th>
<th>TAG</th>
<th>wt%</th>
<th>TAG</th>
<th>wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 DB</td>
<td>7.4</td>
<td>1 DB</td>
<td>36.8</td>
<td>2 DB</td>
<td>34.0</td>
<td>3 DB</td>
<td>16.0</td>
<td>&gt;3 DB</td>
<td>5.6</td>
</tr>
<tr>
<td>PPP</td>
<td>5.1</td>
<td>MOP</td>
<td>0.9</td>
<td>POO</td>
<td>20.3</td>
<td>OOO</td>
<td>4.4</td>
<td>LOO</td>
<td>1.8</td>
</tr>
<tr>
<td>PPS</td>
<td>1.2</td>
<td>POP</td>
<td>23.7</td>
<td>SOO</td>
<td>2.4</td>
<td>POL</td>
<td>4.1</td>
<td>OLO</td>
<td>1.2</td>
</tr>
<tr>
<td>PSP</td>
<td>0.3</td>
<td>POS</td>
<td>5.7</td>
<td>OPO</td>
<td>1.0</td>
<td>PLO</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td>4.4</td>
<td>PLP</td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSO</td>
<td>0.2</td>
<td>SLP</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DB = double bonds; M = myristic; P = palmitic; S = stearic; O = oleic; L = linoleic.

2.2.4 Modifying the chemical composition of palm oil

Having reviewed the chemical composition of palm oil, it has been shown that palm oil contains a diverse range of TAGs which largely contribute to its distinct physical properties, one of them being its semi-solid nature at room temperature. Palm oil in its natural state has very limited use and requires modification to its physical and chemical properties to increase suitability for use in a variety of food and non-food applications. Palm oil can be modified through a number of different techniques, namely by (1) blending with other oils and fats to alter the fatty acid composition in the oil, (2) hydrogenation, which reduces the degree of unsaturation of the acyl groups in the oil, thus hardening and increasing its resistance towards oxidation, (3) interesterification, which involves redistributing the fatty acids positions on the TAG molecule and (4) fractionation, a process coupling partial crystallisation of the oil followed by physical separation of the crystallised fraction from the uncrystallised liquid portion (Kellens et al., 2007).

In general, the process of blending different oils and fats is the simplest and cheapest process among the four modification processes previously mentioned and requires two or more types of oils or fats to produce a blend with a specific composition (Djikstra, 2007). Due to the distinct semi-solid characteristic of palm oil, the solid fraction can be added into more liquid oils to enhance the hardness and plasticity in...
margarine and shortening formulations of these more unsaturated oils while unsaturated oils can be added to the liquid fraction of palm oil to increase its resistance against clouding in more temperate countries. Over the last 25 years, the specifications for oil blends have changed substantially to meet consumer demands of fat products with increased nutritional values and functionality. The demand for fat products with specific requirements however, has less chance to be met by merely blending natural oils and fats (Allen, 1998). Moreover, raw material costs play a large part in determining the final product cost and more often the selling cost of the final product from blending would be higher than the original individual raw materials used. Hence, in order to achieve consumer-oriented product specifications while at the same time reducing the on-shelf costs, other modification processes need to be applied or combined with blending.

Hydrogenation has been applied in the modification of oils and fats since the early 1900’s and involves the addition of hydrogen atoms to the double bonds in the unsaturated fatty acid chains of TAGs in oils and fats, in the presence of a metal catalyst, to increase its saturation level. This process is only partially done and is mainly carried out to harden more liquid oils (i.e. olive oil, soybean oil, canola oil) to enable further use in the production of margarines and shortenings. Partial hydrogenation also increases the resistance to oxidation, hence making it stable for use in the manufacturing of food products, thereby prolonging their shelf life. However, this type of modification process also forms trans isomers of the fatty acid chains, which have been proven to increase the risk of coronary heart disease (Willett et al., 1993; Mozaffarian et al., 2006). For the past decade, the oils and fats industry have begun to look into possible alternatives in replacing products containing trans fats due to growing health concerns associated with partially hydrogenated fats. New legislation for the reduction or complete elimination of trans fats from food supplies have already been in enacted in countries such as Denmark, USA and Canada. From this fact, it seems that the future of hydrogenation as a modification process for oils and fats is very limited.

Interesterification was initially introduced into the oils and fats industry to provide an alternative to hydrogenation which would reduce or eliminate the formation of trans fatty acids. This process involves the rearrangement of the fatty acids distribution
within and between the TAGs components either via a chemical route or an enzymatic route. Chemical interesterification (CIE) utilises a metal alcoholate catalyst which randomly distributes fatty acids among all possible TAG positions whereas enzymatic interesterification (EIE) employs lipase catalysts which can either be random or regiospecific (1,3- or 2-specific) or fatty-acid-specific (Marangoni & Rousseau, 1995). Some of the applications of interesterified oils and fats besides replacing hydrogenation to produce zero-trans fats for margarine and shortening applications include (i) the manufacture of cheap substitutes for cocoa butter, (ii) infant formula, (iii) production of nutritionally enhanced food products by the incorporating essential fatty acids (EFAs) such as ω-3 fatty acids into spreadable fats, and (iv) the synthesis of tailor-made TAGs with a specific fatty acid composition and distribution within the TAG molecule (Marangoni & Rousseau, 1995; Osborn & Akoh, 2002). In general, both interesterification processes offer the possibility to produce fats with improved functionality and nutritional values. On the other hand, CIE results in a lack of specificity, production of undesired side products and the uncontrollable distribution of the positions of fatty acids in the TAGs while EIE incurs expensive lipase catalyst costs required for its operation. Due to the lack of research on the behaviour and application of newly structured TAG in food products, the application of interesterification in modifying oils and fats is quite limited.

Fractionation is the most popular of all oils and fats modification processes. Its mechanism largely depends on the ability of TAGs to form crystals of a different composition to the original mother oil when subjected to cooling in a controlled manner. The crystals are then separated from the uncrystallised portion of the oil, thereby producing two different fractions with physical and chemical properties that differ greatly from the original mother oil. Fractionation improves the functionality and characteristics of oils and fats, extending their utilisation in edible and non-edible applications which may have not been possible in its original form. Since the late 19th century, fractionation has been widely applied to the production of feedstocks for margarine, shortenings, frying oils and salad oils. Due to its importance to this thesis, fractionation shall now be dealt with and elaborated upon further in the next section.
2.3 FRACTIONATION PROCESSES

2.3.1 Overview

The fractionation process has been extensively reviewed by several authors in the last decade (Kellens & Krishnamurthy, 1994; Gibon & Tirtaux, 2002; Timms, 2005). The principle of fractionation is the partial crystallisation of the oil followed by separation of the crystallised phase from the remaining liquor by various filtration techniques. This process is largely based on the differences in the melting points of the constituent TAGs in an oil or fat. The resulting products are a liquid phase and a solid phase which are termed olein and stearin respectively.

There are three types of fractionation processes that are commonly encountered within the oils and fats industry. These are solvent fractionation, detergent fractionation and dry fractionation. These methods of fractionation differ in their crystallisation and separation methods, whether using a solvent, a detergent or by purely mechanical means respectively. Each fractionation method shall be reviewed separately in further detail.

2.3.2 Detergent fractionation

In detergent fractionation processes, also known as the Lanza or Alfa-Laval Lipofrac processes, a detergent is added into the bulk oil to aid separation of the olein and stearin phases (Smith, 2001). The oil is initially melted and slowly cooled to crystallise high melting TAGs. At the end of the crystallisation time, an aqueous detergent (surface active agent) solution of 0.5% sodium lauryl sulphate with magnesium or sodium sulphate is added to wet the crystals formed, permitting them to suspend in the aqueous phase. Separation is carried out by centrifugation whereby the olein fraction is separated from the aqueous phase containing the stearin crystals. The aqueous phase and stearin are subsequently heated to melt the crystals. The mixture is later centrifuged to recover the melted oil phase from the aqueous detergent phase, which is cooled and recycled back into the process. Removal of the detergent from the

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respective olein and stearin fractions is achieved by hot water washing followed by drying under vacuum.

Detergent fractionation provides improved separation using the centrifuge compared to the pure dry process. However, its main disadvantage is the use of detergents. The final products require exhaustive washing, which is sometimes inefficient in completely removing these materials. In addition, even trace amounts of detergents in the products pose detrimental effects to health (Bernardini & Bernardini, 1975). The combination of dry fractionation and the membrane filter press (see section 2.3.4) have made the detergent process obsolete due to the huge reduction in operating costs involving effluent problems and it avoids using additives which are harmful to health if present in the final product to a significant extent.

2.3.3 Solvent fractionation

Solvent fractionation involves the addition of a solvent (typically hexane or acetone) to the bulk oil to crystallise the olein phase from the stearin phase. These solvents have high selectivity towards certain components, for example hexane can be utilised to obtain high quality olein where diacylglycerols are removed in the stearin (Smith, 2001).

This process starts out by mixing crude or refined oil with a solvent followed by cooling in a scraped surface heat exchanger. Brine or ammonia is usually the medium used for cooling. When crystallisation is complete, a vacuum is applied to the slurry to ease filtration and the cake is subsequently washed with clean, chilled solvent to remove the entrained olein. The solvent is then removed from the fractions by distillation. This process enables good separation of the solid and liquid phases because the entrained liquid phase in the filter cake is a solution of the olein and the solvent.

Solvent fractionation has a high selectivity and offers a cleaner separation of desired components compared to other fractionation techniques. Although solvent
fractionation offers distinct advantages over dry fractionation in terms of improved product quality, the main disadvantages of this process include post-treatment of finished products and the need for explosion-proof equipment, which both make this fractionation process the most expensive compared to the others. Nevertheless, it still finds popularity in the processing of high value-added products, which require specific compositions of certain components, such as in the production of Saturated-Unsaturated-Saturated (SUS) type fats for specialty fats such as cocoa butter equivalents (CBE).

### 2.3.4 Dry fractionation

Fractionation using the dry method involves heating the feed oil to a temperature of 70-80°C in the crystallizer prior to cooling of the melt. This is done in order to completely melt all solid fat ensuring that no nuclei are present, i.e. erasing any crystals or crystal memory from the oil which would subsequently affect the crystallisation process. The bulk oil is then cooled slowly using chilled water according to a predetermined cooling programme in which the oil is continuously agitated in order to produce a homogeneous slurry of crystals. During this cooling stage, careful control of processing parameters such as oil temperature, agitator speed and the temperature of the cooling medium are carried out. Once the desired final fractionation temperature has been reached, the crystallized slurry containing a mixture of stearin crystals and liquid olein is discharged from the crystallizer into filtration equipment for the subsequent separation stage.

This fractionation technique has the advantage of being a pure modification process without the use of any chemicals or additives compared to its other counterparts. Besides its full reversibility, it is the only fractionation process known to not impose any environmental hazards, thus making it the preferred and most widely practised oil modification method around the world.
2.3.5 Separation

Separation is the second stage involved in fractionation processes. In this stage, solid TAG crystals are separated from the remaining liquid TAGs at the crystallising temperature in order to yield the olein and stearin fractions. It is also important to note that there are also liquid TAGs which remain trapped in the solid TAG crystals which also need to be removed and this is known as entrainment. The problem of entrainment will be elaborated further in the next section. The separation stage is thus as important as the crystallisation stage since the end product quality highly depends on minimising the entrainment of either phase in the other.

There have been several types of separation processes commercially applied and they range from vacuum drum filtration, vacuum belt filtration, decanting, centrifuging, hydraulic press and membrane filter pressing. Filtration of the solid phase from the liquid olein is usually carried out utilizing filters ranging from Florentine filters, rotary drum filters or membrane filters. Florentine filters were developed by S. A. Fractionnement Tirtiaux and employ an endless rotating, stainless steel perforated belt where filtration takes place horizontally and continuously under slight vacuum conditions. Membrane filters consist of plate and frame chambers where the membrane is inflated to apply pressure in order to expel the olein from the stearin cake when the chambers are full of stearin (Deffense, 1985). Nowadays, the membrane filter press is the much preferred separation method for fractionation as higher olein yields can be achieved and entrainment levels are significantly reduced with the increase in squeezing pressure, as observed during the fractionation of palm oil, as shown in Table 2-6 (Timms, 2005; Kellens et al., 2007). The liquid oil entrained in filter cake in this case is defined as the ratio between mass of liquid olein in cake and mass of cake, whereas the yield of olein is the ratio of the mass of uncrystallised liquid olein to the total mass of oil filtered (uncrystallised olein + stearin cake).
Table 2-6  Comparison of different filtration techniques in a palm oil fractionation plant (adapted from Timms, 2005; Kellens et al., 2007)

<table>
<thead>
<tr>
<th>Filtration data</th>
<th>Vacuum filtration (drum/belt)</th>
<th>Centrifugation (nozzle)</th>
<th>Membrane press (6 bar / 16 bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid oil entrained in filter cake (%)</td>
<td>59</td>
<td>53*</td>
<td>45 / 35</td>
</tr>
<tr>
<td>Yield of olein (%)</td>
<td>72</td>
<td>76</td>
<td>78 / 82</td>
</tr>
</tbody>
</table>

*Estimation made by Timms (2005)

Willner (1994) noted that filtration pressure plays a significant role in determining the product quality. By applying a higher squeezing pressure during the filtration step, the separation efficiency can be increased. This is due to the expulsion of entrained olein trapped within the stearin cake thereby increasing the yield and quality of the olein in terms of cold stability. A 15 bar filtration pressure is sufficient for the production of oleins (normal and superoleins) by single and double stage fractionation but for the production of palm-based cocoa butter equivalent products, higher pressures are required (~30 bar) in order to match solvent fractionation (Kellens, 1996).

An important factor that needs to be considered in order to achieve good separation of the olein from the stearin is crystal size and shape. Filtration is best carried out when the crystals are in the $\beta'$ form (Deffense & Tirtaux, 1989). They are easy to filter and tend to stay in suspension in the liquid oil. They consist of extremely small thin needles which develop regular aggregates that gradually grow during the crystallisation process.

2.3.6 Entrainment

Entrainment is the occurrence of trapped liquid within and between solid crystals during a liquid-solid separation process. It is mainly caused by the three-dimensional structure of agglomerated crystal in which liquid is contained by capillary and viscous forces due to crystal fissures (Timms, 1994). There are two forms of entrainment which can occur during the liquid-solid separation stage - intra-particle and inter-
particle entrainment (Bemer & Smits, 1982). The former involves entrapped liquid oil within a particle or aggregates of particles whereas the latter is the occlusion of uncrystallised liquid between agglomerates. Entrainment is largely affected by the process conditions applied during the crystallisation process, the hydrokinetics of the crystalliser as well as the type of separation technique used after the crystallisation stage (Bemer & Smits, 1982).

Much has been done to address the problem of entrainment in edible oil fractionation. Bemer & Smits (1982) demonstrated that during palm oil crystallisation, washing the retentate with an organic solvent was only effective in removing interparticle liquid while the intraparticle liquid was still retained within the retentate and forms up to about 50% of the retentate mass. Improvements in the separation step have moved from the conventional vacuum filtration systems to the membrane filter press that is most commonly used nowadays. Hamm (2005) reported that a cake produced via vacuum filtration typically contained a solid fat content (SFC) of approximately 40% which is equivalent to a porosity or an entrainment level of 60%. The entrainment in this case is defined as the ratio between the mass of uncrystallised olein and the mass of the cake. Kellens (1994) made a comparison between the usage of vacuum filtration and membrane filter press in palm oil fractionation and showed that the SFC of the cake increased from 41% using the former method to 55% in the latter.

The application of pressure in the membrane filter press to the solid cake during the separation stage has been shown to increase the yield of the valuable olein and at the same time reduce the entrainment level in the cake. Kellens et al. (2007) demonstrated that when a 30 bar squeezing pressure was applied to a membrane filter press having a 25 mm filter chamber width, the minimum achievable entrainment in palm oil fractionation is 30%, as depicted in Table 2-7.
Table 2-7  Effect of squeezing pressure and filter chamber width on the separation efficiency in palm oil fractionation (adapted from Kellens et al., 2007)

<table>
<thead>
<tr>
<th>Chamber plate width (mm)</th>
<th>Squeezing pressure (bar)</th>
<th>Liquid oil entrained in filter cake (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stearin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Olein</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>45</td>
<td>23.6 76.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>39</td>
<td>20.0 80.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>35</td>
<td>18.3 81.7</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>40</td>
<td>20.6 79.4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>36</td>
<td>18.8 81.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>30</td>
<td>16.7 83.3</td>
</tr>
</tbody>
</table>

Figure 2-6  Separation efficiency (SE) dependence on pressure (P) in palm oil and palm olein filtration (van den Kommer and Keulemans, 1994)

The effect of pressure during filtration on the final product quality is more prominent in palm oil fractionation than in palm olein fractionation, as shown by van den Kommer and Keulemans (1994) in Figure 2-6. In this case, separation efficiency (SE) is defined as the fraction of the solid phase in the stearin cake. Hence, it has been shown that increasing the squeezing pressure during the filtration stage improves the separation efficiency and substantially reduces the level of entrained olein in the fractionation of edible oils. However, high squeezing pressures may result in partial or
total passage of the stearin cake through the filter cloth as not all crystals can withstand these extreme conditions. One way to overcome this problem is to apply low pressures on thinner stearin cakes (Kellens et al., 2007).

Although many improvements in the filtration process have been developed for the last two decades, entrainment still remains an issue when pursuing high purity of the solid phase. It has been repeatedly shown that the post-fractionation separation stage can to no extent be clean and complete. Apart from only highlighting this issue, little effort has been made to include the entrainment factor in post-processing product analysis where quality and quantity is of major concern. Therefore, it is probably about time that entrainment is considered and incorporated each and every time into the determination of product yields and quality in order to obtain the true characteristics of the filtered solid phase.

2.3.7 Application of fractionation technology

Fractionation has been applied to many types of oils and fats with the purpose of either producing a liquid fraction with enhanced cold stability or a hard fraction with improved melting properties. Major oils and fats that are industrially fractionated include palm oil, palm kernel oil, milk fat, hydrogenated soy bean oil, hydrogenated rapeseed oil, cottonseed oil, hydrogenated fish oil, edible beef tallow and lard (Deffense & Tirtaux, 1989; Kellens & Krishnamurthy, 1994; Hamm, 1995; Gibon & Tirtaux, 2002). In addition, partial glycerides including fatty acids and esters are also fractionated to yield various products for non-food applications. Table 2-8 outlines the products from the fractionation of these oils and their various edible and non-edible applications.

The extensive usage of fractionation as one of the most important fat modification methods available since the last century has rendered it possible to produce a wide variety of food and non-food products alike. This fact, coupled with the abundance of worldwide supply in oils and fats has initiated a lot of research in improving the process to increase the product yield and quality to suit various end-use applications.
With the inherent complexity in the crystallisation of oils and fats, some researchers regard that fractionation is an art rather than a science since the control of the crystallisation stage is more often easier done from experience (Tiriaux, 1989).

Nonetheless, in order to achieve continuous improvement in this process and of the quality of products derived therefrom, it is worthy to explore the crystallisation phenomena more in-depth. That is to determine the underlying behaviour of each component interacting within the oil and to fully understand whether the separation of each component between the liquid and solid phases is purely based upon its equilibrium behaviour or whether other factors prevail. This will eventually facilitate better control over the fractionation process and create opportunities for expanding existing applications. The fundamental aspects with respect to fat crystallisation will now be discussed.
<table>
<thead>
<tr>
<th>Type of oil</th>
<th>Application of product fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olein</td>
</tr>
<tr>
<td>Palm oil</td>
<td>Cooking oils</td>
</tr>
<tr>
<td></td>
<td>Industrial frying oils</td>
</tr>
<tr>
<td></td>
<td>Salad oils</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>Non-dairy coating fat</td>
</tr>
<tr>
<td></td>
<td>Non-dairy coffee whiteners</td>
</tr>
<tr>
<td></td>
<td>Toffees &amp; cream fillings</td>
</tr>
<tr>
<td>Milk fat (butter oil)</td>
<td>Soft butters</td>
</tr>
<tr>
<td></td>
<td>Liquid cooking butter oil</td>
</tr>
<tr>
<td></td>
<td>Ice creams</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogenated soy bean oil and</td>
<td>Salad oils</td>
</tr>
<tr>
<td>rapeseed oil</td>
<td>Cooking oils</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>Salad oils stable at very low temperatures</td>
</tr>
<tr>
<td>Hydrogenated fish oil</td>
<td>Salad oils</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Edible beef tallow</td>
<td>Frying oils</td>
</tr>
<tr>
<td></td>
<td>Natural pourable deep frying shortening</td>
</tr>
<tr>
<td></td>
<td>Block / soft margarine base</td>
</tr>
<tr>
<td>Lard</td>
<td>Lubricant</td>
</tr>
<tr>
<td></td>
<td>Cutting oil</td>
</tr>
<tr>
<td></td>
<td>Animal oil</td>
</tr>
<tr>
<td>Fatty acids and esters,</td>
<td>Cosmetics</td>
</tr>
<tr>
<td>MAG and DAG mixtures</td>
<td>Pharmaceutical products</td>
</tr>
</tbody>
</table>
2.4 FUNDAMENTAL ASPECTS OF FAT CRYSTALLISATION

2.4.1 Driving force for crystallisation

For an oil or fat to crystallise, it must reach a supersaturated or supercooled state. This means that the concentration of the solute to be crystallised needs to be increased above the saturated solution concentration at any given temperature (Timms, 1995). This can be depicted in a solution saturation-supersaturation diagram as illustrated schematically in Figure 2-7.

![Saturation-supersaturation diagram](image)

**Figure 2-7 Saturation-supersaturation diagram**

In Figure 2-7, the continuous line represents the solubility or saturated solution line. Below this line, the solution is in an unsaturated state and is said to be stable, hence making crystallisation impossible ad infinitum. Anywhere above this line, the system is said to be supersaturated. The metastable zone is the region between the solubility line and the dashed line i.e. the metastable limit. In this zone, crystallisation is probable but needs to be prompted by external assistance such as deliberate seeding or agitation even though the solution is supersaturated. Crystal growth in the metastable region is also possible. In the unstable zone, crystallisation will occur spontaneously and nucleation is also possible. The solubility line depends upon the thermodynamic conditions of the system while the metastable limit depends on kinetic factors such as
the supersaturation or cooling rate, the agitation speed, the presence of impurities and the solutions’ thermal history (Mullin, 2001).

In solution crystallisation, supersaturation is usually expressed in terms of solution concentration and is commonly represented by the concentration driving force, $\Delta c$ which can be estimated using the equation below (Mullin, 2001; Jones, 2002)

$$\Delta c = c - c^*$$

(2-1)

where $c$ is the concentration of solute and $c^*$ is the concentration of solute at saturation point. This enables one to relate the chemical potential driving force, $\Delta \mu$ of a system based on solute concentration as follows

$$\Delta \mu \propto \ln \left( \frac{c}{c^*} \right) \equiv \frac{c}{c^*} - 1 = \frac{\Delta c}{c^*} = S - 1 = \sigma$$

(2-2)

where $\Delta \mu$ is the difference in chemical potential, $S$ is the supersaturation ratio and $\sigma$ is the relative or absolute supersaturation (Jones, 2002).

Oils and fats generally crystallise from the melt and supersaturation in this context is more often termed supercooling ($\Delta T$). Supercooling is expressed as the difference between the melting temperature, $T_m$ and the temperature of the system at equilibrium, $T$, written as

$$\Delta T = T_m - T$$

(2-3)

The relative supercooling is given by

$$\sigma = \frac{\Delta T}{T_m}$$

(2-4)

To enable an oil or fat to crystallise, it must be supercooled. Once the system acquires sufficient supercooling, this will initiate nucleation.
Solid-liquid equilibrium (SLE) forms the foundation for many separation processes including crystallisation processes. Thermodynamic equations have been established to relate the equilibrium condition of a system comprising of a liquid phase and multiple solid phases. Himawan et al. (2006) have reviewed in great detail the thermodynamic and kinetic aspects of fat crystallisation. In modelling the solid-liquid equilibrium in fats, the soundest theoretical basis is to start off with the expressions of the chemical potential of components within such system. The basis for equilibrium is that the chemical potential of each component \( i \) in each phase is equal to that in any other phase (Prausnitz et al., 1999). In order to explain this further, consider a fat system at constant pressure consisting of one liquid phase and one solid phase. At equilibrium therefore,

\[
\mu_i^L = \mu_i^S \tag{2-5}
\]

where \( \mu_i^L \) and \( \mu_i^S \) are the chemical potential of a component \( i \) in the liquid and solid phase respectively. For any phase \( P \), be it liquid or solid, the chemical potential of component \( i \) is defined as

\[
\mu_i^P = \mu_{i,0}^P + RT \ln (\gamma_i^P x_i^P) \tag{2-6}
\]

with \( \mu_{i,0}^P \) being the chemical potential of pure component \( i \) in phase \( P \), \( x_i^P \) the mole fraction of component \( i \) in phase \( P \) and \( \gamma_i^P \) the activity coefficient for component \( i \) in phase \( P \). Substitution of equation (2-6) into equation (2-5) yields the SLE equation of component \( i \) as follows:

\[
\mu_{i,0}^S + RT \ln (\gamma_i^S x_i^S) = \mu_{i,0}^L + RT \ln (\gamma_i^L x_i^L) \tag{2-7}
\]

If the chemical potential of component \( i \) of the solid phase falls below that in the liquid phase, then there will be a driving force for a transformation of liquid phase molecules to the solid phase. The thermodynamic driving force for crystallisation of a component \( i \) (\( \Delta \mu_i \)) is the difference in chemical potential of \( i \) between the liquid (\( \mu_i^L \)) and solid (\( \mu_i^S \)) phases (Himawan et al., 2006). Thus,
\[ \Delta \mu_i = \mu_i^L - \mu_i^S = \mu_{i,0}^L - \mu_{i,0}^S + RT \ln \left( \frac{\gamma_i^L x_i^L}{\gamma_i^S x_i^S} \right) \] (2-8)

The variation of the chemical potential of the pure components in each phase \((\mu_{i,0}^L\text{ and } \mu_{i,0}^S)\) with temperature, \(T\) and pressure, \(P\) can be established from the fundamental thermodynamic relationship

\[ d\mu_{i,o}^P = -S_{i,o}^P dT + V_{i,o}^P dP \] (2-9)

where \(S_{i,o}^P\) and \(V_{i,o}^P\) are the molar entropy and molar volume of pure component \(i\) respectively for phase \(P\). For processes at constant pressure, this reduces to

\[ d\mu_{i,o}^P = -S_{i,o}^P dT \] (2-10)

For a system comprising of a liquid and solid phase,

\[ d\left( \mu_i^L - \mu_i^S \right) = -\left( S_{i,o}^L - S_{i,o}^S \right) dT = -\Delta S dT = -\frac{\Delta H}{T} dT \] (2-11)

This expression can be integrated with respect to temperature taking into account that the chemical potentials of the liquid and solid phases are equal at the melting temperature of pure component \(i\) at the system pressure \((Tm,P)\) and that the change in molar enthalpy is delineated by

\[ \Delta H_{i,o} \approx \Delta H_{mi,o} + \Delta C_{pl,o} \left( T - T_{mi} \right) \] (2-12)

with \(\Delta H_{mi,o}\) being the molar enthalpy of melting of pure component \(i\) at the reference temperature \(T_{mi}\) and \(\Delta C_{pl,o}\) the difference in molar heat capacity between the solid and liquid phases for pure component \(i\). Integration of equation (2-11) will generate (Wesdorp et al., 2005; Himawan et al., 2006):

\[ \frac{\Delta \mu_i}{RT} = \frac{\Delta H_{mi}}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) - \frac{\Delta C_{pl}}{R} \left( \frac{T_{mi} - T}{T} \right) + \frac{\Delta C_{pl}}{R} \ln \left( \frac{T_{mi}}{T} \right) + \ln \left( \frac{\gamma_i^L x_i^L}{\gamma_i^S x_i^S} \right) \] (2-13)
With \((T_m - T)\) values lying usually within 0 to 20, the terms with \(\Delta C_p\) are comparatively small and often neglected (Los et al., 2002). This approximation leads to a simplification of equation (2-13):

\[
\frac{\Delta \mu_i}{RT} = \frac{\Delta H_{m,i}}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) + \ln \left( \frac{\gamma_i^L x_i^L}{\gamma_i^S x_i^S} \right)
\]  

(2-14)

As mentioned earlier, the mixing behaviour in the liquid state is known to be almost ideal, hence \(\gamma_i^L = 1\). This reduces the above to

\[
\frac{\Delta \mu_i}{RT} = \frac{\Delta H_{m,i}}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) + \ln \left( \frac{x_i^L}{\gamma_i^S x_i^S} \right)
\]

(2-15)

where \(\gamma_i^S\) is the activity coefficient of component \(i\) in the solid phase at an equilibrium condition of temperature, \(T\). Equation (2-15) corresponds to the crystallisation chemical potential driving force of a component \(i\). To be able to use this equation, a description of the activity coefficient \(\gamma_i^S\) as a function of the composition of the solid phase, \(x_i^S\) is still required.

When there is a large difference between the melting points of the components, for example a fat containing a mixture of high melting saturated TAGs and low melting unsaturated TAGs, the high melting component forms an almost pure crystal, making \(x_i^S = 1\) (Wesdorp et al., 2005; Himawan et al., 2006). Ideal miscibility in the liquid TAGs is assumed and the activity coefficients for both the solid and liquid phases are equal to unity. This reduces (2-15) to the Hildebrand solubility equation:

\[
\ln x_i^L = \frac{\Delta H_m}{R} \left( \frac{1}{T_m} - \frac{1}{T} \right)
\]

(2-16)

Supercooling and supersaturation can be defined within this overall framework.
2.4.2 Nucleation

The nucleation process forms an integral part of the fats crystallisation process and it serves as the starting point in crystal formation. Once a fat is in a supercooled stage, the occurrence of nucleation may be spontaneous or may be assisted by external instigators. Nucleation takes place with the initial formation of nuclei, caused by the aggregation of molecules or ions in a supersaturated solution or melt. Clusters of molecules smaller than nuclei are called embryos and will tend to redissolve if formed (Boistelle, 1988).

The two types of nucleation that mainly occur in fats crystallisation or crystallisation processes in general are primary nucleation and secondary nucleation. The differences between these two types of nucleation are that the former involves direct nucleation from solution whereas the latter is induced by the presence of crystals after a system has reached a supersaturated state (Mullin, 2001). The modes and mechanisms of the different types of nucleation are shown in Figure 2-8 and shall be described in the next sections.

Figure 2-8 Modes and mechanisms of nucleation (Jones, 2002)
2.4.2.1 Primary nucleation

Primary nucleation can be separated into two mechanisms, namely homogeneous nucleation and heterogeneous nucleation. Homogeneous nucleation commonly occurs spontaneously from a clear solution and at high supercooling rates while heterogenous nucleation is often stimulated by the presence of impurities within the solution. In this section, the former shall be initially reviewed followed by the latter.

Homogeneous nucleation begins with the formation of nuclei in a clear supercooled solution. The determinant factor in ensuring its occurrence is that the nuclei formed is stable enough to grow and can withstand dissolution. Nuclei stability is associated with the change in the Gibbs free energy; the free energy of the particle should decrease in order for a stable nucleus to form and subsequently for the nucleation process to evolve. The variation of Gibbs free energy with radius, r (assuming spherical geometry) is shown in Figure 2-9.

![Figure 2-9 Free energy diagram for nucleation (Mullin, 2001)](image)

The overall change in Gibbs free energy of this particle, \( \Delta G \), equals the sum of the surface excess free energy, \( \Delta G_s \) and the volume excess free energy, \( \Delta G_v \) as follows:
\[ \Delta G = \Delta G_S + \Delta G_V = 4\pi r^2 \gamma + \frac{4}{3} \pi r^3 \Delta G_v \]  

(2-17)

where \( \gamma \) is the interfacial energy, the energy required to overcome surface tension due to increasing surface area and \( \Delta G_v \) is the free energy change of transformation per unit volume. From this equation, \( \Delta G_S \) is a positive term and is relative to the surface area, \( r^2 \) whereas \( \Delta G_V \) is a negative term and is associated with the change of free energy between a very large particle and the solute in solution (Mullin, 2001). A stable nucleus is formed when \( \Delta G \) reaches a maximum and this maximum value represents the critical excess free energy, \( \Delta G_{crit} \) required to form a critical nucleus of size, \( r_c \), which can be written as

\[ r_c = \frac{-2\gamma}{\Delta G_v} \]  

(2-18)

Nuclei having a radius higher than \( r_c \) are stable and will continue to grow spontaneously whereas if the radius is smaller than \( r_c \), it will be unstable and redissolve back into the bulk of the solution. Combining equations (2-14) and (2-15), we obtain the critical excess free energy, \( \Delta G_{crit} \) for nucleation from solution expressed as

\[ \Delta G_{crit} = \frac{16\pi \gamma^4}{3(\Delta G_v)^2} = \frac{4\pi \gamma r_c^2}{3} \]  

(2-19)

For homogeneous nucleation occurring from the melt, which is often encountered during fats crystallisation, similar expressions in terms of supercooling can be derived for \( \Delta G_{crit} \) as follows (Himawan et al., 2006):

\[ \Delta G_{crit} = \frac{16\pi \gamma^3 T_m^2}{3(\Delta H_m \Delta T)^2} \]  

(2-20)

with \( \Delta G_v \) given by
\[ \Delta G_v = \frac{\Delta H_m \Delta T}{T} \]  

(2-21)

and the radius of a critical nucleus written as

\[ r_c = \frac{2\gamma T}{\Delta H_m \Delta T} \]  

(2-22)

where \( T, \Delta H_m \) and \( \Delta T = T_m - T \) are the solid-liquid equilibrium temperature, melting enthalpy and supercooling, respectively. From these equations, it is clear that \( r_c \) depends on the temperature of the system, \( T \) and \( \Delta G_r \) is a function of the degree of supercooling, \( \Delta T \). Thus, if the temperature was increased, the critical nucleus size would also increase and a larger change in free energy would be required for the system to nucleate.

The nucleation frequency or rate, \( J \) which represents the number of nuclei formed per unit time per unit volume can be written in the form of the Boltzmann distribution as follows (Aquilano and Sgualdino, 2001):

\[ J = A \exp \left( -\frac{\Delta G}{kT} \right) \]  

(2-23)

where \( A \) represents the global kinetic coefficient and \( k \) is Boltzmann’s constant \((1.3805 \times 10^{-23} \text{J K}^{-1})\). \( A \) can be calculated using the following expression

\[ A = \frac{N_A kT}{h} \]  

(2-24)

with \( N_A \) being the Avogadro number \((6.023 \times 10^{23} \text{mol}^{-1})\) and \( h \) is Planck’s constant \((6.626 \times 10^{-34} \text{J s})\). Hence, inserting equation (2-17) into (2-20) yields the following expression for the nucleation rate in melt crystallisation.
\[ J = A \exp \left[ - \frac{16 \pi \gamma^3 T_m^2}{3kT(\Delta H_m \Delta T)^2} \right] \]  
\[ (2-25) \]

From a theoretical point of view, as the degree of supersaturation or supercooling increases, the nucleation rate also increases following an exponential behaviour as depicted by the solid curve in Figure 2-10. Empirically, this does not occur in nucleation from the melt as the nucleation rate will reach a maximum and subsequently decrease (represented by the dashed curve). This is due to a steep increase in the viscosity of the melt as the degree of supercooling is increased which hinders movement of molecules, thus suppressing the formation of ordered crystal structures (Mullin, 2001).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure_2-10.png}
\caption{Effect of supersaturation on the nucleation rate (Mullin, 2001)}
\end{figure}

Due to this behaviour observed in melts, a viscosity term needs to be included into equation (2-22) to account for the transportation of molecules across the diffusion barrier, giving

\[ J = A' \exp \left[ - \frac{16 \pi \gamma^3 T_m^2}{3kT(\Delta H_m \Delta T)^2} \right] \exp \left[ \frac{\Delta G'}{kT} \right] \]  
\[ (2-26) \]
where $\Delta G'$ is the activation energy for molecular motion across the crystal-melt interface (Mullin, 2001). Equation (2-23) is generally known as the Turnbull-Fisher equation and can be used to quantify the nucleation rate in melts.

Homogeneous nucleation, however, rarely occurs in fats crystallisation. Instead, it is heterogeneous nucleation that most often takes place and this type of nucleation mechanism involves the crystal formation taking place on solid particles such as dust, walls of the container or foreign molecules (Timms, 1995). Heterogeneous nucleation mainly depends upon the wetting angle, $\theta$, which is the angle of contact between the nuclei and the surface of the impurity. The larger the wetting angle, the easier it is for nucleation to occur.

The presence of impurities usually lower the surface energy as a result of lesser molecules needed to induce nucleation. This, in turn, lowers the degree of supercooling required. Heterogeneous nucleation can be expressed similarly to homogeneous nucleation but since the overall free energy change associated with nucleation is lower than that for homogeneous nucleation (hence, $\Delta G'_{\text{crit}} < \Delta G_{\text{crit}}$), then

$$
\Delta G'_{\text{crit}} = \phi \Delta G_{\text{crit}} \quad (2-27)
$$

where $\Delta G'_{\text{crit}}$ is the overall free energy change for heterogeneous nucleation, $\Delta G_{\text{crit}}$ is the overall free energy change for homogeneous nucleation and $\phi$ is a factor where $\phi < 1$ and depends on the wetting angle, $\theta$ as shown in the equation below (Mersmann et al., 2001):

$$
\phi = \frac{(2 + \cos\theta)(1 - \cos\theta)^2}{4} \quad (2-28)
$$

If the wetting angle is 180°, then $\phi = 1$. This corresponds to no contact between the nuclei and the surface of the foreign body and implies that homogeneous nucleation is taking place. A wetting angle of between 0 and 180° will result in $\Delta G'_{\text{crit}} < \Delta G_{\text{crit}}$ and yield heterogeneous nucleation. Complete contact between the nuclei and the foreign
body surface ($\theta=0$) renders $\phi=0$ and subsequently $\Delta G_{\text{crit}} = 0$. This entails that there is no energy is required for nucleation to take place and represents seeding of the solution (Mullin, 2001).

### 2.4.2.2 Secondary nucleation

Secondary nucleation is a class of nucleation mechanism that occurs when a solution or melt already contains crystals, as opposed to primary nucleation which occurs when there are no crystals present in the system. A number of factors that lead to the occurrence of secondary nucleation have been already outlined in Figure 2-8. These include: contact of crystals with other crystals or crystalliser parts, shear induced by fluid flow in the crystalliser, fracture and attrition due to particle impingement or fluid flow and particle disruption caused by detachment of weak fragments from the crystal structure (needle) (Jones, 2002). From these factors, it can be seen that the contact of a particle, be it with other particles or with parts in the crystalliser plays a major role in promoting secondary nucleation.

Agitation, such as by an impeller blade, is considered the main stimulator of secondary nucleation. The speed of agitation in this case is the main factor controlling the generation of secondary nuclei as a high agitation speed results in increased contact between the agitator surface and existing particles. This action in part, causes crystal breakage and consequently smaller nuclei are generated into the bulk of the melt. In the industrial crystallisation of fats, high speed agitation is often avoided. This often reduces heat transfer within the crystalliser thus leading to slow cooling. Slow cooling of the fat is desired in order to ensure that crystals of uniform size are produced which will facilitate the separation stage (Dijkstra, 2007).

When secondary nuclei are produced via crystal-agitator contact, the rate of nucleation can be expressed by the following equation:

$$B = k_b m_f^N c^b$$

(2-29)
where $k_b$ is a constant, $m_T$ is the concentration of crystals in suspension, $N$ is the measure of the fluid mechanics interactions which is a function of the agitation rate and average power input to the solution and $k$ being in the range of 2 to 4. Values of $b$ usually lie between 1 and 2.5 while most $f$ values are close to 1 which indicates that the dominating effect is of collisions between crystals and agitator and between crystals and crystalliser wall (Garside et al., 2002).

The formation of secondary nuclei takes place when small fragments of existing crystals are dislodged from the crystal surface. These small fragments then in turn act as new nuclei, which either start to grow into larger crystals or redissolve back into the melt, depending upon their critical size (Timms, 1995). Not all secondary nuclei generation is caused by crystal interaction. Crystal irregularities such as defects, dislocation or inclusion within and on the crystal surface have been known to provoke crack formation and subsequent production of broken fragments (Mullin, 2001).

Seeding has been demonstrated to be successful in inducing secondary nucleation in industrial practices of fat crystallisation. It is usually employed to accelerate the crystallisation process and also for controlling the crystal size and size distribution within the process (Timms, 1995; Mullin, 2001). It is thought that seed crystals assist in generating secondary nuclei when part of the crystals which are smaller than the critical size dissolve and the remaining seed crystal, which is smaller than the original seed size, act as new nuclei. Seed crystal size also may effect secondary nucleation as larger seed crystals tend to produce more secondary nuclei compared to smaller seed crystals due to the increased contact and impact energies (Mullin, 2001).

2.4.3 Crystal growth

Once the nucleation process has commenced in a supercooled melt, nuclei larger than the critical size then start to grow. Crystal growth is governed by the incorporation of molecules into the crystal surface from the adjacent liquid layer. This layer is then continuously replenished from the surrounding supersaturated liquid (Timms, 1995). According to Mullin (2001), a number of theories have been developed to describe the mechanism of crystal growth such as surface integration, adsorption layer, kinematic
theories and the diffusion-reaction model. However, only the diffusion-reaction model shall be described here due to its ready applicability to fats crystallisation and reference should be made to Mullin (2001) for a detailed discussion on the other theories on crystal growth mechanisms stated above.

2.4.3.1 Diffusion-reaction theory

The diffusion-reaction theory of crystal growth suggests that there are two steps that take place during the mass deposition onto a growing crystal face over time. The first step is governed by a diffusion process which involves the transportation of solute molecules from the bulk of the fluid phase to the solid surface. Subsequently, a first order reaction process follows with the arrangement of the solute molecules into the crystal lattice (Mullin, 2001). These two mechanisms can be expressed by the following equations for diffusion

\[ \frac{dm}{dt} = k_d A(c - c_l) \]  \hspace{1cm} (2-30)

and reaction

\[ \frac{dm}{dt} = k_r A(c_i - c*) \]  \hspace{1cm} (2-31)

where \( m \) is the mass of solid deposited in time \( t \); \( A \) is the surface area of the crystal; \( k_d \) is the coefficient of mass transfer by diffusion; \( k_r \) is the rate constant for the surface reaction process; \( c_l \) is the solute concentration in the solution at the solution-crystal interface and \( c* \) is the equilibrium saturation concentration. It is implicit in these equations that the assumed driving force for crystallisation is the supersaturation.

From the above equations, the solute concentration at solution-crystal interface or the interfacial concentration, \( c_l \) is often difficult to measure and this term is usually eliminated and substituted by the overall concentration driving force, \( c - c* \). Hence, crystal growth from a solution can then be expressed as
\[
\frac{dm}{dt} = K_G A (c - c^*)^g
\]  

(2-32)

where \(K_G\) is the overall crystal growth coefficient and \(g\) is the exponential term which denotes the order of the overall growth process. Figure 2-11 represents the concentration driving forces at the crystal-solution interface during the crystal growth process in crystallisation from solution.

![Figure 2-11](image-url)

**Figure 2-11** Concentration driving force during the crystal growth process for crystallisation from solution (Mullin, 2001)

The relationship between \(K_G\), \(k_d\) and \(k_r\) is represented by

\[
\frac{1}{K_G} = \frac{1}{k_d} + \frac{1}{k_r}
\]

(2-33)

### 2.4.3.2 Crystal growth from the melt

Fat crystallisation almost always occurs from a melt, except for the few cases in which fats are crystallised from an organic solvent when a high degree of component selection is required. According to Mullin (2001), crystallisation from a melt depends on the heat transfer rate from the crystal surface to the liquid bulk. The temperature at
the crystal-melt interface during crystallisation, \( T_i \) will be higher than that of the supercooled melt, \( T \) due to the release of the heat of crystallisation. This is depicted in Figure 2-12 with the \( T^* \) being the melting point of the crystal.

![Temperature gradients at the crystal-melt interface (Mullin, 2001)](image)

Figure 2-12  Temperature gradients at the crystal-melt interface (Mullin, 2001)

Thus, from Figure 2-12, the overall degree of supercooling is \( \Delta T = T^* - T \) and the driving force for heat transfer across the stagnant film of liquid adjacent to the crystal face is \( T_i - T \). An expression similar to the equation used to describe the crystal growth through the diffusion-reaction mechanism (see equation 2-29) can be written for the crystal growth rate from a melt as follows

\[
\frac{dm}{dt} = K_G^* A (T^* - T)^{g^*} \tag{2-34}
\]

where \( K_G^* \) is the overall growth mass transfer coefficient and \( g^* \) has a value generally between 1.5 and 2.5 (Mullin, 2001).

### 2.4.3.3 Other crystallisation growth rate expressions

Crystal growth rates are usually controlled by a number of factors which include system temperature, the degree of supersaturation, crystal size and habit and the
degree of agitation. There are a number of ways to express crystal growth rate in carefully defined conditions namely as mass deposition rate $R_D$ (kg·m⁻²·s⁻¹), a mean linear velocity $\bar{v}$ (ms⁻¹) or as an overall linear growth rate $G$ (ms⁻¹) (Mullin, 2001). These quantities are related through the following equation

$$R_D = K_D \Delta c^e = \frac{1}{A} \cdot \frac{dm}{dt} = \frac{3\alpha}{\beta} \cdot \rho_c G = \frac{3\alpha}{\beta} \rho_c \frac{dL}{dt} = \frac{6\alpha}{\beta} \rho_c \frac{dr}{dt} = \frac{6\alpha}{\beta} \cdot \rho_c \bar{v} \quad (2-35)$$

where $m$ and $A$ are the particle mass and area, $\alpha$ and $\beta$ are the volume and surface shape factors, respectively, $\rho_c$ is the crystal density, $L$ is a characteristic size of the crystal and $r$ is the radius which corresponds to the equivalent sphere.

Another way to express crystal growth is via the overall crystal growth rate. This is measured in terms of mass deposited per unit time per unit area of crystal surface instead of individual face growth rates. The following equations may be used to quantify the overall linear growth rate, $G$.

$$G = \frac{M_f^{1/3} - M_i^{1/3}}{(\alpha \rho N)^{1/3} t} \quad (2-36)$$

or

$$G = \frac{\beta}{3\alpha \rho} K_D \Delta c^e \quad (2-37)$$

where $M_i$ and $M_f$ are the initial and final crystal masses (kg) in that order, $N$ is the number of individual crystals, $\alpha$ and $\beta$ are the volume and surface shape factors of the crystals respectively, $\rho$ is their density, $t$ is time and $\Delta c$ is the mean supersaturation over the entire run expressed as kg solute per kg solvent. The value of $g$ represents the slope of a plot of log $G$ versus log $\Delta c$ for which then $K_D$ can be measured.

Kirwan and Pigford (1969) have developed a theory for expressing the crystallisation rates of species from a multi-component melt. In their theory, they assumed that the
growth of a crystal is subject to the addition rate of molecules to growth sites on the surface and by the density of said growth sites on the crystal face. The net crystallisation flux of species \( i \) in absolute rate theory terms may be written in the form of a rate coefficient multiplied by a driving force expressed in terms of the chemical potential difference of species \( i \) across a liquid-solid interface as follows:

\[
N_i = f \rho^S (D_i / \lambda) y_i \left[ 1 - \exp\left( -\Delta \mu_i / RT \right) \right]
\]  

(2-38)

whereby \( f \) is the surface step density which is the fraction of the crystal face available for attachment, \( \rho^S \) the molar density, \( D_i \) the interfacial transport coefficient for movement across the liquid-solid interface and \( \lambda \) being the distance the interface advances when one molecular layer is added to the solid (Kirwan & Pigford, 1969). Using this expression, they managed to predict the growth rates for the crystallisation of a component from the melt within an order of a magnitude for an array of pure materials including water, glycerine, salol, tin. Their research group also correlated growth rates of salol and thymol from their binary melts.

An analogous expression of Eq. (2-26) in terms of the composition of component \( i \) in the liquid phase, \( x_i^L \) and solid phase, \( x_i^S \) may be written as

\[
g_i = k_i \left[ x_i^L - x_i^S \exp\left( \frac{\Delta H_{m,i}(T_{m,i} - T)}{RT_{m,i}T} \right) \right]
\]  

(2-39)

where \( g_i \) is the crystallisation or growth rate of species \( i \) across the liquid-solid interface and the driving force term may be expressed as a crystallisation rate coefficient, \( k_i \) as follows:

\[
k_i = f \rho^S (D_i / \lambda)
\]  

(2-40)

The growth rate values in Eq. (2-39) may be divided by the term in the brackets to allow for comparison between \( k_i \) values of different species and its variation with time, depending upon available growth area.
2.4.4 Agglomeration and attrition

Agglomeration is defined as the coalescence of primary particles that are subsequently cemented by chemical forces, for example by a crystalline bridge between two or more particles (Mersmann & Braun, 2001). It takes place following the formation of particles by primary nucleation. Agglomerates differ from flocculates and aggregates in that they can only be generated in supersaturated solutions while the two latter can occur in undersaturated or saturated solutions and are easily destroyed. The strength of the bonding forces i.e. van der Waals forces in agglomerates is the highest compared to the forces that hold together particles in flocculates and aggregates and go from the highest to the lowest in that order (Mersmann & Braun, 2001).

Two types of agglomeration that can occur in crystallisation are primary agglomeration and secondary agglomeration. The former results from the malgrowth of polycrystals, dendrites and twins while the latter occurs due to particle collision in supersaturated, particle suspended systems (Mersmann & Braun, 2001). Figure 2-13 illustrates how particles are formed by agglomeration. Secondary agglomeration or simply agglomeration depends on factors such as mechanical, fluid dynamic and kinetic processes occurring within the system, as well as the properties of the constituent particles. It is further divided into two forms, i.e. perikinetic agglomeration which is diffusion-controlled and occurs due to the Brownian motion of particles in a static fluid and the other form is orthokinetic agglomeration which is shear rate-controlled and occurs to particles in agitated solutions (Mersmann & Braun, 2001; Mullin, 2001; Jones, 2002).

The process of agglomeration starts with particles colliding by the action of diffusion and/or convection forces. If the attractive forces are stronger than repulsive forces, these particles will remain aggregated even after a collision. The tensile strength of agglomerates plays an important role in determining its stability. Disruption of agglomerates depends on the tensile strength between particles that make it up. Among the main factors that control agglomeration are: the level of supersaturation, suspension density, particle size, agitation speed, ionic strength and the presence of impurities (Jones, 2002). Agglomeration can be promoted by the following
conditions; elevated system temperatures, low viscosities, small particle sizes, high particle concentration and high diffusivities within the particulate systems (Mersmann & Braun, 2001).

![Diagram of Particle Formation via Agglomeration](image)

Figure 2-13  Particle formation via agglomeration (Jones, 2002)

Van Putte & Bakker (1987) have demonstrated in their study that palm oil crystals tend to agglomerate under stirring conditions, as portrayed in Figure 2-14. Agglomeration in palm oil crystallisation highly depends on agitation, as demonstrated by Bemer and Smits (1982). They experimented on different stirring conditions and found that agglomeration prevailed during conditions when the concentration of particles and the collision frequency between crystals were high.

The opposite of agglomeration is attrition. Breakage of crystals via attrition is usually governed by collision and fluid mechanics factors within the system. Collisional attrition mainly occurs in agitated systems and is due to the collision of crystals with other crystals, the impeller and parts of the vessel (i.e. crystalliser wall) while fluid mechanical attrition is induced by turbulent fluid flow which is contributed by shear, drag and pressure stresses (Jones, 2002). Attrition is the main contributor towards the generation of secondary nuclei. It prevails during the crystallisation of large crystals (with lengths exceeding 100 \( \mu m \)) that are formed in systems with high solubility. As
the collision velocity and parent crystals size increases, the attrition rate also increases. Crystals most prone to attrition have a high settling velocity and attrition fragments with sizes in the range between 2 $\mu$m to 150 $\mu$m are to a certain extent, bonded to large crystals and may perhaps contribute to their growth (Mersmann & Braun, 2001). As a conclusion, the combination of the various successive stages in crystallisation i.e. nucleation, crystal growth, agglomeration and/or attrition greatly contribute to the final mean crystal size and most importantly, the overall crystal size distribution within the crystallised system. These stages form a large part of the fractionation process and thus studies on palm oil fractionation, which much of this research is based upon, shall now be surveyed.

![Figure 2-14 Electron photomicrographs of the polymorphs of palm oil trisaturated TAGs crystals. (A) $\beta$ (non-stirred); (B) $\beta'$ (non-stirred); (C) $\beta'$ (stirred) (Van Putte & Bakker, 1987)]
2.5 STUDIES ON PALM OIL FRACTIONATION

2.5.1 Overview

Palm oil is the most fractionated oil worldwide and the majority is fractionated via the dry fractionation process. This process has been reviewed as the cheapest and most natural modification process compared to hydrogenation and interesterification (Kellens, 1996; Deffense, 1998). It has dominated other fractionation processes due to the low processing costs involved and also the yield of liquid olein obtained which is comparable to the solvent process. The crystallisation process, separation (filtration) and TAG partitioning aspects of palm oil fractionation will now be covered with an initial introduction to the polymorphism of TAGs.

2.5.2 Polymorphism of TAGs

Polymorphism is the ability of compounds to crystallise in different crystalline structures. They result from the stereochemical configuration of the molecules of the crystal which exhibits different orientations of the zigzag arrangement of the glyceridic chains (Jacobsberg & Oh, 1976). TAGs are assumed to have a tuning fork or chair structure, depending on the differences in the chain lengths and positions of the component fatty acids.

Fats and TAGs exhibit at least three polymorphs termed alpha (α), beta prime (β') and beta (β) (Larsson, 1966). The structure of all three polymorphs mainly differ in their subcell configuration. The α structure is made up of a hexagonal subcell (H) where zig-zag chains are oscillating and are perpendicular to the basal plane. It therefore resembles cylindrical rods packed closely together (Timms, 1978). The β' subcell structure is orthorhombic, with a perpendicular arrangement of alternating zig-zag chain planes (O1) while the β subcell is of the triclinic chain packing type, the zig-zag chain planes being in a parallel arrangement (Sato, 2001). These polymorphs are in order of increasing stability, packing density and melting point. Figure 2-15 illustrates the three main polymorphic forms of TAGs.
Chapter 2 Literature Review

Figure 2-15 Polymorphic forms of TAGs viewed “end on” to the acyl chains (adapted from Wesdorp et al., 2005)

The polymorphism of TAGs has been extensively reviewed (Sato et al., 1999; Sato, 2001). Polymorphs can exhibit one of two distinguishable types of chain length structure - double and triple chain packing (illustrated in Figure 2-16). The double chain packing structure is formed when the three fatty acids in the TAG molecule are the same or very similar in chemical properties (for example in a mono-acid TAG) while the triple chain packing structure will occur when at least two of the three fatty acid chains are largely different (either in length or degree of saturation), thus causing chain sorting (Sato, 2001). The triple chain packing usually occurs within TAGs containing cis-unsaturated fatty acids and those where the fatty acid chains differ by 6 or more. The double and triple chain packing are represented by a -2 or -3 suffix to the respective polymorphic form symbol. For example, a $\beta$ form exhibiting triple chain packing is indicated by $\beta$-3.

Figure 2-16 Chain length structure of TAGs

Upon fast cooling of TAGs, the $\alpha$ polymorph will appear and is able to transform to the $\beta'$ polymorph and subsequently to the $\beta$ when heat or energy is supplied. This

Partitioning of triacylglycerols in the fractional crystallisation of palm oil
transition is slow for TAGs having fatty acids of different chain lengths or asymmetric TAGs. The transition rate increases with increasing similarity in the fatty acid chain length and symmetry (Deffense & Tirtaux, 1989). This transformation is, however, irreversible as the higher melting polymorphs are thermodynamically more stable. In some cases, a form which is lower in melting point and stability compared to the $\alpha$ form may exist. This form is often referred to in the literature as the sub-$\alpha$ form and is assigned the $\beta_2'(\text{sub-}$\alpha$)$ polymorph. The nomenclature $\beta_2'(\text{sub-}$\alpha$)$ is suggested if it is required to indicate its position relative to the $\alpha$ form (Timms, 1984).

### 2.5.3 Crystallisation of palm oil

Crystallisation is a process which involves three stages, namely nucleation, crystal growth and agglomeration of crystals as described in detail in Section 2.4. It is an exothermic process where heat is released in order to allow formation and growth of crystals. Palm oil crystallisation is known to be a rather complex process and many authors have reviewed this topic (Jacobsberg & Oh, 1976; Kawamura, 1979 & 1980; van Putte & Bakker, 1987; Berger, 1989).

The crystallisation of palm oil can be measured by differential scanning calorimetry (DSC) and it is well characterised by two easily distinguishable peak areas in the exotherms (Kawamura, 1979; Kawamura, 1980; Ng & Oh, 1986; Ng, 1990; Zaliha et al., 2004) (see Figure 2-17). These two corresponding peaks correspond to two different groups of TAGs crystallising at different temperatures. One group is designated as the higher melting TAGs which comprise mainly of saturated TAGs such as PPP, PPS and MPP. These higher melting TAGs start to crystallise at around 28.5 °C. The other group crystallising at lower temperatures mainly consists of lower melting TAGs which start crystallising at around 12.4 °C. The melting thermogram also shows two broader peaks in the endotherm. These depict the same two groups of TAGs which have different melting temperatures. The first endothermal peak represents the lower melting TAGs completely melted at 29.5 °C while the second broader peak signifies the higher melting TAGs melting between 36.9 °C to 54.0 °C. The presence of these two types of TAG groups is apparent when the oil forms a semi-solid solution when left at room temperature.
Due to the complexity and large number of components present in palm oil, more and more studies have been carried out in order to clearly understand the underlying factors contributing to its crystallisation behaviour as a whole. The many factors contributing to this phenomenon include intersolubility and polymorphism, presence of minor components, impurities, additives and crystallisation conditions. These factors shall be described here in further detail.

2.5.4 Polymorphism and phase behaviour of palm oil

Intersolubility and polymorphism have by far the greatest influence on palm oil crystallisation. These two phenomena are closely linked together and a description of one of them is usually incomplete without the other. Intersolubility is known as the mutual solubility of a TAG in another leading to the formation of solid solutions (Timms, 1994). The formation of a solid solution is highly dependant on the chemical composition and the crystal structure of different TAGs within a certain mixture. The phase behaviour of the different TAGs in the solid state usually affects the efficiency of the crystallisation process (Timms, 1984).
The phase behaviour of palm oil has been reviewed to a certain extent (Persmark et al., 1976; Timms, 1984; Berger, 1989). It is well established that palm oil is $\beta'$ stable and of this type, the stable sub-polymorph being $\beta'_1$. Persmark et al. (1976) conducted an X-ray diffraction study on the phase behaviour of palm oil and found the existence of three polymorphs, namely $\alpha$, $\beta'_1$, and $\beta'_2$. This study was conducted by rapidly cooling palm oil from 70 °C to temperatures below -10 °C at a cooling rate of 10 °C/min and subsequently heating the oil at 0.5 °C/min until reaching 37 °C. The oil was then slowly cooled therefrom at a rate of 0.3 °C/min to 0 °C. Utilizing the diffraction-pattern-temperature (DPT) camera, they observed X-ray diffraction patterns of the lower melting $\beta'$, $\beta'_2$ prevailing at subzero temperatures below -10 °C upon rapid cooling. They further discovered a mixture of $\alpha$ and $\beta'_1$ polymorphs existing between -10 °C and -5 °C when the oil was further heated slowly. At 7 °C, the $\beta'_1$ form was found to dominate. No diffraction patterns were observed after continued heating to about 37 °C due to the solid content being below 5%. However, on slow cooling of the melt, a $\beta'_1$ form appeared at 22 °C. Figure 2-18 illustrates the polymorphic transformation pathways.

Figure 2-18 Polymorphic transitions of palm oil (Permark et al., 1976)

Berger and Wright (1976) detected a $\beta$ form melting between 21 and 30 °C and suggested that this was mainly attributed to the existence of a PLinP-rich phase showing a stable sub-$\beta$ polymorph (cited by Timms, 1984). Another possibility was that this could be due to their observation of a $\beta$-2 polymorph due to the formation of
a POP/PPO compound but as noted by Timms (1984), no X-ray data were reported, hence the failure to distinguish these two suggestions. Van Putte & Bakker (1987) investigated the kinetics of palm oil crystallisation and showed that palm oil crystallizes in the $\beta'$-modification when cooled to between 21 and 29 °C and into the $\beta$-modification when crystallised between 34 and 46 °C under non-stirring conditions. The amount of SSS components present in the washed filtrate was more than 55% for the former temperature conditions and exceeded 75% for the latter. They found that palm oil crystals formed agglomerates of the $\beta'$ type, consisting of spherulites with numerous needles growing from the center, when stirring conditions were applied.

In a separate study carried out by Deffense & Tirtaux (1989), $\beta$ type crystals were observed when palm oil was maintained or cooled slowly around temperatures between 40 and 45 °C. These types of crystals were thought to originate from handling and storage, thus necessitating melting the oil at high temperatures to destroy all crystal memory upon proceeding with crystallisation. Palm oil also forms mixed crystals or solid solutions (Timms, 1984; Berger, 1989). This tendency is ascribed partly to the relatively high amount of symmetric monooleic disaturated TAGs such as POP (Jacobsberg & Oh, 1976). It is also strongly enhanced by the presence of asymmetric TAGs such as partial glycerides as these act in increasing the $\alpha$-lifetime and decreasing the solid fat content (Knoester et al., 1972; Persmark et al., 1976).

Mazzanti et al. (2005) studied the influence of shear rates on phase transitions during palm oil crystallisation at 17 °C and 22 °C. Applying different shear rates from 0 to 2880 s$^{-1}$, they discovered that the acceleration of the phase transition from $\alpha$ to $\beta'$ increased with increasing shear at both temperatures of study. A simple model was developed in which they describe that initially the $\alpha$ phase nucleates from the melt followed by nucleation of the $\beta'$ phase on the $\alpha$ crystallites. They concluded that under shear, small crystallites are formed from the $\alpha$ nuclei which easily transform to the $\beta'$ form. A lower shear retards the nucleation of the $\beta'$ crystals since this causes the $\alpha$ nuclei to aggregate and provide less nucleation sites per unit volume for the $\beta'$ form to nucleate.
A very recent study conducted by Chong et al. (2007) using coupled time-resolved synchrotron X-ray diffraction with high sensitivity differential scanning calorimetry found that palm oil in its crude form crystallises into two different $\beta'$ polymorphs of double and triple chain length stacking when a cooling rate of 0.1 °C/min was applied. This was evident at temperatures below 27 °C and was revealed through the XRD peaks which displayed two $\beta''$ subcells developing successfully. They also suggested that palm oil is composed of a three-phase system involving a $\beta'$, a $\beta$ and a liquid phase (two solids and one liquid) rather than a single solid and single liquid system due to the coexistence of stable $\beta'$ plus $\beta$ forms at high temperatures.

### 2.5.5 TAG partitioning

The most comprehensive review on palm oil fractionation with respect to TAG partitioning was carried out by Deffense (1985). He concluded that trisaturated TAGs (SSS), namely PPS, PPP and MPP had a very high tendency to enrich in the stearin and favoured low iodine values with a considerable reduction occurring in the olein. Another major tendency that was observed was that the monosaturated type (SUU) would preferably be enriched in the olein fractions while the disaturated TAGs (SSU) would crystallize in the stearin and olein depending on the asymmetrical and symmetrical isomers respectively. However, the crystallisation of the SSU types would only occur once the SSS TAGs have completely disappeared (Deffense et al., 1989).

In the work of Sulaiman et al. (1997), a fractionation experiment conducted on palm oil found that the percentage concentration of three SSS TAGs, i.e. PPP, MPP and PPS in the crystal relative to their initial composition in the starting palm oil decreased with the reduction in temperature. Their study suggested that these three TAGs were responsible for initiating nucleation during the crystallisation process due to their higher concentration in the solid fraction at the clouding point. This study was in agreement with Deffense (1985) where the high melting TAGs would crystallise out in the intial stages of crystallisation. However, they argued that the concentration another high melting SSS TAG, i.e. PSS remained unchanged in the
nuclei and completely formed crystals despite its high melting point, hence further supporting their suggestion on the importance of PPP, PPS and MPP in the nucleation stage of palm oil crystallisation.

2.5.6 Effects of minor components

Jacobsberg & Oh (1976) carried out studies on the influence of free fatty acids (FFA), partial glycerides content and degree of oxidation on palm oil crystallisation and concluded that an increase in all these factors lowered the solid fat content (SFC) of palm oil. This conclusion was based on the observed decrease in SFC measurements and from DSC thermograms which showed a shift towards the lower melting range with increasing FFA, partial glyceride and oxidation levels. Where partial glycerides are concerned, this was mainly attributed to the extension of the $\alpha$-crystals lifetime in the presence of partial glycerides, thereby delaying the transition to $\beta'$ and also due to eutectic or mixed-crystals formation (Persmark et al., 1976). Therefore, it is imperative to ensure that the initial oil quality prior to crystallisation should bear a low FFA, partial glyceride and oxidation level in order to improve the crystallisation properties of palm oil.

A later study conducted by Siew & Ng (1999) showed that the effect of DAGs on the crystallisation of palm olein depended on the type of DAGs present in the oil. Dipalmitoylglycerol (PP), particularly of the 1, 3-isomer caused the oil to crystallise rapidly while palmitoyloleoylglycerol (PO) was found to exhibit a retardation effect in the crystallisation of the oil. The dioleoylglycerol (OO) glyceride, however, showed no significant effect with respect to the crystallisation of palm oil. They later found that DAGs also inhibited the nucleation process and retarded crystal growth rates of TAGs at low degrees of supercooling (Siew & Ng, 1999).

Additives also have been shown to play some inhibitory effects in palm oil crystallisation. Kawamura (1980) investigated the retardation effect of sorbitan esters on the crystal polymorphic transformation in palm oil and found that sorbitan tristearate was the most effective in retarding the transformation from the unstable $\alpha$
and $\beta'$ crystal forms to the stable $\beta$ at concentrations and heating rates greater than 1.5% and 5 K/min, respectively.

### 2.6 THERMODYNAMICS OF FATS CRYSTALLISATION

#### 2.6.1 Thermal properties of pure TAGs

The physical and chemical behaviour of a specific fat mainly depends on the properties of the constituent TAGs and the phase behaviour of mixtures of TAGs (Sato, 2001). A good starting point to predict the melting property of a fat is by analysing the thermal properties of its component TAGs. The thermal properties of TAGs have been critically reviewed by various authors (Malkin, 1954; Knoester et al., 1972; Timms, 1978). For this work, the melting points and heats of fusions of TAGs are of major importance in determining the solid-liquid behaviour of palm oil and shall be reviewed here in detail. For approximations, the melting point of a TAG can be estimated by averaging the melting points of its fatty acid components. The melting points of some palm oil TAGs relevant to this work in their different crystalline forms are presented in Table 2-9. It can be seen that the melting points generally increase from $\alpha$ to $\beta'$ to $\beta$ (but not always) and with increasing degree of saturation.

Timms (1978) has summarised the most reliable data of heats of fusions of TAGs based on experimental data. Some selected values of heats of fusions of palm oil TAGs in the $\alpha$, $\beta'$ and $\beta$ forms are given in Table 2-10, Table 2-11 and Table 2-12. It can be seen that the enthalpy values increase from from $\alpha$ to $\beta'$ to $\beta$, reflecting the closer packing and greater thermodynamic stability of the higher melting forms.
### Table 2-9 Melting points of some palm oil TAG crystal forms

<table>
<thead>
<tr>
<th>TAG</th>
<th>Melting point (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>β</td>
</tr>
<tr>
<td>PPP</td>
<td>-</td>
<td>56.7</td>
</tr>
<tr>
<td></td>
<td>44.7</td>
<td>55.7</td>
</tr>
<tr>
<td>OOO</td>
<td>-32</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-11.8</td>
</tr>
<tr>
<td></td>
<td>-33.7</td>
<td>-10</td>
</tr>
<tr>
<td>POP</td>
<td>15.2</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td>16.6</td>
<td>33.2</td>
</tr>
<tr>
<td>PPO</td>
<td>18.5</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td>18.4</td>
<td>34.6</td>
</tr>
<tr>
<td>POO</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>14.2</td>
</tr>
<tr>
<td>OPO</td>
<td>-18.3</td>
<td>11.7</td>
</tr>
<tr>
<td>POS</td>
<td>18.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>19.6</td>
<td>31</td>
</tr>
<tr>
<td>PPS</td>
<td>47.4</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>46.5</td>
<td>59.5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>59.9</td>
</tr>
<tr>
<td></td>
<td>46.4</td>
<td>58.7</td>
</tr>
<tr>
<td>PSP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td>47.2</td>
<td>67.7</td>
</tr>
<tr>
<td>MPP</td>
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<td>52</td>
</tr>
<tr>
<td>SOS</td>
<td>-</td>
<td>36.7</td>
</tr>
<tr>
<td>SOO</td>
<td>-</td>
<td>8.8</td>
</tr>
<tr>
<td>OOL</td>
<td>-</td>
<td>-28.3</td>
</tr>
<tr>
<td>OLL</td>
<td>-</td>
<td>-3.02</td>
</tr>
<tr>
<td>PLP</td>
<td>-</td>
<td>18.6</td>
</tr>
</tbody>
</table>
### Table 2-10  Heats of fusions of TAGs in the α form

<table>
<thead>
<tr>
<th>Triacylglycerol (TAG)</th>
<th>ΔH&lt;sub&gt;f&lt;/sub&gt; (kJ/mol)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP</td>
<td>95.8</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PSP</td>
<td>112.2</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPS</td>
<td>100</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PSS</td>
<td>106.0</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PMP</td>
<td>79.0</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>MPP</td>
<td>89.0</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>POP</td>
<td>70</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPO</td>
<td>53</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>POS</td>
<td>71.5</td>
<td>Rousset et al. (1998)</td>
</tr>
<tr>
<td>SOO</td>
<td>109.7</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>PSO</td>
<td>111.0</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>PLinP</td>
<td>99.6</td>
<td>Timms (1978)</td>
</tr>
</tbody>
</table>

### Table 2-11  Heats of fusions of TAGs in the β' form

<table>
<thead>
<tr>
<th>Triacylglycerol (TAG)</th>
<th>ΔH&lt;sub&gt;f&lt;/sub&gt; (kJ/mol)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP</td>
<td>126.5</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PSP</td>
<td>165.5</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPS</td>
<td>124.0</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPO</td>
<td>113.5</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>POO</td>
<td>94.6</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>SOO</td>
<td>109.7</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>PSO</td>
<td>111.0</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>PLinP</td>
<td>99.6</td>
<td>Timms (1978)</td>
</tr>
</tbody>
</table>
Table 2-12  Heats of fusion of TAGs in the β form

<table>
<thead>
<tr>
<th>Triacylglycerol (TAG)</th>
<th>ΔH_f (kJ/mol)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPP</td>
<td>171.7</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>Knoester et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>171.3</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PSP</td>
<td>166.2</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>166</td>
<td>Knoester et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>166</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPS</td>
<td>169.1</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>Knoester et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>166.3</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>POP</td>
<td>149.9</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PPO</td>
<td>116.4</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>POS</td>
<td>146.5</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>149</td>
<td>Rousset et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PSO</td>
<td>111.0</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>SPO</td>
<td>126.0</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>POO</td>
<td>94.6</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>OPO</td>
<td>125.6</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>SOO</td>
<td>109.7</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>OOO</td>
<td>95.5</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>PLinP</td>
<td>99.6</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>MPP</td>
<td>140</td>
<td>Wesdorp et al. (2005)</td>
</tr>
<tr>
<td>P(OH)P</td>
<td>111.8</td>
<td>Timms (1978)</td>
</tr>
<tr>
<td>P(OH)(OH)</td>
<td>70.3</td>
<td>Timms (1978)</td>
</tr>
</tbody>
</table>
2.6.2 Phase diagrams of binary TAG systems

In order to fully comprehend and understand the crystallisation behaviour of a fat system such as palm oil, it is useful to next study the interaction of pure TAGs in smaller systems i.e. binary or ternary mixtures of specific TAG components of that particular fat. A phase diagram is a convenient way to describe the phase behaviour of binary TAG mixtures. As noted by Timms (1984), there are four types of phase diagrams occurring in binary TAG systems. The types of phase diagrams that have been observed from studies conducted previously are (a) monotectic with continuous solid solution, (b) eutectic, (c) monotectic with partial solid solution and (d) peritectic as illustrated in Figure 2-19.

The most common type of phase behaviour demonstrated by TAG binary systems is of the eutectic type (Timms, 1984). This type of behaviour tends to occur when the TAGs differ in molecular volume, shape or polymorph and slightly in melting point. Examples of these are PPO/POO and POO/OPO, portrayed in Figure 2-20. A TAG mixture will exhibit continuous solid solution behaviour when the TAGs are alike in terms of melting point, molecular volume and polymorph. Typical systems displaying this behaviour are PPP/000 (Rossell, 1967) and POS/POP (Wesdorp et al., 2005), as depicted in Figure 2-21.

If the difference in melting point of the TAGs is increased, the eutectic system is likely to shift to the monotectic system (Timms, 1984). Monotectic systems can be observed in PPP/POO (Wesdorp et al., 2005) as further illustrated in Figure 2-22. The only observed peritectic behaviour was found to be in the SOS/SOO system. In all cases, except for SOS/SOO, typical examples presented here of TAGs showing specific types of phase behaviour shall consist of palm oil TAGs.
Figure 2-19 Types of phase diagrams of binary TAG systems (Timms, 1984)

![Figure 2-19](image)

Figure 2-20 Phase diagrams showing eutectic behaviour (Wesdorp et al., 2005)

![Figure 2-20](image)
Figure 2-21  Phase diagrams showing monotectic with continuous solid solution behaviour (a) PPP/OO (Rossell, 1967) and (b) POS/POP (Wesdorp et al., 2005)

Figure 2-22  Phase diagram of PPP/POO showing monotectic behaviour with partial solid solution (Wesdorp et al., 2005)
The most comprehensive review on phase diagrams of TAG systems was conducted by Rossell (1967). He compiled, correlated and checked the reliability of available information on previous work done on TAG systems until up to 1966. His work presented four types of systems which included systems involving only simple mono-
acid TAGs, mixed-acid TAGs, ternary mixtures of TAGS and systems with partial glycerides. He also reported that from his review of all the existing binary phase diagrams up to that date, there were a number of problems which occur during the construction of a binary phase diagram. These include the presence of impurities, incomplete or incorrect stabilisation and other experimental errors. However, he did not give any quantitative interpretation of the phase diagrams, thus most of these phase diagrams were open to further investigation.

In later work, Knoester et al. (1972) investigated the solid-liquid phase behaviour of six TAGs containing palmitic and stearic acids using a microcalorimeter and found that the phase diagrams of 10 out of 15 mixtures showed eutectic behaviour while the rest showed high miscibility in the solid state. All mixtures containing PPP and PSP demonstrated eutectic behaviour except with PPS and SSP respectively. Their study depicted that the presence of an asymmetric TAG in a binary mixture will result in a high mutual solubility in the solid phase. They also concluded that the mutual solubility of TAG in stable crystals critically depends on the exact conformation of the molecules and even small differences in total chain length or simple rearrangement of chains makes it impossible for mixed crystals to form.

The miscibility of TAG in the liquid state has been shown to be ideal where there are no changes in heat or volume during mixing (Knoester et al., 1972). In such cases, the ideal solubility equation or the Hildebrand equation can be employed. However, this is only the case when trying to express the equilibrium between crystals of a pure component within a liquid mixture. A different expression is required to relate the equilibrium of a liquid mixture and a mixed crystal, which was discussed earlier in Section 2.4.1.
Chapter 2

Later work by Rossel (1973) reviewed the interaction of various types of TAGs and concluded that similar TAGs do not necessarily show compatibility (see Table 2-13). He concluded that the existence of these different systems were mainly due to polymorphism of the crystals and overlapping of the glyceridic chains in the crystal lattice. His work also found that a molecular compound formation was observed in the systems of SSO/SOS and POP/OPO. These compound crystals form when the TAGs are in an exact stoichiometric ratio of 1:1 as they neatly pair together in a crystal lattice.

**Table 2-13 Interaction of triglycerides (Rossel, 1973)**

<table>
<thead>
<tr>
<th>Continuous Solid Solutions</th>
<th>Eutectics / Monotectics</th>
<th>Compound Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSS / ESS</td>
<td>PPP / SSS</td>
<td>SSO / SOS</td>
</tr>
<tr>
<td>POS / SOS</td>
<td>EEE / SOS</td>
<td>POP / OPO</td>
</tr>
<tr>
<td>POP / POS</td>
<td>POS / PSO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPP / LLL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PPP / SOO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POS / SPO</td>
<td></td>
</tr>
</tbody>
</table>

Molecular compounds were also observed by Koyano et al. (1992) who investigated the phase behaviour of SOS and OSO with X-ray diffractometry and thermal analysis. They found that a molecular compound was formed when the two components were in equal concentrations (1:1), causing two monotectic phases of SOS/compound and OSO/compound positioned side-by side. Figure 2-23 illustrates this behaviour. Their investigation found that this compound possessed a double chain length structure opposed to SOS and OSO in their stable forms. They also observed this similar compound formation in other binary mixtures of POP/OSO and POS/OSO.
A study by Minato et al. (1996) on the phase behaviour of PPP/POP using synchrotron radiation X-ray diffraction and DSC showed that this binary system exhibited monotectic properties in both metastable and stable forms. They also observed an \( \alpha \) to \( \beta \) solid-state transformation of PPP below 40% POP concentration while above POP concentrations of 50%, the same polymorphic transformation went through an intermediate \( \beta' \) form for both POP and PPP. Figure 2-24 illustrates the binary phase diagram obtained by Minato et al. (1996).
A year later, Minato and his co-workers extended their study on the binary mixture of POP and OPO using the same technique. They observed similar results as in Koyano et al. (1992) where they discovered the formation of a molecular compound ($\beta_c$) at a 1:1 concentration ratio of POP and OPO (Minato et al., 1997a). This behaviour was also observed in a separate study within the same year with a slightly different binary mixture of POP and PPO (Minato et al., 1997b). Figure 2-25 gives a representation of the observed behaviours in both POP/OPO and POP/PPO systems.

![Phase diagrams of POP/OPO (Minato et al., 1997a) and POP/PPO (Minato et al., 1997b)](image)

**Figure 2-25** Phase diagrams of POP/OPO (Minato et al., 1997a) and POP/PPO (Minato et al., 1997b)

A more recent study on the phase behaviour of two major TAGs present in palm oil i.e. POP and OOP was conducted by Zhang et al. (2007). They found that mixtures of POP/OOP were immiscible and displayed eutectic behaviour in both their most stable and metastable states. This finding was in contrast with the studies on POP/OPO and POP/PPO by Minato et al. (1997) where a molecular compound was formed. Zhang et al. (2007) concluded that the immiscibility nature of the POP/OOP mixture was mainly due to the destabilisation effect of the glycerol conformation, acyl chain packing and methyl end stacking within the molecule arrangement, thus yielding an absence of a molecular compound formation, as depicted in Figure 2-26.
Having reviewed all the available literature on phase behaviour of lipids up to this date, it is surprising that there are limited studies relating to the chemical potential driving forces of TAG components present in palm oil as the composition and/or temperature of the system changes, particularly during the crystallisation process. Thus, this work is intended to explore the evolution of the driving forces for crystallisation of individual components in palm oil. It is hoped that this will deepen our understanding of the phase behaviour of lipid mixtures in palm oil which could later be extended to other fats systems in general.

2.7 CONCLUSIONS

Palm oil has been shown to be an important source of feedstock for edible and non-edible applications alike in the oils and fats industry due to its versatility and distinct physical and chemical characteristics. The dry fractionation process is widely used to modify palm oil to produce subfractions with enhanced properties, which further expands palm oils' usage. Crystallisation plays a major role in the fractionation process as the final product quality will largely depend upon the crystallisation conditions applied prior to the separation stage. Thus it is necessary to understand the crystallisation behaviour of the components that form the very nature of palm oil in order to effectively control the crystallisation process to yield the product characteristics desired in the end.
Having reviewed all the available literature on phase behaviour of lipids up to this date, it is surprising that there have not been many studies relating to the chemical potential driving forces of TAG components present in a fat system as the composition and/or temperature of the system changes, particularly during the crystallisation process of palm oil. The theory on this subject has long been established, however few attempts have actually been made to try and fit experimental data with the thermodynamic models outlined in the preceding sections. It has also been shown that most crystallisation studies on palm oil reviewed in this chapter have been conducted on static (non-stirring) systems such as differential scanning calorimetry (DSC) and X-ray diffraction (XRD), using very small amounts of sample which is far from representing the actual crystallisation process applied on an industrial scale. This may be due to the limited number of instruments available which provide continuous monitoring during the study of the crystallisation process.

The unceasing entrainment problem encountered during the separation stage of the fractionation process of edible oils and fats, particularly in the fats processing industry and at laboratory scale alike, has been brought up in the past literature time and time again. Even so, this issue has yet to be addressed and resolved as after surveying the current literature, most researchers take the composition of the ST fraction containing the entrained OL as such in the absence of any correction for entrainment. This may not reveal the true ST composition as entrainment largely remains the main cause for the SUU and UUU TAGs to be present in the composition of the ST even though their melting properties suggests that they ought to remain uncrystallised in the OL.

Hence, in order to fill in the gaps within this research area, this work is intended to study the partitioning behaviour of palm oil TAG components and to explore how they relate to the evolution of the driving forces for crystallisation. This study also seeks to investigate to what extent the role of entrainment plays in studying the true crystallisation behaviour of palm oil TAGs. It is hoped that this will deepen our understanding of the phase behaviour of lipid mixtures in palm oil which could later be extended to other fat systems in general.
3 EXPERIMENTAL METHODOLOGY

3.1 INTRODUCTION

This chapter shall present a detailed description of the experimental and analytical techniques used in this work. The chapter begins by briefly describing how the palm oil samples are prepared followed by how the fractional crystallisation experiments are performed. The main monitoring and analytical tools used in this research comprise the focused beam reflectance measurement (FBRM) technique and non-aqueous reversed-phase high performance liquid chromatography (NARP-HPLC). These shall be introduced and their operation and application shall be elaborated upon together with a discussion on how the data obtained from these two techniques were treated. The chapter will end with an introduction to differential scanning calorimetry (DSC) and its application in this research.

3.2 FRACTIONATION

3.2.1 Sample preparation procedure

Refined, bleached and deodorised (RBD) palm oil was supplied by Golden Jomalina Sdn. Bhd. (Sepang, Malaysia) and shipped via the Malaysian Palm Oil Board (MPOB). Sample preparation involved melting the palm oil container to 70 °C either in an oven or temperature-controlled water bath until it was completely liquefied. This step ensures that the oil and all crystals present in it are completely melted. This is followed by stirring or vigorous shaking of the palm oil container in order to ensure homogeneity throughout. Approximately 700 mL of the homogenised palm oil was then transferred to a pre-weighed 1000 mL beaker and subsequently introduced into the crystalliser system used in this study. This sample preparation procedure was carried out for all the crystallisation experiments involved in this research.
3.2.2 Experimental rig setup

Fractionation experiments were carried out in an 800 mL or similar scale stirred cylindrical round-bottomed jacketed glass vessel (see Figure 3-1). Approximately 700 mL of palm oil was charged into the jacketed glass vessel for every experiment. The oil temperature in the vessel was controlled and monitored using a PT100 platinum resistance thermometer directly connected to a programmable circulating bath (Model ministat 125, Huber Technology, Offenburg, Germany) linked to a PC control station. The thermal fluid used in the cirulator was silicone oil (Dow Corning® 200/20 cS). Three thermocouples (TC0, TC1 and TC2) were installed at the top, middle and bottom of the vessel to monitor the temperature in the crystallising oil.

Figure 3-1 A schematic representation of the experimental set-up for the crystallisation experiments
Experimental Methodology

Figure 3-2  Photograph of the actual crystallisation rig

A stainless steel agitator comprising of two 3-blade marine impellers fixed to the agitator shaft was utilised to agitate the system in study. The agitator speed was held at 200 rpm throughout all experiments to avoid any differences in crystallisation behaviour of the oil that may occur due to any changes in agitator speed. A focused beam reflectance measurement (FBRM) probe (Model A100, Laser Sensor Technology Inc., Washington, USA) was installed through a flanged port at the top of the vessel, providing continuous real time monitoring of the particle count and size distribution, as shown in Figure 3-2. A detailed description of the principles and measurement method of the FBRM shall be discussed in further detail in Section 3.3.

All readings of the vessel temperature, thermostat temperature, agitation speed and FBRM particle counts were recorded and saved by the PC via a LabVIEW data acquisition interface (National Instruments Corp., Texas, USA). This also provided feedback control of the agitator speed and crystalliser temperature, which could be controlled according to a preset temperature-time profile. The system could also be configured to control the vessel jacket temperature rather than the temperature of the

Partitioning of triacylglycerols in the fractional crystallisation of palm oil

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palm oil in the vessel. The jacket temperature control has a faster response. In this work, three types of fractionation experiments were conducted on palm oil, namely isothermal crystallisation, non-isothermal crystallisation and remelting studies. The design of the temperature profiles for all of these shall be discussed in the following subsections.

3.2.3 Isothermal cooling profile

The isothermal cooling profile designed in this work was tailored to ensure that stable isothermal conditions were reached in as short a time as possible with minimal “undershoot” of temperature and thus prevent any nucleation occurring before reaching the desired isothermal temperature \(T_1\). This was done in order so that any effect on the composition and partitioning behaviour of the TAGs in the oil was simply due to the variation in time only. The control strategy during the cooling stage was based on the jacket temperature \((T_j)\) which was then switched to the oil temperature \((T_{oil})\) for the isothermal hold period. This provided the fastest and most reproducible control of temperature during the cooling periods, whilst ensuring that the oil temperature maintained its correct value during the isothermal hold period.

The following temperature set point programme was applied for all isothermal crystallisation experiments: heat \((T_j)\) to 85 °C at heating rate of 2 °C/min, cooling to 75 °C at a cooling rate of 2 °C/min, further cooling to 72 °C at a cooling rate of 0.3 °C/min, holding at 72 °C for 45 minutes, cooling to 60 °C at a cooling rate of 2.4 °C/min, further cooling to 3 °C below \(T_i\) at a rate of 1.18 °C/min, heating to \(T_i\) at a rate of 0.2 °C/min and finally holding the temperature (now \(T_{oil}\)) isothermally at \(T_i\) until crystallisation is deemed complete. The basis for identifying that the crystallisation stage was complete and that system equilibrium has been reached is when the particle counts reading measured by the FBRM and the product yields no longer changed with time. Hence, the isothermal time, \(t_i\) in these experiments varied according to the point when system equilibrium was attained.

Figure 3-3 illustrates a typical example of the isothermal cooling profile applied at 30 °C. Five isothermal holding temperatures of 24 °C, 26 °C, 28 °C, 30 °C and 32 °C
were studied in this work. The cooling profiles of all temperatures were exactly the same during the initial part of the experiment and only differed in the $T_i$. During each of these studies, the oil was sampled and vacuum filtered at regular time intervals of between 7 to 10 minutes after approximately 15 to 20 minutes from the occurrence of nucleation until the yield of the solid fraction filtered and FBRM particle counts remained constant. Filtration times shall be designated as $t_f$ throughout this work.

![Isothermal cooling profile for palm oil crystallisation at 30 °C ($T_{sp} = \text{setpoint temperature; } T_{oil} = \text{oil temperature}$)](image)

**3.2.4 Non-isothermal cooling profile**

In the non-isothermal cooling experiments, different cooling rates were applied to the palm oil crystallisation process. The oil was initially heated to 70 °C at a heating rate of 1 °C/min and maintained at that temperature for 20 minutes until the oil was fully melted to remove any crystal effects. This was followed by cooling the oil to 40 °C at a cooling rate of 1 °C/min and further cooling to a final temperature of 10 °C at a cooling rate of 0.1, 0.3 and 0.5 °C/min as depicted in Figure 3-4. The circulator control mode for the non-isothermal experiments was the $T_{oil}$ setpoint for the entire
duration. The oil was then sampled and filtered at regular time intervals of between 10 to 15 minutes after the nucleation took place until the oil temperature reached 10 °C.

![Graph showing non-isothermal cooling profiles for palm oil crystallisation](image)

**Figure 3-4** Non-isothermal cooling profiles for palm oil crystallisation

### 3.2.5 Post-crystallisation remelting study temperature profile

The post-crystallisation remelt study conducted in this work was carried out to examine the melting behaviour of palm oil when a subsequent sequential melting step was applied immediately after isothermal crystallisation at a certain $T_i$. This would allow for the possibility of analysing the different TAG components or groups that may start to melt as the temperature of the system is raised in a successive manner. The initial isothermal cooling profile implemented in this study prior to the remelting stage was identical to the isothermal cooling profile applied at 24 °C (please see previous subsection 3.2.3). The circulating bath controller mode was set to 'internal' at the start of the experiment to permit the control of $T_j$ to follow $T_{sp}$. As soon as the setpoint reached 24 °C, it was manually switched to the 'control' setting to allow for the regulation of the $T_j$ that will enable $T_{oil}$ to reach the desired setpoint temperature, $T_{sp}$. When the isothermal time, $t_i$ reached the end of the cooling profile at 24 °C, the...
$T_i$ was then increased by as much as 2 °C ($\approx T_i + 2$ °C) at a heating rate of 0.4 °C/min. The $T_{sp}$ was maintained at the new $T_i$ for 30 minutes before further increase to the next temperature ($T_i + 2$ °C). This melting method was carried out in the same way from $T_i = 24$ °C until a final $T_i$ was reached of 46 °C where the oil had fully melted and the total particle counts decreased to a minimum. Figure 3-5 depicts the setpoint profile employed for the post-crystallisation remelt experiment.

Samples of the remelted oil were extracted and filtered at every $T_i$ at approximately 5 minutes before the fixed $t_i$ of 30 minutes lapsed. This ensures that the crystallising oil has reached equilibrium at that particular $T_i$. The yields of the filtered products were weighed and calculated and samples were then analysed further by RP-HPLC.

![Figure 3-5 Setpoint profile for the post-crystallisation remelting experiment](image)

### 3.2.6 Sampling and filtration

The crystallising oil was sampled using a 5 mL plastic pipettor tip (ART 5000, Sigma-Aldrich Co., Dorset, UK) connected to a single-channel piston-driven air replacement pipettor (Pipetman P5000, Gilson Inc., Wisconsin, USA). The pipettor tip was immersed in the crystallising oil for 30 seconds before sampling in order to
maintain the pipette tip at the same temperature as the crystallising oil. Once the desired filtration temperature or time was reached, approximately 5 mL of the slurry was sampled and immediately injected onto a filter paper (Whatman Grade 541, Fisher Scientific, Loughborough, UK) used in the vacuum filtration system. The vacuum filtration apparatus used in this work is shown in Figure 3-6. The separated solid and liquid fractions were then weighed and their yields determined. Each fraction was then subjected to compositional analysis by high performance liquid chromatography (HPLC).

**Figure 3-6  Filtration apparatus**

For experiments involving washing of the retentate, laboratory-grade acetone (Fisher Scientific, Loughborough, UK) contained in a washing bottle was weighed prior to washing. Samples were either washed simultaneously during filtration or washed after normal (without washing) vacuum filtration, depending on the study performed. Acetone was squirted onto all areas of the sampled oil on the filter paper to ensure that almost every crystal is washed and the majority of entrained oil is expelled. The acetone bottle was then weighed immediately after each filtration and the acetone usage was calculated. The acetone-washed liquid fraction was then left to evaporate.
3.3 FOCUSED BEAM REFLECTANCE MEASUREMENT (FBRM)

3.3.1 Introduction

Monitoring the characteristics of particles, particularly in the crystallisation of fats is important in developing an understanding of crystallisation behaviour and to facilitate the design of downstream processes. A key aspect in characterising particles during crystallisation is particle population and size distribution. Also vital are the detection of events occurring during the progression of crystallisation. Various types of devices have been employed in the past for particulate characterisation in suspensions in general; the most common are optical measurement techniques ranging from image analysis, laser particle counters, particle size analysers, electrical sensing zones and laser diffraction. However, these conventional methods have drawbacks which require sampling the system under study and/or diluting the sample thus yielding results which may not be a representation of the actual conditions during the process.

The focused beam reflectance measurement (FBRM) method has become a popular tool in recent years for the real time and in-situ monitoring of particulates in suspension systems. This technique prevails over other techniques due to the elimination of the need for sampling and dilution of the system in study as required by most conventional particle analysing methods. The FBRM has the capability of detecting the onset of nucleation and analysing in-situ changes in particle dimension, concentration and population. The next subsection will describe the measurement principle of FBRM in further detail.

3.3.2 Principles of FBRM

The principle of measurement of the FBRM is laser backscattering. The FBRM consists of a cylindrical probe in which a scanning laser beam is passed through a sapphire window at the end of the probe. A set of optical components rotating at high speed causes this beam to move in a circular path (see Figure 3-7(a)). As the beam passes through the suspension system in study within the specified measurement zone, it can cross through the surface of a particle in its pathway. As this happens, the light...
from the laser beam will be reflected back into the probe. The duration the laser beam
takes to cross through a particle ($\Delta t$) is then multiplied by the velocity of the scanning
beam ($v_b$) resulting in a chord length ($s$), which is defined as the distance of the path
of the laser beam crossing the particle (Ruf et al., 2000). This is illustrated in Figure
3-7(b).

The chord length depends on the orientation and geometry of the particle at the time
of measurement. At any one time, thousands of chord lengths are measured and these
are sorted according to different size channels. The measurement range of the FBRM
is between 0.8 to 1000 $\mu$m and measurements can be grouped into 38 intervals or 90
intervals, depending on the FBRM model used. All recorded chord length
measurements in their respective intervals are collected to produce a real-time chord
length distribution (CLD) depicted as a histogram, as shown in Figure 3-7(c). The
count-based distributions provided by the FBRM software can either be weighted or
unweighted to analyse changes occurring within the process. The former is more
susceptible to changes in coarse particles while the latter is sensitive to small changes
in finer particles, thus allowing one to monitor events such as nucleation, crystal
growth or agglomeration during the crystallisation process.

![FBRM measurement principle](image)

**Figure 3-7** FBRM measurement principle (a) FBRM probe, (b) chord length
measurement and (c) histogram of the chord length counts (Ruf et al., 2000)
The number of counts and chord lengths reported is largely influenced by the solids concentration and the particle diameter and shape, respectively (Barrett and Glennon, 1999). However, CLD cannot only be used as a definitive representation of the actual particle size. It gives only an indication of the actual particle size distribution (PSD). To transform CLD to PSD a relationship must be developed to correlate the two based upon some assumption of the particle geometry (the simplest assumes a spherical geometry). A straight-forward method for quantifying real-time PSD is coupling the FBRM with an online image analyser such as the LASENTEC particle and vision measurement (PVM) system, which provides in-process images of particle size and structure. Nevertheless, this does not limit the usage of the FBRM for other monitoring purposes such as quantifying particle population, detection of multiple events during a process and as a means to assess the dynamic changes in particle size distribution during the crystallisation process which can eventually allow prediction of the trends of the final product size.

### 3.3.3 FBRM monitoring procedure

In this study, a focused beam reflectance measurement (FBRM) probe (model A100, LASENTEC Inc., Washington, USA) was installed at a 45° angle through a flanged port in the top of the vessel to follow closely the real time particle count and size distribution as the crystallisation process progresses (see Figure 3-2). The sapphire window at the probe tip was cleaned with general laboratory-grade acetone (Fisher Scientific, Loughborough, UK) prior to use in order to remove any unwanted fine particles or dust that may affect the measurements taken. The crystallisation vessel was further filled with general laboratory-grade acetone (Fisher Scientific, Loughborough, UK) and brought to boil to thoroughly clean the entire system. The precision micrometer was adjusted to a focal point position of 0.020 mm, the distance which is recommended by the instrument supplier. Measurement duration was set to 24.75 seconds and an average of 10 measurements was made during the specified measurement duration. Particle counts and chord lengths were measured, recorded and converted into real-time trends using the LASENTEC software (LASENTEC Inc., Washington, USA). These statistics include the total particle counts, particle counts according to different size ranges, square weighted counts, mean chord lengths.
and length-cubed mean weights. APPENDIX A provides proof of mean chord length calculations executed by the LASENTEC software.

Heath *et al.* (2000) conducted a study comparing different conventional particle sizing techniques i.e. laser diffraction and electric sensing zone with the FBRM response on sieved aluminium and calcite suspensions. They found that the square-weighted chord length FBRM results were comparable to other sizing techniques for sizes between 50 to 400 μm. From their study, it can be said that the best way to follow the particle size evolution throughout the entire crystallisation experiment is either by observing the square-weighted chord distribution (SWCD) at different points in time (i.e. filtration points) or from monitoring the change in the square-weighted mean chord length over time. The former statistic is defined as the square-weighted chord lengths in individual bins divided by the sum of the square-weighted chord lengths in all bins and is calculated using the following equation at different time intervals:

\[
SWCD = \frac{n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2} \tag{3-1}
\]

where \(n_i\) is the counts in an individual measurement channel, \(M_i\) is the midpoint of an individual channel and \(k\) is the upper channel number (\(2 \leq k \leq 38\)). The latter shows the evolving average or mean chord size of the crystals throughout the entire experiment and is calculated using the following equation:

\[
\bar{C}_s = \frac{\sum_{i=1}^{k} Y_{i,s} M_i}{\sum_{i=1}^{k} Y_{i,s}} = \frac{\sum_{i=1}^{k} \left[ \frac{n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2} \right] M_i}{\sum_{i=1}^{k} \left[ \frac{n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2} \right]} = \frac{\sum_{i=1}^{k} n_i M_i^3}{\sum_{i=1}^{k} n_i M_i^2} \tag{3-2}
\]

where \(\bar{C}\) is the average chord, \(s\) is the length square weight and \(Y_i\) is the percentage (%) per channel.
3.4 REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

3.4.1 Principle of RP-HPLC

High performance liquid chromatography is a liquid chromatographic technique which is commonly used in lipid analysis. The separation of compounds in HPLC is based on the selective interactions of solutes between a mobile phase (a liquid) and a stationary phase (a solid) (Buchgraber et al., 2004). There are two types of HPLC methods; normal phase and reversed phase HPLC. The difference between the two is that the former employs a stationary phase which is more polar than the mobile phase while this condition is reversed for the latter. In this work, reversed phase high performance liquid chromatography (RP-HPLC) was used to determine the TAG composition of palm oil. It separates complex mixtures of TAG components of lipids based on the chain length and degree of unsaturation of the fatty acid components in the TAGs. Separation of TAG species by RP-HPLC will only take into account regiospecific positions without distinguishing between fatty acid positions of 1 and 3 of the glycerol molecule.

3.4.2 Sample preparation for RP-HPLC

Samples from all experiments consisted of the original palm oil (PO) and the respective olein (OL) and stearin (ST) fractions from each filtration. Samples were melted thoroughly in an oven at 60 °C until completely liquefied. Approximately 0.02500 ± 0.00010 g of sample was pipetted into 2 mL glass vials (Supelco, Bellefonte, USA). The sample was then diluted with 0.5 mL of chloroform of HPLC grade (Fisher Scientific, Loughborough, UK), forming a 5% (w/v) solution and screw capped with a fitted Teflon-lined septum. Samples were shaken vigorously to ensure complete dilution of the oil sample within the solubilisation solvent. Sample preparation was carried out in the same manner for all samples unless otherwise stated.
3.4.3 TAG analysis procedure via NARP-HPLC

A HPLC instrumentation system (see Figure 3-8) equipped with a Hewlett Packard HPLC binary pump system (model HP 1100 Series, Waldbronn, Germany), a variable loop injector system, a column oven and an Agilent refractive index detector (Agilent 1100 Series, Waldbronn, Germany) was used to analyse TAGs in the palm oil, palm olein and palm stearin samples. TAGs were separated on two identical Waters NovaPak® C18 (Waters Corp., Darmstadt, Germany) columns connected in series with column dimensions of 3.9 mm internal diameter by 300 mm length and preloaded with silica with a particle size of 4 μm. Both columns were maintained at a temperature of 25 °C. The mobile phase used was a mixture of HPLC-grade acetone (Fisher Scientific, Loughborough, UK) and HPLC-grade acetonitrile (Fisher Scientific, Loughborough, UK) in a volume-to-volume (%v/v) ratio of 63.5:36.5 and the flowrate was fixed at 1 mL/min. The injection volume was set to 10 μL of 5% (wt/volume) of oil in chloroform. Attenuation was fixed at 500 x 10^3 RI units and the refractive index detector was maintained at 35 °C. The total runtime for a single injection was 130 minutes and at any one time, only four samples could be analysed as the elution solvent reservoir did not permit any volume exceeding 1 L. The total run time for four samples was 15 hours.

Identification of TAGs was made based on the retention time of TAG standards using the same column, solvent and flowrate conditions in the work of Haryati et al. (1998) and by comparison with earlier literature (Ghazali et al., 1995; Swe et al., 1995; Chen et al., 2007). Integration of chromatographic peaks was carried out manually using the HPLC ChemStation integration software. Integrated peak areas of TAGs and other minor components were normalised based on the total area of all peaks present in the chromatogram, as follows:

\[ \% \text{ component } i = \frac{A_i}{\sum_{i=1}^{n} A_i} \times 100 \quad (3-3) \]

where \( i \) is any component, \( A \) is the area of the chromatographic peak and \( n \) is the number of components present in the sample. Other minor components such as MAGs...
and DAGs were not quantified individually and their concentrations were collocated together and their total concentration expressed as ‘Others’. In this work, compositional HPLC results were expressed in weight percentages (%wt) based on Christie (2003).

![HPLC instrument used in this study](image)

**Figure 3-8**  HPLC instrument used in this study

The TAG analysis procedure was initially performed on a single Waters Nova-Pak© C\textsubscript{18} column. However, the chromatogram of analysed samples showed several peaks which were incompletely resolved and highly overlapped, as shown in Figure 3-9 for a representative chromatogram of palm oil. It can be seen that overlapping occurs between the areas of major palm oil TAGs of PPP, POP, POO and MPP, between OOL, POL and PPL and between peaks of OLL, PLL and MLP. Using a longer column or arranging two columns in series is one way to improve the resolution of the eluted peaks in the chromatogram (Meyer, 1998). Thus, an additional identical Waters Nova-Pak© C\textsubscript{18} column was connected in series with the existing column and resulted in improved resolution of the TAG peaks with minimum overlap between them, as depicted in Figure 3-10. Although the overlaps between the peaks identified earlier have been minimised, there still remains some degree of overlap which needs to be addressed in order to obtain the true compositions of TAG components. To further assist peak separation, a method for resolving overlapping peaks in MATLAB was developed which shall be discussed in detail in the next section.

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Figure 3-9  Representative chromatogram of palm oil TAGs using a single Novapak C18© column

Figure 3-10  Representative chromatogram of palm oil TAGs using two Novapak C18© columns connected in series
3.4.4 Method for resolving overlapping peak areas

Quantitative information obtained from HPLC chromatograms are usually the area and height of the peaks of eluted compounds. When successive peaks overlap, this may cause inaccurate quantitation in the respective peak areas. Peak integration within chromatographic systems usually tackle overlapping peaks by imposing a cut off where the signal passes through a minimum, which may cause some parts of the areas of these peaks to be erroneously gained by the area of the neighbouring overlapped peak (Meyer, 1998). This method of separation of overlapping peaks is only error-free when the peaks are symmetrical and identical in size. However, this is not the case when dealing with TAG components of an oil or fat which may contain various components of differing concentrations, giving different peak sizes. Thus, it is important to ensure correct peak area calculation or integration in order to obtain the true concentration of a specific component when analysed by chromatographic techniques.

In this work, an overlapping problem occurred between several major TAGs within palm oil, palm olein and palm stearin, namely between OLL, PLL and MLP, between OOL, POL and PPL and between MPP, OOO, POO and POP. This was resolved by devising a MATLAB constrained nonlinear optimisation procedure which enables fitting sum of Gaussians to these overlapping peaks. Prior to this optimisation procedure, a preliminary procedure involving correcting the baseline of the chromatograms generated from HPLC was carried out. Often the baseline of chromatograms from HPLC do not lie in a perfectly horizontal line and there is always some degree of baseline drift occurring. This drift can either be upwards or downwards, depending on different factors such as solvent changing, solvent leaks, mixing problems etc. Although many attempts and precautions have been done to ensure that the HPLC system & solvents are prepared and set-up properly, the baseline drift problem still persists. Hence, the method of baseline correction is generally carried out by shifting the base of the chromatogram into a horizontal position, ensuring that it remains at a value of 0 of the y-axis at both ends. This was performed on different parts of the chromatogram at a time. Figure 3-11 shows illustrates the baseline correction procedure on the group of peaks comprising of MPP, OOO, POO and POP in the chromatogram of palm oil.
Subject to:

\[ x_{\text{min}} \leq x \leq x_{\text{max}} \]
\[ \tilde{f}_{w,j} = \sum_{i=1}^{Ng} w_i N_i(\mu_i, \sigma_i) \]  

(3-6)

where \( x = [w_1, \sigma_1, \mu_1, \ldots, w_{Ng}, \sigma_{Ng}, \mu_{Ng}] \) with \( N_i \) being the Gaussian distribution function where \( i = 1, 2, 3, \ldots, Ng \), \( w_i \) the weight of each Gaussian, \( \sigma_i \) the standard deviation of the Gaussian, \( \mu_i \) the mean of each Gaussian, \( N \) the retention time range, \( Nd \) the number of retention time steps, \( f_{w,i} \) the experimental RIU obtained from HPLC, \( \tilde{f}_{w,j} \) the simulated RIU, where \( i = 1, 2, 3, \ldots, Nd \), \( x_{min} \) is the vector of the lower boundaries and \( x_{max} \) is the vector of the upper boundaries for the parameters of each Gaussian.

This technique was implemented only on the overlapping peaks mentioned earlier (i.e. between OLL, PLL and MLP, between OOL, POL and PPL and between MPP, OOO, POO and POP) and carried out separately for each of these groups. The number of iterations was set to a maximum of 200 iterations as it was shown that the error function value had decreased to a minimum and no longer changed when the iteration number was increased further. The flowchart for the algorithm shown in Figure 3-12 below outlines the steps taken in performing this optimisation procedure. This optimisation procedure was a very time-consuming task as the time taken for a single optimisation programme with 200 iterations to run on just one set of overlapping peaks was approximately 30 minutes. Hence, in a 24 hour period, only 15 HPLC data of samples could be analysed in this programme.
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3.5 TREATMENT OF DATA

3.5.1 Overview

This section shall present a detailed description on the methods and equations used in the treatment of data obtained from the various experimental and analytical techniques explained in the preceding sections of this chapter. The procedure for calculating the yields of the fractionation products and mass fraction of TAGs will be discussed followed by the method used for calculating the crystallisation rates and chemical potential of individual TAGs based on HPLC and TAG thermal properties, treatment of data with respect to the different growth models available from the literature and finally a discussion on the estimation of the entrainment level within the retentate and recalculation of the true ST composition based on the entrainment factor.

Figure 3-12 Flowchart for solving the nonlinear constrained optimisation problem in MATLAB for resolving overlapping peak areas in HPLC
3.5.2 *Product yields*

The crystallisation product yields, mass of TAG and normalised TAG compositions in the OL and ST fraction derived from the experimental and analytical procedures outlined in previous sections were determined in order to allow for the comparison of each TAG with time.

The yield of each olein (OL) or stearin (ST) fraction from each filtration step is expressed as a percentage (%) of the overall sample weight:

\[
Yield \text{ of olein (\%)} = \frac{M_{OL}}{M_{OL} + M_{ST}} \times 100\% \tag{3-7}
\]

\[
Yield \text{ of stearin (\%)} = \frac{M_{ST}}{M_{OL} + M_{ST}} \times 100\% \tag{3-8}
\]

where \(M_{OL}\) and \(M_{ST}\) are the mass of OL and ST fractions in grams respectively.

3.5.3 *Mass fraction of TAGs*

The partitioning of TAG components between the OL and ST phases during the crystallisation of palm oil can be quantified using the equation below:

\[
m_{i}^{OL} = x_{i}^{OL} \times \frac{M_{OL}}{M_{OL} + M_{ST}} \tag{3-9}
\]

\[
m_{i}^{ST} = x_{i}^{ST} \times \frac{M_{ST}}{M_{OL} + M_{ST}} \tag{3-10}
\]

where \(m_{i}^{OL}\) and \(m_{i}^{ST}\) are the mass fractions of TAG component \(i\) in the OL and ST fractions respectively and is expressed in units of grams TAG in OL or ST per 100 g of crystallising palm oil.
An alternative method of describing partitioning is:

\[ m_i^{\prime} = \frac{m_i^{\prime}}{m_i^{\prime\prime}} \]  

(3-11)

where \( m_i^{\prime} \) is the mass of TAG, \( i \) in fraction, \( f \) expressed in units of grams of TAG in OL or ST per total mass of TAG in palm oil and \( m_i^{\prime\prime} \) is the total mass of TAG, \( i \) in 100 g of the starting palm oil which is the sum of the masses of TAG \( i \) in all fractions, \( P \) as follows:

\[ m_i^{\prime\prime} = \sum_{f=1}^{P} m_i \]  

(3-12)

### 3.5.4 Calculation of crystallisation rates of TAGs based on HPLC data

One of the aims of this research was to quantify the crystallisation or growth rates of individual TAGs during the isothermal and non-isothermal crystallisation of palm oil. This was done by taking the masses of TAGs equated in the previous equation and calculating the difference in the masses over time. For all crystallisation methods, the crystallisation rates of TAGs were calculated as follows:

\[ \frac{dm}{dt}_i = \frac{(m_i^{\prime})_n - (m_i^{\prime})_{n-1}}{t_n - t_{n-1}} \]  

(3-13)

where \( \frac{dm}{dt}_i \) is the crystallisation rate of TAG component \( i \), in units of grams of TAG in fraction, \( f \) per 100 g palm oil per unit time and \( t \) is the filtration time in minutes and \( n \) is the filtration number. This rate does not distinguish between nucleation and growth rates of the individual TAGs. Hence, the above equation can be considered as a way to quantify the overall crystallisation rate of a component \( i \) over the time interval, \( t_n - t_{n-1} \).
3.5.5 Calculation of the chemical potential of TAGs based on HPLC data

The chemical potential driving force for the crystallisation of palm oil TAGs was quantified using a rearrangement of equation (2-15) (see section 2.4.1). Here the assumption has been made that assuming that the solid phase behaves ideally during crystallisation, the activity coefficient of component $i$ for the solid phase will equal to unity ($\gamma_i^S = 1$) and this gives the following expression for calculating the chemical potential of each TAG:

$$\mu_L - \mu_S = \frac{\Delta H_{m0} (T_{m0} - T)}{T_{m0}} + RT \ln \frac{x_L}{x_S} \quad (3-14)$$

In some cases the assumption of ideality may obviously be incorrect but this serves as a useful quantity to evaluate, in the absence of activity coefficient data. This calculation was done for all the solid and liquid fractions collected at all filtration times, $t_f$. It has been shown that palm oil tends to crystallise in the $\beta'$ form below 34°C (Persmark et al., 1976; Van Putte & Bakker, 1987). For this reason, it can be said that only the $\beta'$ polymorph was formed during the isothermal and non-isothermal crystallisation experiments conducted. Thus, values for $\Delta H_{m0}$ for the $\beta'$ polymorph were taken from values compiled from various literatures as tabulated in Table 2-11. $T_{m0}$ values were obtained from values compiled in Table 2-9 in section 2.6.1.

3.5.6 Calculation of TAGs growth rate based on selected models

A modified version of the equation from Kirwan & Pigford (1969), i.e. equation (2-39) as outlined in section 2.4.3.3 was chosen to evaluate the experimental data from the crystallisation of palm oil. This was selected in order to ascertain whether the model could explain the experimental data and give insight into the overall growth behaviour of a mixture of palm oil TAGs during crystallisation. The term $g_i$ in equation (2-39) is taken from the value of $(dm/dr)_i$ calculated from equation (3-13) in subsection 3.5.4. A plot of the growth rate, $g_i$ versus the crystallisation driving force term on the right-hand side of equation (2-39) for every species of TAG is plotted and
would yield a slope of $k$. This is done to ascertain whether palm oil TAGs growth rates follow a linear or non-linear relationship based on the applied driving force of the system.

### 3.5.7 Entrainment estimation and composition correction

Few literature studies have provided ways of determining the entrainment level in the solid fraction post-filtration. Timms (1994) proposed a way to approximate entrainment by incorporating the iodine value (IV) of the liquid and solid fractions in the mass balance equation of the ST cake. The IV is an indication of the amount of unsaturated fatty acids contained in an oil or fat. Double bonds present in the constituent fatty acids of a fat react with iodine compounds and raise the IV, increasing the degree of unsaturation of the fat. The equations used by Timms (1994) were:

$$ S = E + C \quad (3-15) $$

$$ S \cdot IV^S = C \cdot IV^C + E \cdot IV^O \quad (3-16) $$

where $S$ is the percentage of ST in oil, $C$ is the percentage of crystals in oil, $E$ is the entrainment which is defined as the liquid (OL) trapped in the crystals, expressed as a percentage of the ST and $IV^S$, $IV^C$ and $IV^O$ are the iodine values of OL, ST and crystals respectively. Rearrangement of equation (3-16) and substituting equation (3-16) into equation (3-15) yields:

$$ IV^O - IV^S = \frac{1}{S} \cdot C \cdot (IV^O - IV^C) \quad (3-17) $$

A plot of $(IV^O - IV^S)$ versus $1/S$ should yield a straight line with a slope of $C \cdot (IV^O - IV^S)$. 

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Another method for quantifying entrainment was suggested by Hamm (1986). He proposed that entrainment could be calculated based on the composition of the OL fractions obtained from filtration and values obtained via this method had provided similar results with that of Timms (1994). However, they did not describe in further detail the actual entrainment calculation method via the OL composition route. Hamm (2005) later equated entrainment with the degree of porosity in the filter cake produced. This was calculated by subtracting the percentage of the solid fat content (SFC) measured by nuclear magnetic resonance (NMR) from the total fat content (100%). This method involves a direct measurement of the fraction of the SFC by NMR immediately post-filtration and provides a quick and accurate way of estimating the level of entrainment.

In our study, we have derived two ways of estimating the level of entrainment in the ST. The first method is based on a simple overall mass balance involving the mass and TAG compositions of the OL and ST products obtained from filtration, similar to that of Timms (1994) but instead of using the IV, the TAG compositions of the products are used. This method assumes that the UUU TAGs do not crystallise at all due to their respective melting points being below 0 °C and thus making it likely that these TAGs do not crystallise into the solid phase to any significant extent under the conditions of the crystallisation experiments. Therefore, any UUU TAGs present in the retentate is assumed to be entirely contributed by entrainment of the OL between the ST crystals. To avoid confusion to the reader, throughout this work, the term 'ST' shall refer to the retentate collected which contains ST crystals and entrained OL while the term 'corrected ST' will refer to the the composition of ST after entrainment correction (pure ST crystals without entrainment of the OL).

In the case of palm oil, there are three UUU TAGs identified to have melting points far below zero, namely OOO, OOL and OLL having β' form melting points of -11.8 °C, -28.3 °C and -30.2 °C in that order (de Man, 1999). The mass balance equation for the ST is

$$M_{x}x + M_{y}y = (M_{x} + M_{y})z$$  (3-18)
where $M_E$ is the mass (g) of entrained OL in the ST fraction, $M_{ST}$ the mass (g) of crystals in the ST fraction, $z$ the composition (g TAG/g ST) of corresponding TAG in the total ST collected (entrained OL plus pure ST crystals) after filtration and $x$ and $y$ are the compositions (g TAG/g OL and g TAG/g ST) of the TAGs in the corresponding OL fraction and ST fraction respectively. Rearrangement of the above equation yields

$$\frac{M_E}{M_E + M_{ST}} = \frac{z - \frac{M_{ST}}{M_{E} + M_{ST}} y}{x}$$  \hspace{1cm} (3-19)

If we assume that OOO, OOL or OLL do not crystallise, then their composition, $y$ in the solid fraction ST would then be nil, hence reducing the above equation to

$$\frac{M_E}{M_{E} + M_{ST}} = \frac{z}{x}$$  \hspace{1cm} (3-20)

The term on the right hand side of equation (3-20) is the entrainment level that is being quantified and is expressed as the ratio of entrained OL to the total ST retentate collected on the filter paper. Another way of expressing the level of entrainment is the entrained liquid to solid ratio of the ST fraction ($L : S$):

$$\frac{M_E}{M_{ST}} = \frac{z}{x - z}$$ \hspace{1cm} (3-21)

Equations (3-20) and (3-21) can be used for a single UUU TAG or for a combination of UUU TAGs.

The second method for entrainment correction involves using the overall mass balance equation on the sample collected during each filtration as outputs and taking into account the original palm oil composition as the input. This equation involves the mass and composition of the original palm oil sampled prior to filtration, as follows:
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\[ M_{PO} w = M_{OL} \phi x + \left( M_{OL} (1 - \phi) x + M_{ST} y \right) \]

(3-22)

where \( M_{PO} \) is the total mass of the sample of crystallising palm oil collected in the pipette tip before filtration, \( M_{OL} \) the mass of OL collected at the bottom of the vacuum flask after filtration, \( M_{ST} \) the mass of entrained OL plus ST collected on the filter paper, \( \phi \) being the fraction of pure OL that is in the filtrate and \( w \) being the composition (g TAG/g PO) of the corresponding TAG in the initial PO sample. If we rearrange the above equation, we obtain

\[ w = \frac{M_{OL} \phi x}{M_{PO}} + \left( \frac{M_{OL} (1 - \phi) x + M_{ST} y}{M_{PO}} \right) \]

(3-23)

In order to solve this equation, we would have to assume an initial guess for \( f \) that satisfies \( w \) in the above equation.

3.6 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

3.6.1 Principles of DSC

DSC is a thermoanalytical technique whereby the difference in the amount of heat required to increase the temperature of a sample compared to an empty reference pan is measured as a function of temperature. The amount of heat required will mainly depend upon the type of physical transformation (e.g. crystallisation or melting) that the sample will undergo. This in turn allows one to measure the energy absorbed or released during such transitions. When the difference in the amount of energy required to heat the sample and reference at the same rate is plotted as a function of temperature, the curve generated is called a DSC thermogram.

There are two types of DSC systems; power compensated and heat flux. In this work, a heat flux DSC was used to analyse the samples. In a heat flux DSC, the same heating power is supplied to both the sample and the empty reference pan. The
temperature difference between the pans is measured and is then calibrated to the enthalpy change in the pan by an equivalent calibration factor. This type of DSC set-up consists of two sealed aluminium pans: one pan containing about 10 to 15 mg of the sample and the other is generally an empty pan acting as a reference pan, as shown schematically in Figure 3-13. The two pans are heated or cooled simultaneously in a microfurnace and the calculated difference in heat flow between the two is plotted as a function of time.

DSC has been long established as one of the primary methods of characterising the thermal behaviour of edible oils and fats, especially in palm oil and its products. The DSC crystallisation curve is only influenced by the chemical composition of the oil (and cooling conditions) while the melting curve depends upon the previous thermal history of the sample. Application of DSC in edible oils and fats includes measurement of crystallisation and melting temperatures, heats of fusions and crystallisation, monitoring phase behaviour of TAG mixtures as well as their polymorphic transformations (Tan & Che Man, 2000).

Figure 3-13 Schematic view of a heat flux DSC with $\Delta T$ being the platform temperature difference and $T_0$ the body (furnace) temperature (Hohne et al., 2003)
3.6.2 DSC experimental procedure

The main purpose of using DSC in this work was to confirm that the palm oil samples used in this study were behaving normally in terms of their crystallisation and melting properties thus further providing an indication of the quality of the sample. All palm oil samples used for this study originated from the same batch; hence a representative sample was obtained for this analysis. A heat flux differential scanning calorimetry (DSC) (model DSC Q10, TA Instruments, Crawley, UK) equipped with a thermal analysis data station was used (see Figure 3-14). Nitrogen (99.999% purity) was used as a purge gas and flowed at approximately 50 mL/min. The DSC instrument was calibrated for temperature and enthalpy using an indium standard. Samples of between 10 to 15 mg were weighed into aluminium pans to the nearest 0.1 mg and covers were hermetically sealed into place. An empty sealed aluminium pan was used as a reference pan. Samples were placed in the microfurnace and were subjected to the following temperature programme: isothermal at 70 °C for 10 minutes, cooled to -60 °C at a rate of 5 °C/min and held for 10 minutes, before heating from -60 °C to 70 °C at a rate of 10 °C/min. All thermal analysis data were recorded by the manufacturer's software (TA Instruments Universal Analysis, TA Instruments, Crawley, UK).

Figure 3-14 DSC instrument used in this study

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DSC thermograms can be characterised by a number of different parameters which are distinct for any type of oil or fat. The crystallisation thermogram provides the onset temperature ($T_{on}$) which is the point where the extrapolated tangent line of the exothermic peak intersects with the baseline while melting thermograms show the offset temperature ($T_{off}$) in the last endotherm in the same way $T_{on}$ is identified (Tan & Che Man, 2002). Figure 3-15 illustrates these two different parameters. Various peak temperatures between $T_{on}$ and $T_{off}$ are termed transition temperatures and represent the temperatures of maximum differential heat flow throughout the thermograms. In this study, the DSC manufacturer's software (TA Instruments Universal Analysis 2000, Version 4.3A, TA Instruments, Crawley, UK) was used to determine these different parameters within the thermograms generated from the DSC analysis, namely the $T_{on}$, $T_{off}$ and transition temperatures between $T_{on}$ and $T_{off}$.

Other useful parameters that can be measured from the DSC thermogram are the enthalpies associated with the cooling and melting process. This can be obtained by determining the area under the crystallisation or melting peaks and is expressed in units of J·g/K. However, since the baseline of the curves did not remain horizontal throughout the duration of the analysis, it was difficult to determine the area under the curves. Hence, only temperature parameters previously mentioned shall be considered here for comparison purposes with established results from the literature.

![Figure 3-15](image.png)

**Figure 3-15** Offset and onset temperatures in the DSC crystallisation and melting thermograms of RBDPO at scanning rate of 20 °C/min (Tan & Che Man, 2002)
3.6.3 DSC thermograms of palm oil used in this study

The DSC crystallisation curve of the representative sample of the RBD palm oil used in this study is presented in Figure 3-16. A sharp exothermal peak in the curve can be identified, occurring at the higher temperature range of between 12 °C and 20 °C. The $T_{off}$ of this higher temperature peak is seen to start at 17.70 °C, reaching a maximum of 16.74 °C. This peak corresponds to the crystallisation of the higher melting fraction of palm oil, namely the stearin fraction. Upon further cooling, a second exotherm was visible around 0.46 °C and this can be ascribed to the crystallisation of the lower melting fraction in palm oil i.e. olein fraction (Tan & Che Man, 2002). Another two smaller peaks occurring within this second exotherm was also observed at temperatures of -7.55 °C and -45.44 °C. The two main large peaks are the main characteristics associated with the two dissimilar fractions contained in palm oil. This result agrees well with previous studies and reports which have shown very similar values (Haryati et al., 1999; Tan & Che Man, 2000; Tan & Che Man, 2002).

Figure 3-16  DSC crystallisation curve of the RBD palm oil used in this study
Figure 3-17 DSC melting curve of the RBD palm oil used in this study

Figure 3-17 illustrates the melting curve obtained from the DSC analysis of the same representative sample of the RBD palm oil upon cooling to -60 °C. The melting curve shows the two distinct endotherms occurring at high and low temperature ranges which represent the main melting characteristics of palm oil. The melting endotherm at the high temperature range corresponds to the high melting fraction of TAGs (stearin), consisting of a plateau with twin shoulder peaks that lie between 16 °C and 44 °C. The lower melting endotherm corresponds to the lower melting fraction of TAGs (olein) and comprised of four overlapping peaks with temperatures ranging from -20 °C to 16 °C. This result agrees well with other established findings reported for palm oil samples (Haryati et al., 1997; Tan & Che Man, 2000; Tan & Che Man, 2002).

It is worth noting that no two palm oil samples are exactly alike and even palm oil samples from the same country of origin will not show exactly the same characteristics in the DSC analysis. Nevertheless, it is evident that the $T_{on}$ and $T_{off}$ as well as the temperature ranges of the peaks observed in the respective endotherm and exotherms of the RBD palm oil used in this study, as shown in Figure 3-16 and Figure
3.17 lie very close to the values reported in the previously published studies. Hence it is reasonable to conclude that the palm oil samples used in this study are of good quality and suitable for use in the crystallisation and melting experiments that make up the bulk of this work.
4 ISOTHERMAL CRYSTALLISATION OF PALM OIL UNDER SHEAR

4.1 INTRODUCTION

This chapter will present and discuss the results of the isothermal crystallisation study of palm oil under shear. The effects of the different isothermal holding temperatures on the FBRM response shall be presented followed by effects on the composition of TAGs. The composition will then be subjected to correction for entrainment and the corrected ST composition shall be compared with the uncorrected version. Estimation of the crystallisation rates and chemical potential of individual TAGs and their variation with time shall be presented and the chapter will end with a conclusion on the crystallisation behaviour of TAGs during the isothermal crystallisation of palm oil.

4.2 EXPERIMENTS PERFORMED

The sample preparation for the isothermal crystallisation experiments were carried out according to section 3.2.1. Isothermal experiments were performed in accordance with the method described in section 3.2.3. Five different isothermal crystallisation temperatures were studied in this work: 24, 26, 28, 30 and 32 °C.

The FBRM response was extracted from the LASENTEC software and compared between each isothermal crystallisation temperature. Other treatment of the FBRM data is described in subsection 3.3.3. The filtration products were subjected to compositional analysis by HPLC as outlined in section 3.4. The methods for further correction and treatment of compositional data are described in detail in section 3.5.
4.3 EXPERIMENTAL RESULTS AND DISCUSSION

4.3.1 Monitoring of the isothermal crystallisation of palm oil using FBRM

Figure 4-1 shows the total counts profile as a function of time for isothermal crystallisation temperatures ($T_i$) between 24 °C and 32 °C as measured by the FBRM. The results for each isothermal crystallisation temperature are plotted from the point at which the setpoint temperature ($T_{sp}$) reaches 72 °C in the setpoint programme (see section 3.2.3). This allows for comparison between the different $T_i$ and ensures that the initial particle count starts at a minimum, indicating that the palm oil has completely melted.

![Figure 4-1](image)

Figure 4-1 Influence of isothermal crystallisation temperature on the onset of nucleation and total counts profile as measured by FBRM

Initially the total counts start at a minimum value and did not change for some time which shows that the oil has fully melted and that no crystals are present. The FBRM response then shows the onset of nucleation (formation of enough particles with detectable size) which is represented by a sudden surge in the total particle counts.
profile and this observation is simultaneously confirmed by visual observation. From Figure 4-1, it can be seen that as the $T_i$ increases, the time taken for the onset of nucleation (or the induction time for nucleation) also increases, with the longest time for nucleation to occur observed at 32°C. From the slopes of the total counts curve, the nucleation rate is seen to decrease with increasing $T_i$.

Once nucleation has occurred, the total particle count increases rapidly and steadily for most of the $T_i$ (with the exception of 28°C and 30°C). This steady increase shows that the number of nuclei or crystals being generated is increasing with time, indicating continuous formation of crystals during the isothermal hold. A comparison of the nucleation rates between different $T_i$'s show that the nucleation rates of experiments conducted at $T_i$'s between 24°C and 28°C were broadly similar, as depicted in Figure 4-1. At 30°C, the nucleation rate was observed to be much less and at 32°C, it was observed that the nucleation rate was initially much lower than at other $T_i$ but then increases afterwards. This may be due to secondary nucleation. It was also observed that there was a lack of smoothness in the total counts data obtained at 28°C and 30°C. Reasons for this are unclear.

In general, the total particle count continues to increase further until it levels off and reaches a final plateau. This plateau indicates that no new crystals are being formed and suggests that the crystallisation process has reached equilibrium. It also suggests that there is little or no longer any change in the amount of crystals produced. To support and confirm this theory, the yield of the filter cake collected during the filtration stage is also plotted against time as illustrated in Figure 4-2 for the isothermal crystallisation of palm oil at 32°C.

The figure shows that the filter cake yield correlates well the results provided by the FBRM total counts. Filter cake yield curves comparable to the one obtained at 32°C, were also observed for all $T_i$ between 24°C and 30°C. As the total counts increase, the filter cake yield correspondingly increases in amount and this trend appears similar as the total counts level out and the filter cake yield correspondingly becomes stable. This confirms the earlier assumption that the plateauing of the total counts signals that the system has reached isothermal equilibrium and that crystallisation is no longer taking place.
Figure 4-2  Isothermal crystallisation of palm oil at 32 °C: ST fraction (%) and total particle counts as measured by FBRM

From these results, it can be concluded that the isothermal crystallisation of palm oil measured by FBRM exhibits a sigmoidal shape similar to plots characterised by other measurement techniques such as DSC, pNMR, transmittance/turbidity, time-resolved XRD, polarised light microscopy, changes in viscosity and ultrasound velocity measurements (Foubert et al., 2003). This demonstrates the FBRM’s capability in giving direct observation or monitoring particulate concentration and population during the isothermal crystallisation of palm oil under shear. Even though the total particle counts is directly correlated with the amount of fat crystallising as evident in Figure 4-2, it does not however reflect the actual quantity of solid fat during crystallisation and only serves as an indication; FBRM serves only as a tool for monitoring purposes and hence cannot stand alone. Further analysis on extracted samples from the crystallisation process or other methods for quantifying the actual crystal concentration such as the pNMR (for solid fat content measurements) and HPLC (for TAGs concentration measurement) is required to supplement FBRM data in order to confirm the events taking place as illustrated in this example. Nevertheless, FBRM’s advantage in providing in-situ measurements during the crystallisation process makes it an important monitoring tool.
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Figure 4-3  Effect of isothermal crystallisation time on the square-weighted chord length distribution at 24 °C

Figure 4-4  Effect of isothermal crystallisation temperature on the mean chord length of palm oil crystals
FBRM also provides additional data in the form of chord length distributions which can be weighted in various ways (see sections 3.3.2 and Appendix A). Heath et al. (2002) evaluated the effect of applying length weightings to FBRM data for a series of sieved aluminium fractions and made a comparison between the FBRM response data and the conventional laser diffraction method. They found that square-weighted chord distribution agreed well with the volume distribution obtained using the laser diffraction method. On this basis, only the square-weighted chord distribution shall be considered in this work.

Figure 4-3 illustrates the effect of isothermal crystallisation time on the square-weighted chord distribution of palm oil crystallising at 24 °C. Each time value shown represents the time when filtration was carried out. Initially the mean chord length of the crystals observed during the first filtration was around 90 microns. As the crystallisation process progressed, the chord lengths of the crystals started to shift towards higher values with increasing crystallisation time. This suggests that crystal growth and agglomeration were taking place as crystallisation went along. Towards the end of the isothermal crystallisation period, the chord length of crystals at 24 °C remained constant and the final crystal mean chord length was observed to be in the 240 microns region.

A more convenient way to compare the changes in crystal size between different experiments is by observing the mean chord length of palm oil crystals over time. Figure 4-4 shows the evolution of the mean chord length of palm oil crystals at temperatures between 24 °C and 32 °C. At 24 °C and 26 °C, the mean chord length of palm oil crystals initially increases rapidly as the crystallisation time increases. This suggests that crystal growth and/or agglomeration are taking place at the same time. The trend then reaches a maximum and becomes stable suggesting that the occurrence of these two phenomena has completed.

At 28 °C, the mean chord length of palm oil crystals initially increases gradually to a maximum size of approximately 400 μm. However, instead of levelling off, the crystal size starts to decrease gradually starting from the 205th minute until it reaches a final value of 370 μm. Once this final lower value has been reached, the trend then plateaus and remains constant at this lower value. The same trend is observed at 30 °C.
whereby a maximum crystal chord size of nearly 540 μm is achieved but drops down to 315 μm. As for the isothermal crystallisation experiment at 32 °C, it can be seen from Figure 4-4 that there was only a small increase in the mean chord length of crystals from 190 μm near the beginning to 220 μm maximum at the end. The trends in the mean chord lengths observed at 28 °C and 30 °C were not seen to occur at 32 °C, suggesting that there was only a small amount of crystal growth or agglomeration occurring and that no secondary nucleation had taken place within the crystallising oil. This was presumably due to the less number of TAGs that are able to crystallise at this temperature. An analysis of the ST fraction collected during filtration would be able to confirm this presumption.

One can investigate the cause of the decrease in the average particle size by comparing the mean chord length data with the total counts profile. If we look back at Figure 4-1 showing the total particle counts curves for 28 °C and 30 °C, we can see that during the 220th minute, the total particle counts was still increasing for both $T_i$. An increase in the total counts with the mean chord length simultaneously decreasing now strongly suggests that secondary nucleation events are taking place. This is because newly formed nuclei will contribute to the increase in the total particle counts in the system while their size will impart a reduction in the overall average size of particles within the system.

Himawan et al. (2006) in their review noted some nucleation mechanisms resulting from secondary nucleation caused by (1) mechanically chipped off fragments of growing crystals which in turn act as new nuclei, (2) the collision of crystals with other crystals and also with parts of the crystalliser which in turn generate small crystals, and (3) the enhancement of the nucleation event due to static condition disruption of the liquid lamellae by the existence of crystal lattices. One of these mechanisms in their review may explain what took place during this event at both 28 °C and 30 °C.

As palm oil crystallisation progresses, there is a rapid increase in the viscosity of the oil slurry due to the combination of nucleation and parallel crystal growth events occurring at the same time. When the degree of supercooling is high, the nucleation rate also increases until a maximum is achieved. This subsequently decreases as the
increase in viscosity of the slurry with time often restricts the motion of particles, suppressing the formation of ordered structures (Mullin, 2001). Diffusivity is also decreased at high viscosity conditions and leads to a reduced overall growth rate due to mass transport limitations (Himawan et al., 2006). As a result, the only movement within the crystallising slurry is localised around the agitator and is a direct contribution of the agitation of the system, which then may cause severe disruption in the crystal structures leading to crystals attrition. As near motionless crystals are agitated, the impact of the agitator results in crystal impingement and this may have caused the generation of new smaller crystals from broken fragments of existing crystals, thereby leading to a decrease in the crystal mean chord length.

4.3.2 Measured HPLC composition

Figure 4-5 to Figure 4-8 illustrate the raw TAG compositions of the OL and ST products obtained during filtration which were measured by HPLC at isothermal crystallisation temperatures of 24 °C and 32 °C respectively, expressed as mass fractions (%wt) (see Appendix B.1 for the measured TAG compositions of the OL and ST products at all other temperatures between 24 °C and 32 °C). For the composition of the ST fractions collected at 32 °C, the results from the first five samples are not reported as there were insufficient amounts of sample to allow HPLC analysis to be carried out. To aid interpretation of the graphs in this chapter and the rest of the thesis, the line type varies according to the TAG saturation, i.e. black continuous = SSS, black dashed = SSU, grey continuous = SUU and grey thin continuous = UUU.

At all $T$, the same trend for SSS TAGs is observed where SSS TAGs deplete entirely from the OL fraction, confirming earlier studies by Deffense (1985) and Sulaiman et al. (1997). The compositions of all other TAGs in the OL however, do not change significantly with time. The same behaviour is also observed for all TAGs in the ST, except for PPP which initially shows a higher concentration at the start of the experiment at 32 °C. From Figure 4-8, it can be seen that the initial amount of PPP in the ST at 32 °C (which is in fact the 6th filtration point) is nearly twice as much as that at 24 °C (Figure 4-6). It is likely that this amount would be higher in the first five ST
samples at 32 °C. This suggests that it may be possible to enrich PPP to more than 30% at Tc's higher than 32 °C.

Figure 4-5 Raw TAG composition of OL fraction at 24 °C

Figure 4-6 Raw TAG composition of ST fraction at 24 °C
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Figure 4-7 Raw TAG composition of OL fraction at 32 °C

Figure 4-8 Raw TAG composition of ST fraction at 32 °C
The effects of temperature on TAGs concentration was monitored by plotting the final concentration of TAGs in the OL and ST as a function of $T_i$. These are shown in Figure 4-9 and Figure 4-10, respectively. Most noticeable is the substantial increase in the amount of PPP in the ST fraction from about 15% at 24 °C to nearly 25% at 32 °C as $T_i$ is increased, as illustrated in Figure 4-10. A probable explanation for this is that when higher crystallisation temperatures are employed during palm oil crystallisation, only the more saturated TAG species can survive in the crystalline form due to their higher pure component melting temperatures (see Table 2-9) while other TAGs containing more unsaturated fatty acids remain uncrystallised due to their lower melting points. This is reflected in the unchanging compositional trends of the SUU and UUU TAGs in the OL fraction at all $T_i$ as depicted in Figure 4-9.

A comparable observation with past literature is the appreciable amount of the more unsaturated TAGs, i.e. SUU (i.e. mainly POO and POL) and UUU TAGs present in the ST fractions at all $T_i$. This is depicted in Figure 4-12 which shows the change in the final concentration of TAG groups (i.e. UUU, SUU, SUS and SSS) in the ST fraction as the $T_i$ increases. The presence of SUU and UUU TAGs in the ST may be attributed to the amount of liquid OL entrained within and between the ST crystals in the retentate. In general, only the SSS TAGs experience an increase in the ST while the SUS, SUU and UUU TAGs decrease in the ST as $T_i$ increases. However, an increase in $T_i$ did not show any significant change in the trends of all TAG groups in the OL, as illustrated in Figure 4-11.

Results obtained by Deffense (1985) showed values slightly higher than 25% and 3% for SUU and UUU TAGs groups in the ST respectively while Kellens et al. (2007) reported a SUU mass fraction of 21.8% and UUU mass fraction of about 3.4% in the ST. Braipson-Danthine and Gibon (2007) reported mass fraction values ranging from 19% to 31% of SUU TAGs and a 2 to 6% of UUU TAGs in different types of ST fractions obtained during the dry fractionation of palm oil using both the membrane and vacuum presses with the vacuum presses yielding a larger amount of SUU in the ST fraction. In this study, the amounts of SUU and UUU TAGs present in the ST fraction ranged from between 24 to 29% for the former and 4 to 5% for the latter, which is comparable to the results obtained by Deffense (1985) and Braipson-Danthine & Gibon (2007).
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Figure 4-9  Effect of isothermal temperature, $T_1$ on the final concentration of TAGs in the OL fraction

Figure 4-10  Effect of isothermal temperature, $T_1$ on the final concentration of TAGs in the ST fraction

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Figure 4-11  Final concentration of TAG groups in the OL fraction at all $T_i$

Figure 4-12  Final concentration of TAG groups in the ST fraction at all $T_i$
4.3.3 **Partition coefficient of TAGs**

The partition coefficient \( K_d \) describes the equilibrium distribution of a component between two different phases. TAG distribution between the liquid (OL) phase and the solid (ST) phase can be determined by calculating the ratio of the concentration of TAGs in the OL phase and the ST phase at constant temperature and pressure as follows (Siew & Ng, 1995):

\[
K_d = \frac{[TAG]_{OL}}{[TAG]_{ST}} \quad (4-1)
\]

\( K_d \) values for individual TAGs at all \( T_i \) were quantified using the above equation using the OL and ST compositional data obtained from HPLC and were plotted as a function of time. Figure 4-13 illustrates this at a \( T_i \) of 24 °C while similar plots at all other \( T_i \) can be found in Appendix B.2.

![Graph showing partition coefficient values of palm oil TAGs](image)

**Figure 4-13**  Partition coefficient \( (K_d) \) values of palm oil TAGs as a function of time at 24 °C
In general, it can be deduced that at all \( T_1 \), SSS TAGs (PPP, PPS and MPP) showed higher preference for the ST phase as depicted by their \( K_d \) values which lie below the equipartition partition line \((K_d = 1)\). The preferential complete partitioning of PPS into the ST phase in particular was enhanced at temperatures below 26 °C. Certain SUS TAGs, namely SOS and MLP showed very slight preference for the ST phase, while all other TAGs showed \( K_d \) values above 1, indicating that they had a higher affinity for the OL phase at all \( T_1 \). It can also be seen that POP and POS started to partition more into the ST phase at lower \( T_1 \) (e.g. 24 °C). This could either be due to the lower crystallisation temperature employed which enabled them to crystallise in larger amounts into the ST or could be linked to the formation of a solid solution (co-crystallisation) of these TAGs below temperatures of 25 °C (Rossell, 1967).

4.3.4 Cumulative concentration of TAGs

An easier way to follow changes in the TAG groups during isothermal crystallisation of palm oil is by following the percentage conversion of each TAG as they crystallise into the solid (ST) fraction with time. With a consistent basis of a fixed mass of palm oil (solid + liquid), the amount of each TAG in each phase can be determined from multiplying the HPLC mass fraction by the filtration yield of the phase (equation 3-9 or 3-10 in Section 3.5.2). When the OL and ST amounts for each TAG are added together, they should produce a constant value per g of palm oil as no TAGs leave the vessel (apart from sampling which does not change the composition of the contents). This is illustrated in Figure 4-14 for the total mass of TAGs in palm oil at 24 °C (see Appendix B.3 for plots of the total mass of TAGs at all other \( T_i \)'s). The concentration of TAGs at the point of nucleation (the first data point in each figure) corresponds to the TAGs concentration in the original palm oil used in each study.
It can be fairly confirmed that at 24 °C, the total amount of each TAG in the system calculated from the two phases remained consistent throughout the entire duration of the isothermal crystallisation experiments, as depicted by the horizontal trends observed for all TAG species in Figure 4-14. Similar trends were also observed at all other $T_i's$ in this study (see Appendix B.2). Very small variations in the straightness of the trends can be attributed to human error during the analytical procedure carried out by HPLC which cannot be avoided; otherwise the data presented herein shows invariable consistency with the concentration of each TAG in the system until the end of each run.

The accumulation of TAGs in the ST phase is now considered, focusing on the two extremes of temperature. Figure 4-15 and Figure 4-16 show the cumulative concentration of TAGs in the ST fraction at 24 °C and 32 °C respectively. These were calculated using the method described in Section 3.5.2 (equation 3-11) and the total counts profile at the respective temperatures is also plotted together for comparison purposes. From these figures, it can be seen that the TAG concentration curves depicted in each graph correlate well with the total counts profile as both show similar
trends. During the initial stage a steep slope can be observed in the total counts indicating that the nucleation process is dominant and that there is also a sharp increase in the concentration of the SSS TAGs. This then progresses further with crystal growth until a maximum concentration of TAGs (mass of TAGs in ST curve) and particles (depicted by the total counts curve) is reached and the two trends reach a plateau at the same time, signifying that the system has reached equilibrium and that the rate of crystallisation has become very slow or nearly non-existent.

An observation which is confirmed by literature studies is that all SSS TAGs crystallise out almost completely into the ST fraction at all $T_i$, as shown by Deffense (1985). The SSS TAGs are seen to remain at the same concentration at the end of the experiments regardless of $T_i$. However, most noticeable is the considerably lower amounts of the other TAGs which tend to group together at lower concentration levels as $T_i$ is raised. The probable reason for this is that at higher crystallisation temperatures, only the more saturated species (SSS) TAGs are able to crystallise in higher concentrations due to their higher pure component melting points compared to other TAG species with lower pure component melting points which a larger amount of these TAGs tend to remain uncrystallised in the OL phase at higher $T_i$.

![Figure 4-15 Cumulative concentration of palm oil TAGs in the ST fraction at an isothermal temperature of 24 °C](image-url)
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Figure 4-16  Cumulative concentration of palm oil TAGs in the ST fraction at an isothermal temperature of 32 °C

Another possible explanation for the higher concentration of SSS TAGs in the ST fraction is due to their importance in the nucleation stage in the crystallisation process. It has been shown that they form nuclei upon which other TAGs will attach themselves to during the crystal growth stage (Sulaiman et al., 1997). A similar basis could also be assumed for the group of the other TAGs crystallising at lower concentrations in the ST. From Figure 4-15 and Figure 4-16, for example SOS showed the highest initial concentration within this group and it may be assumed that during palm oil crystallisation, the most saturated species would crystallise out first followed by other TAGs according to decreasing $T_m$. The highest melting TAG in this lower concentration group, i.e. SOS would probably have provided growth sites for the other TAGs to crystallise on and co-crystallisation of these other TAG species could have taken place.

However, this does not clarify why the SUU and UUU TAGs are even crystallising at all. The pure component melting temperatures of these TAG groups do in fact, lie below 20 °C (see Table 2-9). In theory, this implies that TAGs containing at least two unsaturated fatty acid components in their structure are not supposed to crystallise to
any significant extent and only the SSS and SUS TAGs should prevail at the isothermal crystallisation temperatures used in this study. The considerable amount of the more unsaturated TAGs group in the ST fraction may be caused by two factors. The first factor could be that the filtration process may have occurred at a lower temperature (room temperature) compared to the $T_i$ in study, thus causing the more unsaturated TAGs to crystallise on the filter paper. However in the case of $T_i = 24 \, ^\circ\text{C}$, this will not be very different. The second factor could be due to the presence of entrapped OL between ST particles during the filtration stage (entrainment).

If this is indeed the case, the mass fractions of TAGs in the ST fraction can be deemed to be incorrect as these do not reflect the actual composition of the ST. In order to determine how far entrainment contributes to the error in the TAG composition of the ST fractions in palm oil crystallisation, a calculation method for estimating the level of entrainment has been proposed (see section 3.5.7) and its implementation in this study shall be discussed in the later sections of this chapter. Before the TAGs compositional data are subjected to entrainment correction, results on further analysis relating to the TAG crystallisation rates and chemical potential driving force during crystallisation shall be presented in the next section.

4.4 FURTHER ANALYSIS

4.4.1 Overview

The purpose of this section is to present the results from further analysis with respect to the TAG compositional data acquired from the HPLC. This involves the attempt at quantifying the crystallisation rates of palm oil TAGs and determining the crystallisation driving force of these TAGs based on several selected thermodynamic models as outlined previously in sections 3.5.4 and 3.5.5. To simplify our calculations, the TAGs were assumed to behave ideally throughout the crystallisation process. The results therefrom shall be elaborated upon in the next section.
4.4.2 Crystallisation rates of TAGs

In this work, the crystallisation rates of palm oil TAGs were observed by monitoring the relative change in the mass of TAG components in the solid crystal phase as the crystallisation process progresses with time. The crystallisation rate of each TAG, \( \frac{dm}{dt} \), was initially calculated using equation (3-13) (see Section 3.5.4) for all \( Ti \). However, it was found that this representation was unsuitable in following the change in the mass of TAGs over time. This is because there will always be a small degree of unavoidable error associated with the experimental data. The crystallisation rate calculation is based on calculating a gradient between successive data points, and even small amounts of noise in the data are amplified in such a calculation, hence giving trends which showed large fluctuations. These results are therefore not helpful and are not presented.

Thus, another way to approach this was to plot the percentage conversion of each TAG in the ST phase. This was calculated using equation (3-10) (see Section 3.5.2) and also allows the variation of masses of individual TAGs to be followed with time. The conversion of individual TAGs into the ST phase was quantified for all TAGs at all \( Ti \) and plotted with time. The initial concentration of each TAG at the point of nucleation was taken as zero as it can be said that the very first crystal starts appearing at the onset of nucleation and the oil bulk was in a completely uncrystallised state at any time prior to the nucleation point. Figure 4-17 and Figure 4-18 illustrates this for \( Ti \) of 24 °C and 32 °C respectively. This approach is very similar to that used in Figure 4-15 and Figure 4-16 except that greater emphasis is placed on the major TAGs, the concentrations of which are known with much greater percentage accuracy.
Figure 4-17  Mass of TAG in ST per 100g palm oil at 24 °C

Figure 4-18  Mass of TAG in ST per 100g palm oil at 32 °C
Results here demonstrate that as the $T_1$ increases from 24 °C to 30 °C, PPP remains at a concentration of around 5 g PPP in ST/100g PO, as depicted in Figure 4-17 (see Appendix B.4 for similar plots at $T_1$ between 24 °C to 30 °C). This shows that an increase in $T_1$ within this temperature range does not have a profound effect on the conversion of PPP in the solid phase. However, from Figure 4-18 at 32 °C, PPP showed a much higher conversion into the solid phase with an increase in conversion up to nearly 6 g PPP in ST/100g PO. This may indicate that at temperatures higher that 30 °C, it would be possible to concentrate or purify more PPP into the ST phase above the limit observed between 24 °C and 30 °C as less of the other TAGs are crystallising at these higher temperatures.

The conversion of POP and POO into the solid phase at 24 °C initially showed a concentration of about 10 g POP in ST/100g PO and 5g POO in ST/100g PO. These values then experienced a decrease to slightly below 6g POP in ST/100g PO and slightly above 3g POO in ST/100g PO respectively when $T_1$ was increased from 24 °C to 28 °C and remained at those values at 30 °C. The conversion of POP and POO then suddenly increased at 32 °C with amounts comparable to that at 28 °C. This suggests that there is a possibility of further concentrating these TAGs at temperatures above 32 °C, similar to that of PPP.

The TAGs PLP and POL were found to show similar conversion trends in which these concentrations remained close together throughout the experiments conducted at $T_1$ of 24 °C to 26 °C. This might suggest that these two TAGs were crystallising together as a solid solution during the crystallisation of palm oil. Yet from 28 °C to 32 °C, it seems as though they are slowly separating with PLP showing a higher conversion into the ST phase compared to POL. An explanation for this could be due to the fact that PLP contains more saturated components i.e. 2 palmitic acids on the glycerol backbone and thus as $T_1$ increases further, the TAG containing more saturated components has a tendency to prefer the solid phase.

The lower amount of other minor TAGs concentrating into the ST phase as $T_1$ increases can be confirmed by observing the trends in their amounts from 24 °C to 32 °C. At 24 °C (see Figure 4-17), it was observed that TAGs which had a concentration of less than 2% were seen to decrease to below 1 g in ST/100 PO as the $T_1$ reached 32
°C. A similar explanation with regards to their survival at higher $T_i$ can be suggested here as the $T_m$ of these TAGs are significantly lower than the $T_i$ studied. There is also a possibility that there are fewer sites for lower melting TAGs within this group to crystallise on as the higher melting TAGs in this group i.e. PPS and MPP are present at very low concentrations, i.e. less than 4% for the former and less than 2% for the latter.

### 4.4.3 Crystallisation driving force of TAGs

One of the aims of this research was to determine the crystallisation driving force of each palm oil TAG at different isothermal temperatures ($T_i$). In Section 2.4.1, it has been shown that the driving force for crystallisation of TAG components is the difference in chemical potential ($\Delta \mu$) between the respective liquid and solid phases of a TAG. If we assume that all TAG components are very similar in nature; i.e. $\gamma^L_i = 1$ and $\gamma^S_i = 1$, then we can calculate the crystallisation driving force of TAG components using a simplified version of equation (2-15) as follows:

\[
\frac{\Delta \mu_i}{RT} = \frac{\Delta H_{m,i}}{R} \left( \frac{1}{T} - \frac{1}{T_m} \right) + \ln \left( \frac{x_i^L}{x_i^S} \right)
\]

(4-2)

This form of the driving force takes into account the compositional effects in the multi-component mixture of palm oil TAGs while at the same time assuming that both the liquid and solid phases behave ideally. This may not be completely accurate but does serve as a useful basis for comparison in the absence of activity coefficient information. Van Putte and Bakker (1987) have shown that palm oil crystallises into $\beta'$ aggregates in stirred systems, hence it was assumed that our palm oil system behaved in a similar manner at all $T_i$ in this study. Utilising the TAG concentrations of the OL and ST phases obtained from HPLC and the thermal properties of pure TAGs compiled earlier in Section 2.6.1, the crystallisation driving force was quantified for each TAG at every $T_i$ and their values plotted with time. This was calculated only for TAGs with available thermal properties in the $\beta'$ form as tabulated in Table 2-9 and Table 2-11.
Figure 4-19 and Figure 4-20 shows plots of the crystallisation driving force ($\Delta \mu$) of selected palm oil TAGs at $T_i$ of 24 $^\circ$C and 32 $^\circ$C respectively while similar plots at all other $T_i$'s are shown in Appendix B.5. A similar trend observed at all $T_i$ is the larger $\Delta \mu$ of the SSS TAGs compared with other TAGs at the beginning of the isothermal crystallisation runs which later gradually decrease with time and as the system approaches a state of equilibrium. There is however, no change in the $\Delta \mu$ of UUU, SUU and SSU TAGs and they more or less remain constant throughout the run. The UUU TAGs show the lowest $\Delta \mu$ compared to all TAGs while the $\Delta \mu$ of SUS and SUU lie intermediate between the $\Delta \mu$ of SSS and UUU TAGs.

The initial large $\Delta \mu$ associated with the SSS TAGs is primarily due to the dominant effect of their higher melting temperatures ($T_m$). Mid-experiment, the decreasing $\Delta \mu$ of PPP and PPS begin to drop lower than that of POS and SOS as the OL compositions fall. A probable explanation for this is that the SUS TAGs only start to crystallise once the SSS TAGs have completely crystallised out of the OL as suggested by Deffense (1985). So as the amount of SSS TAGs deplete in the OL, they can no longer sustain their initial $\Delta \mu$ and are succeeded by the $\Delta \mu$ of SUS TAGs.

![Crystallisation driving force versus time of palm oil TAGs at 24 $^\circ$C](image)

**Figure 4-19** Crystallisation driving force versus time of palm oil TAGs at 24 $^\circ$C
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Figure 4-20  Crystallisation driving force versus time of palm oil TAGs at 32 °C

A noticeable effect of increasing $T_i$ on the $\Delta \mu$ of palm oil TAGs is the decrease in the starting value of $\Delta \mu$ at the beginning of each run, except at 32 °C where the calculation of $\Delta \mu$ was only possible from the sixth sampling point onwards. The reason for this can be seen by inspection of equation (4-2), i.e. in differences between the $T_m$ of TAGs with $T_i$ ($T_m - T$) and also a decrease in TAGs concentration in the solid phase at higher $T_i$ as depicted in Figure 4-16.

As a rule of thumb, for a TAG component to crystallise out into the ST phase, its chemical potential in the liquid phase ($\mu^L$) must be higher than the corresponding chemical potential in the solid phase ($\mu^S$), hence $\mu^L > \mu^S$. In our case, since system ideality is assumed, the first term on the right-hand side of equation (4-2) i.e. the term which involves the melting temperature ($T_m$) of the TAG will have the largest contributing effect towards the overall chemical potential driving force of TAGs compared to the second term on the right-hand side of equation (4-2) which contains the compositional effect in logarithmic form. Therefore, TAGs with higher $T_m$ compared to the crystallisation temperature which in this case is $T$ will always portray a higher driving force compared to TAGs with $T_m$ lower than the crystallisation temperature.

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4.4.4 Preliminary conclusions from isothermal crystallisation study

The isothermal crystallisation study on palm oil at various $T_i$ have shown that as $T_i$ increases, SSS TAGs are enriched further in the ST phase while SUS, SUU and UUU TAGs lie grouped together at very low concentrations. The crystallisation driving forces of TAGs is $T_m$ dominant when ideality is assumed, with the SSS TAGs having the highest chemical potential difference which drives their conversion into the solid phase. It has been shown that the overall crystallisation rate of palm oil TAGs occur in a non-linear manner and depends significantly on the degree of supercooling. However, this does not explain the tendency of the SUU and UUU TAGs to be present in the ST fractions collected even though they should not be crystallising at $T_i$’s higher than their $T_m$.

A potential way to unravel this problem is to look into a correction method which can consolidate the effect of entrainment into the compositions of the filtered products. Research has shown that the occurrence of entrainment during filtration in the fractionation of oils and fats is the main reason why there are considerable amounts of SUU and UUU TAGs still being present in the ST even though their $T_m$ suggests otherwise. The following section shall present the results obtained from the implementation of the proposed entrainment calculation described earlier in section 3.5.7 and its effect on the ST composition and crystallisation rates shall be discussed.

4.5 CORRECTION CALCULATION FOR ENTRAINMENT

4.5.1 Introduction

Entrainment calculations were performed according to the methods outlined in section 3.5.7. Two methods of calculation were proposed for each filtration sample; the first method is based on the mass balance within the filtered products alone (the OL fraction and the filter cake containing the entrapped OL) and the second method involves the overall mass balance between the filtered products (outputs) and the starting palm oil (inputs). In this work, only the results from the former method shall
be examined and reported as an attempt on the latter method showed inconsistent results, presumably as a consequence of the greater number of experimental variables required (each contributing additional experimental error and uncertainty).

4.5.2 Entrainment level calculation

The entrainment level in the filter cake was determined using the first calculation method based on the mass balance of the filtered products alone (see section 3.5.7). This involves the assumption that one or all of the UUU TAGs do not crystallise, so the composition of that particular TAG in the solid is equal to zero ($y = 0$). Since the contribution of the composition of OOO towards the overall composition is larger than both OOL and OLL, only the results from the entrainment calculation using the OOO composition and the overall UUU composition shall be considered here. The measured concentration values of OOL and OLL are subject to a considerable amount of percentage error.

Figure 4-21 shows the calculated entrainment level in the filter cake at all $T_i$ based on the assumption that only OOO does not crystallise while Figure 4-22 shows the entrainment level in the ST fraction at all $T_i$ with the assumption that all UUU TAGs do not crystallise into the ST fraction. In both figures, the entrainment level is defined as the ratio between the mass of entrained liquid in filter cake and total mass of filter cake. When comparing these two methods of entrainment calculation, it appears that the latter method shows a lower level of scatter and by implication a lower level of experimental error. Hence, for further data analysis, only the entrainment levels calculated using the latter method shall be used.

It is clear from Figure 4-22 that the levels of entrainment during the initial stages of the experiments were quite high, ranging from 70% to 90% of entrained liquid per total mass of filter cake collected. This may be due to the fact that during this initial period, nucleation of the mother phase was taking place, producing tiny crystals. Even though a large surface area is being generated by the nucleation process, the entrainment level during this stage is still high as these tiny crystals could still form small agglomerates, thus occluding a considerable amount of liquid within the crystal.
structure. As the isothermal holding time progresses, the entrainment level gradually decreases to between 54% and 59% of the total mass of filter cake and remains constant at this value. The entrainment values calculated here were found to be very similar to the values reported by Kellens et al. (2007) using vacuum filtration as tabulated earlier in Table 2-6 (see section 2.3.5 in Chapter 2). It was also found that on average, only about 10% of palm oil was crystallising as evident from the ST yields which have been corrected for entrainment as illustrated in Figure 4-23. This percentage was found to decrease to less than 9% solids as $T_i$ was increased further, except at 32 °C where the level of solids increased to nearly 12%.

According to Bemer and Smits (1982), separation of fat crystals from the mother liquor is much easier done when the crystals increase to 10 to 50 times of their original size due to agglomeration. As the crystallisation time progresses, crystal growth and agglomeration both essentially contribute to the increase in the crystal chord length. As evident from Figure 4-4, the mean chord length initially increases but then becomes stagnant towards the end of the experiment signifying that there is no longer any change in the crystal size (suggesting that the growth process and crystal agglomeration has completed) and that process equilibrium has been reached. The larger crystal chord size at the end of the experiment compared to the initial size shows the relationship between crystal size and filtration efficiency, as the larger the crystals or crystal agglomerates, the more efficient the filtration process and hence the lower the level of entrainment. This correlates well with the entrainment levels of the ST fractions at all $T_i$ shown in Figure 4-22.
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Figure 4-21  Entrainment level in ST fraction at all $T_i$ based on the assumption that OOO TAGs do not crystallise

Figure 4-22  Entrainment level in ST fraction at all $T_i$ based on the assumption that UUU TAGs do not crystallise
4.5.3 Corrected ST composition

Upon determining the entrainment level in the filtrate for all $T_n$, the corrected ST composition (dry ST crystals) was quantified using the mass balance equation on the collected retentate (see equation (3-18) in section 3.5.7). Figure 4-24 and Figure 4-25 shows the corrected ST compositions at 24 °C and 32 °C, both calculated based on the entrainment level when UUU was taken to be equal to zero. If these results were compared to the raw ST composition from the uncorrected data (see Figure 4-6 and Figure 4-8 for the raw ST compositions at 24 °C and 32 °C respectively), the main difference would be that PPP showed a much higher composition in the corrected ST followed by POP, PPS and the rest of the other TAGs. These results illustrate the dominant effect of the $T_m$ during palm oil crystallisation where PPP is expected to crystallise in a higher amount compared to POP due to its higher $T_m$. This differs greatly from results obtained with the raw ST data which showed that the TAG with the highest composition was POP followed by POO, PPP, POL and PLP at 24 °C and at 32 °C, the sequence was POP, PPP, POO, PLP and POL. Similar results for the
corrected ST data were observed at other $T_i$ between 24 °C and 32 °C studied in this work and thus are not reported here. Only results from these two $T_i$ shall be discussed for comparison purposes.

Apart from the difference in the TAG with the highest concentration in the ST, results from the entrainment correction also shows that incorporating the entrainment level in the calculation of the corrected ST enriches the SSS TAGs in the ST phase by more than two-folds compared to the raw ST data. The effect of increasing $T_i$ from 24 °C to 32 °C proved that more SSS TAGs can be concentrated in the ST at higher temperatures. This may confirm our earlier assumption that it may be possible to purify or separate SSS TAGs further at higher crystallisation temperatures due to their higher melting temperatures. Besides that, POP showed an increase in concentration in the corrected ST at 24 °C while at 32 °C, it remains more or less the same throughout the run. PPS crystallises up to 10% in the ST and this value is similar at both $T_i$ while all other TAGs only crystallise in very little quantities i.e. below 4% at 24 °C and this value increases to 6% at 32 °C. The separation between TAGs that lie below 6% also improved when $T_i$ was increased.

![Graph showing corrected ST composition at 24 °C using entrainment calculation based on UUU compositions](image)

**Figure 4-24** Corrected ST composition at 24 °C using entrainment calculation based on UUU compositions
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4.5.4 Partition coefficient of TAGs based on corrected ST composition

The partition coefficient ($K_d$) of SSS and major SUS TAGs (concentrations >2%) based on the corrected ST composition at $T_1$ of between 24 °C and 32 °C were calculated using equation (4-1). Only the plots of $K_d$ versus time at 24 °C (Figure 4-26) and 32 °C (Figure 4-27) shall be presented here for comparison of the influence of $T_1$ on $K_d$. The same partitioning behaviour with respect to the SSS TAGs as observed earlier with the uncorrected data can be observed here from Figure 4-26 and Figure 4-27 where all SSS TAGs show a high tendency to partition into the ST phase regardless of the $T_1$. It can also be seen that as $T_1$ is increased, the partitioning of SUS TAGs into the ST phase, namely POP and POS decrease and they are preferentially retained in the OL phase at higher temperatures. This is depicted by the $K_d$ values of POP and POS which lie below 1 towards the end of the experiment at 24 °C (Figure 4-26) and their $K_d$ values being above 1 at 32 °C (Figure 4-27) throughout the entire duration of the experiment.
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Figure 4-26  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 24 °C (based on corrected ST composition)

Figure 4-27  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 32 °C (based on corrected ST composition)
4.5.5 Crystallisation rates of TAGs based on corrected ST composition

Similar to the method described earlier in Section 4.4.2, the crystallisation rates of TAG species when UUU TAGs were assumed not to crystallise was observed by monitoring the percentage of conversion of each TAG in the ST phase, \( m_i^{ST} \). The corrected ST composition obtained from the preceding section was used in equation (3-10) (see Section 3.5.2) in order to calculate the conversion of individual TAGs into the ST phase so as to look at their variation with time. The conversion of individual TAGs into the ST phase was only quantified for TAGs having a concentration larger than 3% as from Figure 4-24 and Figure 4-25, it was observed that only the SSS and SUS TAGs (except for SOS), namely PPP, PPS, MPP, POP, PLP and POS crystallised in large amounts compared to the other unsaturated TAGs which remained at concentration levels below 3%. Figure 4-28 and Figure 4-29 illustrates \( m_i^{ST} \) of the SSS and SUS TAGs based on the corrected ST composition in units of mass of TAG in ST per 100g palm oil at \( T_i \) of 24 °C and 32 °C respectively.

Figure 4-28  Mass of TAG in ST per 100g palm oil at 24 °C (based on corrected ST composition)
Figure 4-29  Mass of TAG in ST per 100g palm oil at 32 °C (based on corrected ST composition)

From these figures, it can be emphasised that when the entrainment factor was taken into account in quantifying the conversion of palm oil TAGs into the ST phase, all SSS TAGs generally showed higher conversion into the ST compared to the SUS TAGs. This forms the major difference between uncorrected data (raw ST composition) and data corrected for entrainment (corrected ST composition) since the uncorrected ST composition depicted POP and POO having a higher conversion into the solid phase compared to PPP at 24 °C (see Figure 4-17) while at 32 °C (see Figure 4-18), the conversion of PPP into the solid phase was much higher than POO but still lower than POP.

In order to ascertain whether the initial assumption with regards to the UUU TAG group not crystallising at all in the proposed entrainment calculation was correct, the conversion of TAGs into the OL phase was calculated using the same procedure as with the ST and plotted with time. The only revision in this calculation was that the corrected OL mass was taken as the total of the mass of OL collected from sample filtration at the bottom of the flask plus the mass of entrained OL which was
calculated based on a mass balance of the retentate using the entrainment values calculated earlier.

Figure 4-30 illustrates the conversion of TAGs with a concentration >5% into the OL phase while Figure 4-31 represents the conversion of TAGs with a concentration <5% into the OL, both at 24 °C. If the assumption that the UUU TAGs are not supposed to crystallise due to their $T_m$ being lower than the $T_i$ in study were true, then their concentrations in the OL phase should remain constant throughout the whole crystallisation duration until the end. From Figure 4-31, it is clear that the conversion trends depicted by OOO, OOL and OLL remain unchanged throughout the whole course of the isothermal crystallisation experiment at 24 °C, further supporting the assumption that UUU TAGs do not crystallise at this $T_i$. Also noticeable are the unchanging trends in the SUU TAGs namely POO, POL, PLL and SOO. This indicates that the SUU TAGs too do not to crystallise at 24 °C as the $T_m$ of these TAGs also lie below 24 °C (refer to Table 2-9), which is expected. The conversion of the SSS TAGs in the OL is seen to be decreasing with time until they reach nearly zero, signifying their depletion from the OL and subsequent conversion into the ST phase. The same trend is observed for SUS TAGs.

Figure 4-32 illustrates the conversion of TAGs with a concentration >5% into the OL phase at 32 °C of these TAGs. Only POP showed a very slight decrease in conversion, suggesting that only a very small amount of POP is crystallising into the solid phase (this is also observed at 24 °C). PLP, POO and POL show constant trends through to the end, signifying that they do not convert or crystallise into the solid phase. As the $T_i$ increases from 24 °C to 32 °C, the conversion of SSS TAGs showed considerable reduction in their concentrations as depicted in Figure 4-33 for TAGs with a concentration <5% at 32 °C. This trend is similar to that at 24 °C. However, the final conversions of these TAGs in the OL phase at the end of the experiment were seen to be slightly higher at 32 °C compared to at 24 °C. All other TAG with concentrations <5% showed no conversion into the solid phase as depicted by their trends which remained unchanged throughout the whole duration of the experiment. It can be deduced from these results that only PPP and POP crystallise in significant amounts at both 24 °C and 32 °C.
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Figure 4-30  Mass of TAG in OL per 100g palm oil for TAGs >5% at 24 °C  
(based on the corrected OL mass and corrected ST composition)

Figure 4-31  Mass of TAG in OL per 100g palm oil for TAGs <5% at 24 °C  
(based on the corrected OL fraction and corrected ST composition)
Figure 4-32  Mass of TAG in OL per 100g palm oil for TAGs >5% at 32 °C (based on the corrected OL fraction and corrected ST composition)

Figure 4-33  Mass of TAG in OL per 100g palm oil for TAGs <5% at 32 °C (based on the corrected OL fraction and corrected ST composition)
4.5.6 Crystallisation driving forces of TAGs based on corrected ST composition

In the previous section, it was shown that the only TAGs that crystallised in significant amounts into the ST fraction based on the corrected ST composition were PPP and POP. Based on this finding, the crystallisation driving forces ($\Delta\mu$) of only these TAGs shall be considered for comparison purposes in this section. The $\Delta\mu$ of PPP and POP based on the corrected ST composition were quantified using the same method previously applied to the uncorrected data and were plotted as a function of time. Figure 4-34 and Figure 4-35 illustrate the evolution of the $\Delta\mu$ of these TAGs at $T_i$'s of 24 °C and 32 °C respectively. From Figure 4-34, the $\Delta\mu$ of PPP is seen to decrease significantly with time when palm oil was crystallised at 24 °C, indicating that PPP is depleting almost entirely from the OL. This observation corresponds well with the considerable reduction in the concentration of PPP in the OL as depicted earlier in Figure 4-31. As crystallisation progresses, the $\Delta\mu$ of PPP is then succeeded by that of POP, which is also seen to decrease with time albeit at a much slower rate compared to PPP. This behaviour was not observed in the uncorrected data where the $\Delta\mu$ of POP did not show any significant change throughout the entire experiment (Figure 4-19).

At 32 °C, the $\Delta\mu$ of PPP was seen to similarly decrease with time, although at a much faster rate than that observed at 24 °C, as shown in Figure 4-35. This can presumably be attributed to the higher crystallisation temperature employed here, where only the SSS TAGs were likely to crystallise. The same ‘crossover’ behaviour between the $\Delta\mu$ of PPP and POP which occurred at 24 °C could be also observed at 32 °C, where the $\Delta\mu$ of PPP drops to below that of POP, bearing negative driving force values towards the end of the experiment. This is probably due to the large difference between the concentrations of PPP in the OL which decrease to below 1% (Figure 4-7) and the corrected ST (Figure 4-25) with values above 50%. POP showed only a very minor decrease in $\Delta\mu$, indicating that POP still crystallises at 32 °C but is slightly delayed compared to PPP.
Figure 4-34  Crystallisation driving force versus temperature for PPP and POP at 24 °C (based on corrected ST composition)

Figure 4-35  Crystallisation driving force versus temperature for PPP and POP at 32 °C (based on corrected ST composition)
4.5.7 Conclusion from proposed entrainment calculation

The entrainment calculation exercise has demonstrated that SSS TAGs will have an overall higher conversion into the solid phase compared to SUS TAGs as depicted by their higher concentration in the corrected ST compositions reported here. When the entrainment factor was considered in the analysis of the compositional data obtained from HPLC, results show that SUU and UUU TAGs do not convert into the solid phase at all and they remain uncrystallised in the OL at $T_i$'s higher than their $T_m$. This confirms the true behaviour of TAGs if an ideal system were assumed where $T_m$ plays a major determining role in whether a TAG will crystallise or not. It also suggests that the ST compositions often obtained from fractional crystallisation processes do not represent the actual composition of the ST crystals as entrainment greatly affects these values to a large extent.

There are some evidences which support the proposed entrainment correction method. The entrainment levels estimated by this method have proven to be similar to those reported earlier in the literature by other workers (Kellens et al., 2007). This is also supported by the entrainment graph (Figure 4-22) which shows a physical trend that appears realistic. An examination of the differential scanning calorimetry (DSC) crystallisation thermogram of palm oil from Figure 2-17 indicated two distinct peaks, i.e. one peak occurring at a higher temperature range while the other occurring at lower temperature ranges. The majority of SUU and UUU TAGs must predominantly crystallise in the lower peak as their pure component melting temperatures lie within this temperature region. Although the method "coerces" the concentration values of the UUU in the ST fraction to zero, it does not force the values of SUU, SUS and SSS TAGs and these make the corrected data appear much more realistic compared to the uncorrected data. The entrainment correction is only useful in decoupling the crystallisation from filtration effects in order to study the crystallisation process in isolation.

The proposed entrainment calculation procedure may provide a convenient way to correct ST composition from HPLC in order to obtain the corrected ST composition by assuming the UUU TAGs do not crystallise. It does however, still require verification with the actual crystals composition for it to be able to be accepted as a
general method for entrainment quantification. The only way to gauge the actual level of entrainment and the true crystals fraction would be via the usage of a pulsed nuclear magnetic resonance (pNMR) instrument which can measure the true solid fraction of crystallised material without the problem of the entrapped liquid interfering with the measurement. Due to instrument limitations in this research, this verification method was not available. The only other way to approach this problem is by trying to wash the retentate (ST) with an organic solvent to expel entrapped liquid between crystals. The next section shall be presented with a detailed discussion on the retentate washing procedure conducted in order to test the proposed entrainment calculation method.

4.6 RETENTATE WASHING EXERCISE

4.6.1 Experiment performed

The retentate washing exercise was conducted in order to verify whether the actual entrainment level in the retentate collected during palm oil crystallisation was in fact within the range that has been obtained using the entrainment calculation method proposed (i.e. assuming UUU TAGs = 0 in the ST fraction). This is carried out with the assumption that washing the retentate would efficiently expel entrapped OL from within and between ST crystals. Another purpose of this exercise was to see whether the washed products would provide an indication of the actual true ST composition. This may provide valuable answers as to why there are UUU and SUU TAGs still being present in the ST fraction even at crystallisation temperatures above their \( T_m \).

A replication of the isothermal crystallisation experiment conducted at 24 °C was carried out according to the method described earlier in section 3.2.3. The sampling points were determined by referring to the FBRM response and product yields from the previous experiment conducted at 24 °C (see total counts profile in Figure 4-1). Two points were chosen as the filtration points during the experiment; 30 minutes after \( T_i \) was reached (during the nucleation and crystal growth stage) and at 120 minutes after \( T_i \) was reached (i.e. at the end of the experiment when the system had reached equilibrium and the weights of the samples and total counts no longer
changed with time). For each of these sampling points, three samples of the crystallising slurry were extracted from the crystalliser in a consecutive manner and within 6 to 7 minutes so as to minimise as much as possible the difference in time and composition between them. An additional sampling and filtration exercise was conducted on a sample extracted 10 minutes after the second filtration point (at approximately 130 minutes after $T_i$ was reached) in order to investigate the effect the washing procedure had on the weight of ST crystals and to assess the actual level of entrainment from the yields of the filtration products.

Four different filtration methods were employed (see Figure 4-36): (1) filtration with simultaneous washing of the retentate, (2) filtration of the sample, isolation of the first filtrate and retentate, followed by washing of the first retentate and collection of the second filtrate and retentate which contains the entrained liquid and washing solvent, (3) dry filtration (normal vacuum filtration which involves no washing of the retentate), and (4) the same filtration procedure as mentioned in (2) but including weighing the first retentate before the subsequent washing exercise. The washing solvent used in this exercise was general laboratory grade acetone. The first three types of filtration methods were carried out one after the other onto the 3 slurry samples sampled at the first 2 filtration points. This allows for the comparison of the TAG compositions of the filtration products between different filtration methods and mainly to assess how the washing method applied to the retentate effects the composition of the true crystals collected during each sampling point.

![Figure 4-36](image)

**Figure 4-36  Filtration methods employed for retentate washing exercise**
The fourth filtration method was applied to the sample from the final sampling point (at 130 minutes after $T$ was reached). The first filter retentate was weighed before being washed with acetone. After the washing procedure, the second retentate was weighed again and it was assumed that any difference in weight between the two was purely a contribution of the entrained OL expelled by the washing step. Filtrates which contained a mixture of entrained OL and acetone were left dry to allow the acetone to fully evaporate, leaving only the entrained OL. All filtrate and retentate products were weighed and subjected to further compositional analysis by HPLC.

### 4.6.2 FBRM response

Figure 4-37 illustrates the temperature and total counts profile obtained during the original isothermal crystallisation experiment conducted at 24 °C while Figure 4-38 shows the same profiles obtained during a replicate of this experiment for the retentate washing exercise. From both these figures, it is clear that the temperature profiles are identical in both experiments with nucleation occurring at about the same time with only a difference of 4 minutes between these two experiments. The FBRM response of the total counts shows the same trend although slightly higher counts are observed in the latter experiment. The filtration points carried out in this exercise are also indicated in Figure 4-38. It can be fairly confirmed that the experiments showed good reproducibility and that the FBRM can provide consistent results relating to nucleation detection and the trend in the population of particles in the system.
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Figure 4-37  Temperature profile and FBRM response for total particle counts at 24 °C (original experiment)

Figure 4-38  Temperature profile and FBRM response for total particle counts at 24 °C (retentate washing experiment)
4.6.3 Measured HPLC composition

Table 4-1 tabulates the TAG compositions of the four main TAG groups, i.e. UUU, SUU, SUS and SSS of the 6 retentates collected from the three different filtration methods applied. ST1 and ST4 involved simultaneous filtration and washing of the retentate, ST2 and ST5 involved normal vacuum filtration then washing the retentate afterwards while ST3 and ST6 involved only normal vacuum filtration without any acetone wash. ST5 represents the TAG compositions of ST5 obtained from HPLC analysis whereas ST5 shows the predicted TAG compositions of ST5 calculated from the entrainment correction method. This was predicted based on the mass balance of the filtered products and assuming that the entrained OL composition in this experiment was similar to the composition of the final OL product obtained in the original isothermal experiment at 24 °C (Figure 4-5).

From Table 4-1, it can be seen that there is a significant increase in the overall SSS TAGs in the retentates when washing was done, as depicted by their larger concentrations in the washed retentates. Simultaneous washing while filtering the retentate yielded the highest amount of SSS TAGs as shown by ST1 and ST4 when compared to washing the retentate after normal vacuum filtration (ST2 and ST5). Correspondingly, the UUU and SUU TAGs experienced a reduction in concentrations within washed retentates compared to the unwashed version. The concentrations of the SUS TAGs however, were shown to decrease in the washed retentate samples.

It can be concluded that washing the retentates was successful in expelling most of the UUU and SUU TAGs whilst causing the concentration of the SSS TAGs to correspondingly increase in the retentate. Although this was expected, it is not clear why the concentration of SUS TAGs decreased a little in the retentate despite the fact that their $T_m$'s lie at higher temperature ranges which suggest that they should be crystallising and are preferentially concentrated in the solid phase. A possible explanation for this is that the SUS TAGs were probably diluted by the acetone and were washed together with the entrapped OL which contained the UUU and SUU TAGs. To confirm this assumption, it would be worth to investigate the concentration of the TAGs in the collected filtrate (OL) fraction. If the assumption that some of the SUS TAGs were probably washed away or diluted together with the entrained OL
was true, then the washed OL samples should depict an increase in the concentration of this TAG group.

Table 4-1 TAG composition (%) of the collected retentates (ST)

<table>
<thead>
<tr>
<th>TAG group</th>
<th>ST1*</th>
<th>ST2*</th>
<th>ST3*</th>
<th>ST4^</th>
<th>ST5^a</th>
<th>ST5^b</th>
<th>ST6^</th>
<th>ST7^</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>4.2</td>
<td>4.3</td>
<td>4.8</td>
<td>3.4</td>
<td>3.8</td>
<td>3.2</td>
<td>4.6</td>
<td>3.8</td>
</tr>
<tr>
<td>SUU</td>
<td>26.1</td>
<td>27.4</td>
<td>30.6</td>
<td>22.1</td>
<td>22.5</td>
<td>20.1</td>
<td>28.0</td>
<td>21.7</td>
</tr>
<tr>
<td>SUS</td>
<td>38.6</td>
<td>39.2</td>
<td>40.3</td>
<td>41.7</td>
<td>42.1</td>
<td>42.9</td>
<td>42.8</td>
<td>42.2</td>
</tr>
<tr>
<td>SSS</td>
<td>26.4</td>
<td>24.2</td>
<td>19.2</td>
<td>28.7</td>
<td>27.3</td>
<td>30.0</td>
<td>20.0</td>
<td>28.4</td>
</tr>
</tbody>
</table>

* Retentates collected 30 minutes after \( T_i \) was reached
^ Retentates collected 120 minutes after \( T_i \) was reached
a Retentate collected 130 minutes after \( T_i \) was reached
Actual concentration values analysed by HPLC
b Predicted concentration values using the equation (3-18) using the OL composition from the final filtered sample of the original isothermal experiment at 24 °C (Chapter 4)

Table 4-2 TAG composition (%) of the collected filtrates (OL) (120 minutes after \( T_i \) was reached)

<table>
<thead>
<tr>
<th>TAG group</th>
<th>OL4 (F&amp;W)</th>
<th>OL5-1 (F&gt;W)</th>
<th>OL5-2 (F&gt;W)</th>
<th>OL6</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>7.1</td>
<td>7.7</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td>SUU</td>
<td>41.8</td>
<td>42.9</td>
<td>42.1</td>
<td>42.8</td>
</tr>
<tr>
<td>SUS</td>
<td>43.7</td>
<td>42.6</td>
<td>43.3</td>
<td>43.3</td>
</tr>
<tr>
<td>SSS</td>
<td>1.7</td>
<td>1.1</td>
<td>1.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The TAG composition of the filtrates collected after 120 minutes \( T_i \) was reached is given in Table 4-2. Two OL fractions were collected during the fifth filtration point (OL5); the first being the OL collected prior to the washing step while the second was the OL collected after the washing step, i.e. entrained OL. It is evident that washing the retentate had caused the SUS TAGs to increase slightly in the OL (OL4) compared to the OL samples collected from unwashed ST (OL6). But the difference in the amount of SUS between washed and unwashed samples were not the same when their concentrations in the OL fractions were compared with those in the ST
fractions. It is thought that the decrease in the SUS TAGs in the washed ST (Table 4-1) would correspondingly show a similar increase in amount in the OL from washed samples; however this is not the case. It is still unclear why this TAG group is still present in large amounts in the washed and unwashed OL, contributing to more than 40% of the overall TAG concentration. A possible explanation for this could be due to these TAGs being diluted during the washing step, causing them to be expelled together with the entrained OL trapped within the solid crystals.

Surprisingly, a very small amount of SSS TAGs was observed to also increase in the OL samples from the washed retentate. Breitschuh & Windhab (1998) reported that at low crystallisation temperatures, high-melting TAGs showed a tendency to cocrystallise as they are strongly supercooled, further promoting the formation of compound crystals of TAGs bearing similar chainlengths. This may also be one of the reasons for the very small increase in the SSS and SUS TAGs in the OL. It can be concluded that washing the retentate may not be the best option since this additional step may have succeeded in removing the UUU and SUU TAGs but may also have diluted the SSS and SUS crystals, further producing a large variability in the composition of the ST as shown in Table 4-2.

4.6.4 Entrainment level determination

Table 4-3 shows the effect of the washing step on the yields of the filtration products and the level of entrainment in the retentate cake. The entrainment levels in ST3 and ST6 were calculated assuming that the weights of entrained OL in these unwashed samples was a result of the difference between the weights of retentates collected in these unwashed samples and the mass of the previous washed samples (ST3-ST2 and ST6-ST5). This calculation was carried out assuming that no mass change occurred between the two filtration points. The entrainment level in ST7 was quantified by using the actual weight of the entrained OL collected divided by the sum of the entrained OL and the washed ST.
Chapter 4

Isothermal crystallisation of palm oil under shear

Table 4-3  Yield (%) of filtration products and measured entrainment level

<table>
<thead>
<tr>
<th>Filtration products</th>
<th>ST1*</th>
<th>ST2*</th>
<th>ST3*</th>
<th>ST4&lt;</th>
<th>ST5-2&lt;</th>
<th>ST6&lt;</th>
<th>ST7&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>OL</td>
<td>81.8</td>
<td>79.5</td>
<td>68.2</td>
<td>77.9</td>
<td>71.1</td>
<td>63.1</td>
<td>63.3</td>
</tr>
<tr>
<td>ST</td>
<td>18.2</td>
<td>20.6</td>
<td>31.8</td>
<td>22.1</td>
<td>28.9</td>
<td>36.9</td>
<td>36.8</td>
</tr>
</tbody>
</table>

Entrainment†  0.4  0.3  0.3

*Entrainment level, expressed as the ratio of entrained liquid (EnOL) mass to the retentate mass before acetone washing (EnOL:ST)

** and †: please refer to footnotes from Table 4-1.

As expected, the washing step led to an increase in the yield of the OL compared to the unwashed samples. The entrainment level is also higher during the earlier filtrations compared to the samples that were filtered towards the end of the crystallisation time. The higher entrainment level observed during the initial filtration point is thought to be due to the type of process occurring at this stage, which by looking at the FBRM response in Figure 4-38 shows that the oil was still undergoing nucleation and crystal growth as the total counts profile was still increasing at this stage. This shows that entrainment levels improve when new crystals or nuclei are no longer being generated and that the crystal size has reached a maximum. Entrainment levels of ST6 and ST7 are seen to be similar in value, suggesting that the crystallisation process has reached equilibrium and crystallisation of the oil has stopped.

The entrainment levels from the retentate washing exercise at 24 °C as indicated in Table 4-3 show a very large difference compared to the values calculated using the proposed entrainment calculation method described earlier in Section 4.5.2. From Figure 4-22, the calculated entrainment level when palm oil was isothermally crystallised at 24 °C was about 0.80 which later decreased to around 0.65 towards the end of the experiment. These values are quite different compared to the actual value of 0.3 obtained from the washing exercise on the final sample ST7 as shown in Table 4-3. This shows that the actual entrainment level in washed samples was not as high as those calculated from the proposed entrainment calculation and the initial
assumption that the UUU TAGs do not crystallise may not be applicable as these TAGs are still present in the ST fraction, forming a total concentration of nearly 5%.

The only explanation for the cause of this is that maybe the washing exercise had failed to remove these TAGs completely from within the ST crystal agglomerates. Thus, a small amount of these TAGs will still remain entrapped within the structure of crystals (such as between dendrites) and their concentrations in the ST will never decrease to zero despite efforts to wash them away. Bemer & Smits (1982) reported that when crystal agglomerates formed from palm oil crystallisation were repeatedly washed with an organic solvent, the amount of liquid left in the retentate is similar to the amount of intraparticle entrapped liquid, i.e. the liquid entrapped between individual crystals within crystal agglomerates. They concluded that the washing process removes most of the interparticle liquid i.e. the liquid entrapped between crystal agglomerates but fails to remove the intraparticle liquid within the filter cake. The same explanation may be applied in this study.

### 4.7 CONCLUSIONS

The influence of isothermal crystallisation temperature ($T_i$) on the onset of nucleation, TAG concentrations of filtration products, crystallisation rates of TAGs and on the chemical potential driving forces of TAGs during palm oil crystallisation has been studied. In this study, it was shown that the FBRM instrument can be used to detect primary nucleation during palm oil crystallisation. This was indicated by a sudden surge in the total particle counts. An increase in the isothermal crystallisation temperature resulted in a longer induction period required for the onset of nucleation to occur, as detected by FBRM. FBRM also provided an indication of when the system reached equilibrium as observed from the unchanging trend in the total counts within the experiments at each $T_i$. A comparison of the mean chord length of crystals at various $T_i$ showed that the mean size of crystals increases with increasing $T_i$ from 24 °C to 26 °C but then decreases at $T_i$ above 26 °C. This was assumed to be due to a secondary nucleation event occurring at higher $T_i$ of 28 °C and 30 °C as detected by FBRM. However no secondary nucleation event was detected at 32 °C. This study
proved that the FBRM was capable in monitoring the evolution of particle size and population as well as detecting the occurrence of multiple events such as nucleation, crystal growth, agglomeration and secondary nucleation taking place within a crystallising palm oil system.

The effect of temperature on the crystallisation of individual TAGs in the olein and stearin have been determined. The isothermal crystallisation study on palm oil at various $T_1$ have shown that as $T_1$ increases, the crystallisation rates of SSS TAGs in the ST phase prevailed at higher $T_1$, indicating the possibility of enriching them further while SUS, SUU and UUU TAGs lie grouped together at very low concentrations. An estimation of the crystallisation driving forces ($\Delta\mu$) of individual TAGs in their $\beta'$ form have shown that the $\Delta\mu$ for SSS TAGs showed a decrease with time while the $\Delta\mu$ of all other TAGs remained unchanged throughout all experiments. The TAG composition in the respective olein and stearin fractions played a major role in determining the crystallisation driving forces of TAGs as well as the pure component melting temperature ($T_m$). The effect of $T_m$ was dominant when ideality is assumed, with the SSS TAGs having the highest $\Delta\mu$ which mainly drives their conversion into the solid phase. It has been shown that the overall crystallisation rate of palm oil TAGs occurs in a non-linear manner and depends significantly on the degree of supercooling.

Results here have shown that the ST fractions collected from the isothermal crystallisation experiments still contain a substantial amount of UUU TAGs, which when looking at their melting temperatures dictate that they should not have crystallised in the first place. It was assumed that their presence was mainly influenced by the level of entrained liquid between the ST crystals, as reported in literature from previous work. Thus, an entrainment correction procedure was proposed to account for the amount of UUU TAGs in the ST. This method assumed that UUU TAGs do not crystallise to a significant extent in the ST fraction. Calculated entrainment levels revealed values which were very much similar to those reported in earlier studies. The corrected ST yield based on the calculated entrainment levels revealed that only about 12% of palm oil was crystallising compared to the higher yields obtained in the uncorrected data.
According to this correction method, it was also shown that the SSS TAGs in the corrected ST fraction had a higher conversion into the solid phase compared to SUS and SUU which showed the highest conversion into the solid phase in the uncorrected data, which relates well to their thermal properties. The SUU and UUU TAGs showed unchanging trends within the OL fractions, further supporting the earlier assumption that these TAGs do not crystallise at all when the crystallisation temperatures of study were applied. Two TAGs, namely PPP and POP were the only TAGs found to have crystallised in significant amounts at all $T_i$.

The chemical potential driving force ($\Delta \mu$) for crystallisation of TAGs calculated based on the corrected ST composition showed a behaviour which was significantly different compared to those calculated based on the uncorrected ST composition. It was found that the $\Delta \mu$ of PPP showed decreasing values, dropping below the $\Delta \mu$ of POP as the crystallisation proceeded at all $T_i$. This behaviour was not observed in the $\Delta \mu$ of TAGs calculated from the uncorrected data. Results also showed that POP does crystallise in significant amounts but its crystallisation is much more delayed compared to PPP. It is thought that POP will only start to crystallise once the $\Delta \mu$ of PPP started to cease.

In order to verify the proposed entrainment calculation, a repeat of the isothermal experiment at 24 °C was performed whereby the filtration procedure included a washing step in an attempt to expel entrained liquid from within the ST cake. This led to an increase in the SSS TAGs in the ST fraction but still showed the presence of SUU and UUU TAGs. Upon examination of the OL properties, it was observed that the level of SSS and SUS TAGs experienced a slight increase, suggesting that the washing step may have diluted these TAG groups and caused them to be washed together with the entrained OL. The persisting presence of the UUU and SUU TAGs were thought to be attributed to the level of entrapped intraparticle liquid, which, as shown in the past, cannot be removed even if the ST retentate was repeatedly washed. It can be concluded that the washing exercise may have not been the best option and thus, normal vacuum filtration techniques normally employed in fats crystallisation studies were accepted as the best way to the filter samples for all experiments carried out in this study.
To conclude, liquid entrainment within the ST cake has been shown to have a significant influence on the TAG composition in the ST and resultant $\Delta\mu$ of TAGs during the isothermal crystallisation of palm oil. The proposed entrainment correction method was successful in predicting entrainment levels within the ST cake comparable to values reported by previous work and the entrainment levels at all $T_i$ showed physical trends which appeared realistic. The method assumes that UUU TAGs are not present in the ST and that the presence of these TAGs in the ST is mainly due to liquid entrainment. While this assumption “forces” the values of UUU, it does not force the values of SUU, SUS and SSS, in which their concentration values appear much more realistic compared to the uncorrected data. Also, the proposed entrainment calculation method has shown that SUU TAGs too do not crystallise to any significant amount. This is supported by the crystallisation thermogram obtained from differential scanning calorimetry (DSC) (Chapter 2) which shows two distinct peaks occurring at a high and low temperature range respectively where SUU and UUU TAGs must preponderantly crystallise in the peak at the lower temperature range. However, the proposed entrainment correction method is only useful in dissociating the crystallisation process from the effects of the filtration stage to enable one to study the crystallisation process on its own. This study was not intended to find ways of reducing or eliminating the problem of liquid entrainment encountered within the fractionation industry but serves as guidance on how to study the true crystallisation behaviour of TAGs. One would still need to address the entrainment problem in practice.
5 NON-ISOTHERMAL CRYSTALLISATION OF PALM OIL UNDER SHEAR

5.1 INTRODUCTION

This study was conducted in order to investigate the effect of cooling rate variation on the crystallisation of palm oil relating to the differences in the onset of nucleation, the partitioning effect on the TAGs present in palm oil and how the crystallisation rates and chemical potential driving force of each TAG changes with the change in temperature and composition. This is inherently more complicated than the isothermal experiments as the temperature driving forces are constantly changing. This chapter shall begin with a brief description of the experiments performed followed by a presentation of the FBRM response obtained when different cooling rates were applied to palm oil crystallisation. The measured HPLC composition of TAGs shall be presented thereafter followed by further analysis on the crystallisation rates and chemical potential driving forces based on the TAGs composition derived from HPLC. The effect of entrainment on these results shall also be discussed.

5.2 EXPERIMENTS PERFORMED

The sample preparation for the non-isothermal crystallisation experiments were carried out following the general procedure outlined earlier in Section 3.2.1. Non-isothermal experiments were performed in accordance with the method described in section 3.2.4. Three different constant cooling rates were studied in this work, i.e. 0.1 °C/min, 0.3 °C/min and 0.5 °C/min. The FBRM response was extracted from the LASENTEC software and compared between each cooling rate. Other treatment of the FBRM data is described in subsection 3.3.3. The filtration products were subjected to compositional analysis by HPLC as outlined in section 3.4. Further correction and treatment of compositional data is described in detail in section 3.5.
5.3 EXPERIMENTAL RESULTS AND DISCUSSION

5.3.1 Monitoring of the cooling crystallisation of palm oil using FBRM

In this work, the onset of nucleation was detected via two methods, i.e. by visual observation and by the FBRM. Figure 5-1 shows the oil temperature ($T_{oil}$) and particle counts profile obtained when a cooling rate of $0.1 \degree C/min$ was applied during palm oil crystallisation. Times are measured from the point where the setpoint temperature ($T_{sp}$) had reached $40 \degree C$ (immediately before the desired cooling rate started). Nucleation was detected by visual observation at around $30.8 \degree C$ with the formation of a cloud in the oil bulk. This was later detected by the FBRM at $30.1 \degree C$ which was signalled by a sudden surge in the total counts of particles. A delay of about 6 minutes between the two methods of nucleation detection was noted which is thought to be due to the lack of sensitivity of the FBRM which may probably be caused by the size of nuclei which were below the detectable limit of the FBRM of $0.8 \mu m$.

From Figure 5-1, the total counts profile obtained from the FBRM is observed to increase gradually from the point of nucleation, indicating an increase in the crystal population as the system continues to be cooled at a rate of $0.1 \degree C/min$ before levelling off at around 40000 counts per second. When the $T_{oil}$ reached $13.7 \degree C$, the total particle counts suddenly started to increase again to nearly 44000 counts per second. The sudden increase in the total particle counts towards the end of the experiment at a much lower temperature than the nucleation temperature may suggest that a secondary nucleation event is taking place within the crystalliser. This event may either be attributed to a different group of TAGs which start to crystallise at much lower crystallisation temperatures or may have occurred due to the attrition of existing crystals from which small fragments are generated, which in turn contribute to the rise in the total particle counts. This observation is similar to the crystallisation thermogram obtained from differential scanning calorimetry (DSC) showing two exothermal peaks with one peak situated at higher temperatures while the second peak occurred at lower temperature ranges (see Figure 3-17 in Section 3.6.3). This supports the assumption that nucleation of a different group of TAGs may have started to take place within the crystallising slurry.
Chapter 5  Non-isothermal crystallisation of palm oil under shear

![Figure 5-1](image1)

**Figure 5-1** Temperature and total counts curves of palm oil crystallised at a cooling rate of 0.1 °C/min

![Figure 5-2](image2)

**Figure 5-2** Temperature and total counts curves of palm oil crystallised at a cooling rate of 0.3 °C/min

*Partitioning of triacylglycerols in the fractional crystallisation of palm oil*
The temperature and total particle counts profile obtained for the crystallisation of palm oil at a cooling rate of 0.3 °C/min and 0.5 °C/min are shown in Figure 5-2 and Figure 5-3 respectively. From Figure 5-2, a cooling rate of 0.3 °C/min resulted in nucleation taking place at a temperature of 24.7 °C by visual observation with the formation of a cloud in the oil bulk while detection of nucleation by the FBRM occurred at 22.5 °C which was indicated by a sudden increase in the total particle counts per second. When the cooling rate was increased to 0.5 °C/min, the cloudiness in the oil bulk was seen to occur at a much lower temperature of 24.1 °C while the FBRM detected nucleation at 19.4 ºC. At both cooling rate conditions, there was also a delay in the detection of the particles by the FBRM and this period of delay increased with increasing cooling rate. This can be explained as follows: when palm oil is subjected to fast cooling, a large number of small crystals are formed and the faster the cooling rate, the smaller are the crystals formed during nucleation, as observed by Simon et al. (2009).

A comparison of the total counts profile at all cooling rates of study (Figure 5-1 to Figure 5-3) depict that the population of crystals decreases significantly with
increasing cooling rate. This can be seen from the total counts at 0.1 °C/min which show particle counts increasing to more than 40000 counts/second compared to less than 600 counts/second observed when a cooling rate of 0.5 °C/min was employed. The total particle counts profile observed at both 0.3 °C/min and 0.5 °C/min showed a decrease at a certain period of time in the experiment and this subsequently started to increase again. This observation did not occur when a cooling rate of 0.1 °C/min was applied. This may be due to a melt-mediated polymorphic transformation and/or stepwise crystallisation occurring within the crystallising liquid (Marangoni et al., 2005). A further examination of the mean chord size of crystals may give insight into this phenomenon and shall be discussed later in this section.

The results from the FBRM and temperature profiles here have shown that an increase in cooling rate during palm oil crystallisation decreases the temperature of the onset of nucleation. This is in agreement with the study by Marangoni et al. (2005) whereby they showed that a decrease in crystallisation temperature was observed when the cooling rate during crystallisation was increased. A further comparison between the times of the onset of nucleation when different cooling rates of 0.1 °C/min, 0.3 °C/min and 0.5 °C/min is illustrated in Figure 5-4. It is evident that the induction time for the onset of nucleation increased with decreasing cooling rate. This may be explained by the strong dependency of the nucleation rate on the degree of supercooling or supersaturation, according to equation (2-25) (see Section 2.4.2). This was also demonstrated by Kloek et al. (2000) who showed increasing induction time for crystallisation when the initial supersaturation decreased.

An increase in the cooling rate also shows increased nucleation rate of palm oil. This is evident from the slopes of the total counts in Figure 5-4 where the total counts curve for a cooling rate of 0.3 °C/min depicts a steeper slope compared to that at 0.1 °C/min. A higher cooling rate would always result in the production of smaller crystals. Hence to evaluate this further, the final square-weighted chord length distribution of the palm oil crystals at all cooling rates were plotted and compared. A comparison of the chord length distribution (CLD) of palm oil crystals crystallised at cooling rates of 0.1 °C/min, 0.3 °C/min and 0.5 °C/min at the end of each experiment is depicted in Figure 5-5.
Figure 5-4  Comparison of nucleation onsets at various cooling rates during the palm oil crystallisation

Figure 5-5  Comparison on the final chord length distribution of palm oil crystals crystallised at cooling rates of 0.1 °C/min, 0.3 °C/min and 0.5 °C/min
From Figure 5-5, it is evident that smaller crystals are generated when higher cooling rates are employed. The larger crystal CLD observed at 0.1 °C/min is a result of the slower cooling rate applied which may have allowed the nucleation process to progress successfully and provided ample time for the nuclei to subsequently grow into larger crystals and further aggregate, resulting in crystals with a maximum size of 600 μm. Crystals generated at a cooling rate of 0.3 °C/min showed a much smaller size of 300 μm maximum while crystals of a mean size of 150 μm were observed at 0.5 °C/min.

The area under each distribution in Figure 5-5 provides an indication of the population of crystals at the respective chord sizes. A faster cooling rate produces smaller crystals at a lower crystal population compared to the production of large crystal sizes at higher crystal population when slower cooling rates were studied. The reason for this is unclear as there is a possibility that the crystal population indicated by the CLD was generated from a local population of crystals near the FBRM instrument. When cooling rates of 0.3 °C/min and 0.5 °C/min were applied, a rapid increase in the viscosity of the palm oil slurry was observed to occur in the crystalliser. As the viscosity of the slurry increases, this in turn restricts the motion of crystals and causes them to move slower. As a result, fewer particles are scanned by the FBRM during the measurement duration, giving lesser population numbers. This is also confirmed by the lower counts seen at higher cooling rates shown in Figure 5-4.

It was noted earlier that the total counts curve plotted for palm oil crystallised at a cooling rate of 0.1 °C/min (see Figure 5-1) experienced a sudden increase towards the end of the experiment as the oil was cooled down. An increase in the total counts would mean that more crystals were being generated and signified a nucleation event taking place. A way to determine whether this secondary nucleation event was a result of either a different group of components or TAGs that was starting to crystallise or whether it was due to fragments of crystals breaking off from existing crystals due to attrition, the mean chord length and total counts of crystals were plotted together for comparison purposes. This is illustrated in Figure 5-6 for cooling rates of 0.1 °C/min and 0.3 °C/min.
Figure 5-6  Effect of cooling rate on the mean chord length of crystals and total particle counts in palm oil crystallisation

Figure 5-7  Fine and coarse particle counts as a function of time for palm oil crystallised at a cooling rate 0.1 °C/min
As depicted in Figure 5-6, the average chord size of crystals cooled at 0.1 °C/min decreased while the total counts simultaneously increased towards the end of the run. This result implies that there was definitely a secondary nucleation process occurring where newer nuclei were being formed from which their small sizes contributed in lowering the average chord size of crystals while at the same time increasing the crystal population in the crystalliser. This was also confirmed from the CLD plot which showed a bimodal distribution in Figure 5-5. Upon further examination of the fine and coarse particle counts profiles at 0.1 °C/min in Figure 5-7 at this time period, it can be seen that as the fine particles increased, a corresponding decrease in the coarse particle counts is observed. A micrograph of the final ST sample taken by polarised light microscopy which confirms this behaviour is shown in Figure 5-8 where the ST crystals consist of a mixture of small crystals and large agglomerates.

The decrease in population of coarse crystals and parallel increase in fines when palm oil was crystallised at 0.1 °C/min may by due to a genuine secondary nucleation event or the attrition of crystals which produce mechanically chipped off fragments of these existing crystals. This may have occurred because the viscosity of the slurry became too high and thus restricted the motion of crystals. As the system is agitated, collision between the agitator blade and the crystals may have caused crystal attrition resulting in their ends chipping off, thus producing a sudden increase in fine crystals. This results in the final lower average crystal chord size within the crystal population as illustrated in Figure 5-6.

A completely different scenario is observed when a cooling rate of 0.3 °C/min was applied. From Figure 5-6, the mean chord size of crystals is seen to increase when the total particle counts decrease. This could only suggest that an agglomeration process was taking place, where existing crystals start colliding with each other due to the viscosity increase within the slurry. Increased contact between crystals further causes them to aggregate to form larger crystal entities, lowering the number of crystals within the population while increasing their size. This is confirmed by observing the decreasing counts of the fine particles with a concomitant increase in the coarse particles between 100 and 115 minutes, as delineated in Figure 5-9. After 115 minutes, due to further cooling, the slurry shows significant nucleation and this is indicated by the increase in the number of fines.
Figure 5-8  Micrograph of the final ST crystals collected when palm oil was crystallised at a cooling rate of 0.1 °C/min

Figure 5-9  Fine and coarse particle counts as a function of time for palm oil crystallised at a cooling rate 0.3 °C/min
When palm oil was crystallised at a cooling rate of 0.5 °C/min, it can be seen that the fine particle counts initially increased (due to primary nucleation of crystals) until the counts reached a maximum of nearly 120 counts per second at around 12 °C, as shown in Figure 5-10. A corresponding increase in the coarse particle counts is also seen to occur during this period due to the crystal growth process (e.g. agglomeration) but at a much slower rate. As the temperature was lowered further, the fine particles started to decrease in number while the coarse particle counts continued to increase, reaching a peak maximum. These results indicate that an agglomeration process was taking place within the crystallising slurry. Both fine and coarse particle counts then decrease simultaneously. The decrease in coarse particles may suggest that crystal breakage was occurring due to the increased viscosity of the slurry and effect of agitation. A further increase in both trends was observed afterwards, suggesting that both secondary nucleation (increase in fines) and agglomeration (increase in coarse particles) were taking place at the same time. This again may due to a melt-mediated polymorphic transformation and/or a stepwise crystallisation occurring within the liquid, as suggested by Marangoni et al. (2005).
The various peaks in the FBRM signals (e.g. Figure 5-7 to Figure 5-9) correspond to the different events happening (e.g. possible nucleation of different TAG groups, breakage, agglomeration) during the cooling crystallisation of palm oil. A general conclusion from observing the various FBRM responses in this study is that FBRM has the capability of not only providing an indication of the particles population but also in monitoring different crystallisation stages such as detecting the onset of nucleation, agglomeration and attrition during constant cooling experiments of palm oil. FBRM thus provides a valuable tool for detecting and distinguishing multiple events as well as a way to follow in situ changes in the particle concentration during the non-isothermal crystallisation of palm oil as it progresses.

5.3.2 Measured HPLC composition

Figure 5-11 illustrates the raw compositions of all TAGs in the OL fraction from filtered palm oil slurry crystallised at a cooling rate of 0.1 °C/min while Figure 5-12 shows the same for TAGs present at less than 5%. A typical observation in these graphs is the SSS TAGs that are seen to deplete entirely from the OL at the end of the experiment, as depicted in Figure 5-12. As expected, SUS TAGs show a slight decrease in concentration in the OL while the opposite scenario is observed for the SUU and UUU TAGs, confirming earlier studies by Deffense (1985).

The TAG compositions in the corresponding ST fraction are shown in Figure 5-13 for all TAGs and Figure 5-14 for TAGs in the ST fraction with concentrations below 6%. The SSS TAGs are initially seen to bear a high concentration in the ST during the early stage. This can be attributed to their importance in the nucleation process as it has been shown that PPP, MPP and PPS are responsible for the onset of nucleation during the crystallisation of palm oil (Sulaiman et al., 1997). This later becomes constant as the temperature is lowered, suggesting that all of the SSS have crystallised from the liquid into the solid phase. This behaviour is confirmed from Figure 5-12 which depicts their concentrations reducing to zero in the OL phase towards the end of the experiment.
Figure 5-11 Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.1 °C/min

Figure 5-12 Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.1 °C/min (TAGs<5%)
Figure 5-13  Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.1 °C/min

Figure 5-14  Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.1 °C/min (TAGs<6%)
The behaviour of SUS TAGs is reversed where their concentrations increase in the ST with increasing crystallisation time and decreasing temperature, as shown in Figure 5-13. TAGs with concentrations less than 2% do not seem to be experiencing any change in their amounts, showing more or less the same concentration values throughout the experiment as depicted in Figure 5-14.

At a cooling rate of 0.3 °C/min, a similar and typical observation with the TAG concentrations of the OL fraction collected at a cooling rate of 0.1 °C/min is the depletion of the SSS TAGs as the temperature is lowered further (see Appendix C.1 for the measured TAG composition of the OL and ST products at cooling rates of 0.3 °C/min). The only difference the cooling rate effect had on the SSS TAGs in the OL fraction was that there was still about 0.3% in concentration of these TAGs in the OL compared to its total depletion from the OL phase when crystallised at 0.1 °C/min, which is contributed by the concentration of PPP. PPS and MPP however, showed similar behaviour when comparing these two cooling rates where they deplete entirely from the liquid phase and are concentrated in the solid phase.

When a cooling rate of 0.5 °C/min was employed, the retention of the SSS TAGs in the OL increased to 0.7% with some PPP and MPP still remaining whereas PPS depleted completely from the OL (see Appendix C.1 for the measure TAG composition of the OL and ST products at cooling rates of 0.5 °C/min). This may suggest that the SSS TAGs become more difficult to crystallise out completely from the liquid when higher cooling rates are employed and more of these TAGs are retained in the OL as the cooling rate increases. However, a larger degree of supercooling would theoretically initiate the SSS TAGs to crystallise more quickly compared to the less saturated TAGs. A possible reason for this maybe due to the fact that filtration of the samples took place at room temperature which is much higher than the temperature within the crystalliser. This may have caused a very small amount of the SSS TAGs to remelt back into the OL phase during filtration.

It is also observed that as the cooling rate is increased from 0.1 °C/min to 0.5 °C/min, the TAGs that lie below 2% in concentration are more separated at higher cooling rates. The amount of UUU and SUU TAGs in the ST collected also increases. The retention of these TAG groups in the ST suggests increased entrainment level at
higher cooling rates as when the supercooling is high, a greater number of nuclei is generated which result in smaller crystals being produced (Walstra et al. 2001). This makes it more difficult to filter the slurry during the filtration stage and a large portion of the liquid phase is retained together with the ST cake. The ST observed from samples collected when a cooling rate of 0.5 °C/min was studied depicted a wet cake appearing to contain very tiny crystals, further confirming this phenomenon.

5.3.3 Partition coefficient of TAGs

The partition coefficient ($K_d$) of individual palm oil TAGs during the non-isothermal crystallisation of palm oil was calculated using equation (4-1) as previously applied in the isothermal study (see section 4.3.3). $K_d$ values for all TAGs at cooling rates of 0.1 °C/min, 0.3 °C/min and 0.5 °C/min were plotted as a function of temperature. Figure 5-15 and Figure 5-16 show these plots at cooling rates of 0.1 °C/min and 0.5 °C/min respectively while a similar plot at a cooling rate of 0.3 °C/min can be found in Appendix C.2.

It can be seen that at all cooling rates, SSS TAGs showed preferential partitioning into the ST phase depicted by their $K_d$ values being below the equipartition line ($K_d = 1$). All SUU and UUU TAGs had $K_d$ greater than 1, hence higher affinity to be retained within the OL phase. The SUS TAGs however showed increasing preference to partition into the ST phase at lower cooling rates. As the cooling rate increased, the preference for the majority of SUS TAGs to partition into the ST phase decreased as evident by their increasing $K_d$ values which lie above the equipartition line at 0.5 °C/min (Figure 5-16).
Figure 5-15  Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at 0.1 °C/min

Figure 5-16  Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at 0.5 °C/min
It can also be observed that as the cooling rate is increased, the range of the distribution of $K_d$ values of SUS, SUU and UUU TAGs become narrower, suggesting that the applied cooling rate has a profound influence on the separation of TAGs during the cooling crystallisation of palm oil. At lower cooling rates (e.g. 0.1 °C/min in Figure 5-15), the trend of the distributions of TAG groups is more apparent, with SUU and UUU TAGs showing increasing $K_d$ values with decreasing temperature. SUS TAGs (namely POP and POS) correspondingly showed decreasing $K_d$ values as the temperature is lowered further, indicating their increasing crystallisation into the ST phase. These results indicate that the rate of cooling during palm oil crystallisation greatly affects the partitioning behaviour of TAGs.

5.3.4 Cumulative concentration of TAGs

A comparison of the cumulative concentration of palm oil TAGs in the ST fraction at the different cooling rates in study was performed. This allows one to follow the increase in composition of each TAG in the ST phase based on the total amount of TAG present in palm oil. It is evident that an increase in the cooling rate resulted in less SSS TAGs crystallising into the ST phase, as shown by Figure 5-17 and Figure 5-18 for cooling rates of 0.1 °C/min and 0.5 °C/min respectively (see Appendix C.3 for the cumulative concentration of TAGs in the ST fraction at 0.3 °C/min).

Another observable trend is that all other TAGs besides the SSS TAGs crystallise together at lower concentrations. The crystallisation of TAG species from the lower concentration group shows the highest melting TAG in this group, i.e. SOS crystallising out first followed by the other TAGs with decreasing melting point and degree of unsaturation, as shown by the thin (UUU) and thick (SUU) solid gray lines which are located at the lowest concentration level in the graphs. This may suggest that SOS crystallises first due to its higher melting point and creates nucleation sites for other TAGs in this group to attach themselves to and the co-crystallisation of these TAGs may then have taken place.
Figure 5-17  Cumulative concentration of palm oil TAGs in the ST fraction at a cooling rate of 0.1 °C/min

Figure 5-18  Cumulative concentration of palm oil TAGs in the ST fraction at a cooling rate of 0.5 °C/min
Another notable observation is that the separation between the lower concentration TAGs increases at lower cooling rates. Figure 5-17 shows that at 0.1 °C/min, these TAGs crystallised together during the initial stage of the crystallisation process until the middle of the experiment. This was followed by their gradual separation which prevailed during the second half of the experiment. Since the beginning stage is usually governed by the nucleation process, this suggests that the initial supercooling for nucleation to occur resulted in these TAGs crystallising together. Crystal growth during the second half of the experiment at a lower degree of supercooling may have provided enough time for these TAGs to crystallise and grow properly, leading to a better separation between them at 0.1 °C/min compared to when a cooling rate of 0.5 °C/min was employed.

5.4 FURTHER ANALYSIS

5.4.1 Crystallisation rates of TAGs

The crystallisation rates of palm oil TAGs at different cooling rates were determined similar to the method applied in the isothermal studies (see Section 4.4.2 in Chapter 4). When constructing the trend plots of the conversion of TAGs into the solid phase, the nucleation point detected by visual observation was taken as the true onset of nucleation as it was visibly observed that clouding, which is an indication of nucleation in the oil, had occurred even before the detection by FBRM.

Figure 5-19 illustrates the conversion of TAGs into the solid phase, expressed in grams of TAG in ST per 100 g crystallising fat for palm oil crystallised at 0.1 °C/min. In general, the conversion of TAGs into the solid phase showed a gradual increase with time as each TAG component started to nucleate and this was followed by crystal growth as the oil temperature simultaneously decreased. It is clear that POP showed the highest conversion into the solid phase due to its higher concentration in the ST and also in the original palm oil compared to all other TAGs. This was followed by PPP which only showed a higher conversion to the solid phase during the initial stage of the crystallisation process, but was then succeeded by that of POO. The
initial larger conversion rate of PPP into the solid phase is thought to be due to its importance during the nucleation stage (Sulaiman et al., 1997).

**Figure 5-19**  Mass of TAG in ST per 100g palm oil at 0.1 °C/min

**Figure 5-20**  Mass of TAG in ST per 100g palm oil at 0.5 °C/min
PLP and POL showed similar conversion rates into the solid phase from the beginning stage until midway into the experiment. After the 190th minute, they became separated and were seen to crystallise in this manner until the end of the run. It is not clear why this occurred but a probable explanation with regards to their similar conversion rates during the first half of the crystallisation process may be due to a co-crystallisation of these two TAGs from the point of nucleation. All other TAGs showed a conversion of less than 2% into the solid phase with POS having the highest conversion rate among these TAGs when a cooling rate of 0.1 °C/min was applied.

A further observation of the slopes of the crystallisation curves in Figure 5-19 shows a gradual increase in TAG conversion rates into the ST as the temperature is gradually lowered, plateauing towards the end. This is an expected behaviour when a lower degree of supercooling (0.1 °C/min) is employed, resulting in a steady overall growth rate of TAG crystals until the supercooling effect ceases. The almost unchanging trend towards the end may signify that crystal growth of TAGs has stopped and the system has reached a state of equilibrium.

As the cooling rate increases from 0.1 °C/min to 0.3 °C/min, the same behaviour with respect to POP having the highest conversion into the solid phase is observed (see Appendix C.4 for the mass of TAGs in ST per 100 g palm oil at 0.3 °C/min). The conversion rate of PPP however, remained higher than POO from the nucleation point for a much longer period of time until the middle of the run. This may be a result of the higher supercooling employed at a higher cooling rate of 0.3 °C/min compared to when palm oil was crystallised at 0.1 °C/min. POL and PLP are seen to crystallise together throughout the entire duration of the experiment and no separation is observed between these two TAGs compared to their separation during the later stages of the experiment at 0.1 °C/min. The slopes of the crystallisation curves at 0.3 °C/min are much steeper compared to the curves obtained at 0.1 °C/min, implying that the overall crystallisation rate of TAGs increases with increasing degree of supercooling.

When a cooling rate of 0.5 °C/min was employed, POP still showed the highest conversion into the solid phase due to its higher composition within the solid phase, as illustrated in Figure 5-20. However, it is noticeable that PPP did not show a higher
conversion into the solid phase compared to POO during the beginning of the crystallisation process, as observed earlier at 0.1 °C/min and 0.3 °C/min. It was thought that the increase in cooling rate would show an even greater initial conversion of PPP into the solid phase but this was not the case. POO showed a higher rate of conversion into the solid phase compared to PPP most of the time except during the middle stage of the run. This may be due to the increased concentration of POO retained in the ST phase when palm oil was crystallised at 0.5 °C/min as a consequence of the higher entrainment levels in the ST cake collected during filtration. It was found that when higher cooling rates are used, the resultant crystals formed are smaller, similar to the results obtained by Marangoni et al. (2005). This in turn causes difficulty in carrying out the filtration process. It can be concluded that the amount of liquid entrained within the ST cake increases with increasing cooling rate and much of this liquid contains the more unsaturated TAG components.

5.4.2 Crystallisation driving force of TAGs

The crystallisation driving forces of TAGs during the non-isothermal crystallisation of palm oil were quantified similar to the method described earlier in the isothermal studies in Section 4.4.3 (see Chapter 4). Figure 5-21 illustrates the $\Delta \mu$ of palm oil TAGs in their $\beta'$ form as a function of temperature when palm oil was crystallised at 0.1 °C/min (see Appendix C.5 for similar plots of the crystallisation driving forces of palm oil TAGs at 0.3 °C/min and 0.5 °C/min).

At all cooling rates, SSS TAGs showed the highest $\Delta \mu$ among all the TAGs plotted at the start of each experiment. This could primarily be due to their higher pure component melting points which contribute to the higher $\Delta \mu$ of these TAGs (Himawan et al., 2006). TAGs with the highest $T_m$ showed the largest $\Delta \mu$ followed by all other TAGs in decreasing order of $T_m$. This suggests that the overall crystallisation driving force of a TAG decreases as the degree of unsaturation increases in the TAG, as shown by the lower driving force for crystallisation of the UUU TAGs compared to the SUU, SUS and SSS TAGs in that order. Hence, the pure component melting temperature plays an important contributing role in the crystallisation driving force of
a TAG as the degree of supercooling ($\Delta T$) is mainly a result of the difference between the melting temperature ($T_m$) of the TAG and the system temperature, $T$.

![Graph showing crystallisation driving force of palm oil TAGs in the $\beta'$ form versus temperature at 0.1 °C/min.](image)

**Figure 5-21** Crystallisation driving force of palm oil TAGs in the $\beta'$ form versus temperature at 0.1 °C/min

It can also be seen that at all cooling rates, the same decreasing trends of the $\Delta \mu$ of SSS TAGs is observed while the $\Delta \mu$ of TAGs other than SSS TAGs gradually increases as the crystallisation temperature decreases. At 0.1 °C/min, the driving forces of SSS TAGs initially increased during the first half of the experiment (may be due to their importance in the nucleation stage) but then started decreasing as the temperature decreased, as depicted in Figure 5-21. Their driving forces were then superseded by SUS TAGs at around 21 °C, indicating that the crystallisation of the SUS TAGs started to dominate once the SSS TAGs concentration started to deplete from the liquid phase.

When the cooling rate was increased to 0.3 °C/min, the $\Delta \mu$ of SUS TAGs, namely SOS and POS, surpassed the $\Delta \mu$ of PPP and PPS below 19 °C. A further increase in the cooling rate to 0.5 °C/min did not have any effect on the decreasing order of driving force of TAGs according to their $\Delta \mu$. Most notable is the driving forces of
PPP and MPP which do not show any significant change from the start. The driving forces of POS and SOS still show the same behaviour with a steady increase from nucleation and later surpass the driving forces of PPP and PPS at slightly below 20 °C. This shows that the $T_m$ of each TAG still plays an important role in determining their level of supercooling and thus $\Delta \mu$, regardless of the cooling rate employed.

### 5.5 CORRECTION CALCULATION FOR ENTRAINMENT

#### 5.5.1 Entrainment level calculation

The entrainment level for samples collected from the non-isothermal study was calculated using the same method employed for the isothermal study (see section 3.5.7 on the general method for entrainment calculation). Figure 5-22 shows the entrainment levels, uncorrected ST fraction and the corrected ST fraction calculated for ST samples collected at 0.1 °C/min. The most interesting observation is that the fraction of ST crystals in the uncorrected and corrected data showed similar values from the 4 th filtration point onwards. Recalculation of the corrected ST fraction using the quantified entrainment levels showed that the corrected ST fraction was about 30% at the beginning of the experiment and reached a maximum of 37% ST crystals which was similar to the uncorrected ST fraction. A possible reason for this is that at a low cooling rate, the crystals generated were probably very large in size (Kellens et al., 1992). This may have further facilitated the filtration stage. A reference to Figure 5-5 showed that the chord length distribution of crystals had a maximum size of 600 μm and the formation of large agglomerates at 0.1 °C/min was confirmed in Figure 5-8, further supporting this assumption. Hence, it could be deduced that when crystallisation takes place at a low cooling rate, less entrainment occurs due to the larger crystals produced which allows efficient separation of the liquid from the solid phase.
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Figure 5-22  Entrainment level, uncorrected ST and corrected ST fraction as a function of time at 0.1 °C/min

Figure 5-23  Entrainment level, uncorrected ST and corrected ST fraction as a function of time at 0.3 °C/min
Figure 5-24  Entrainment level, corrected ST and corrected ST fraction as a function of time at 0.5 °C/min

Figure 5-23 and Figure 5-24 show the entrainment levels, uncorrected ST and corrected ST fractions obtained when cooling rates of 0.3 °C/min and 0.5 °C/min were employed during the non-isothermal crystallisation of palm oil respectively. An increase in the cooling rate had no effect on the final entrainment level of the ST fractions collected at both of these cooling rates, which showed final ST fractions containing about 70% entrained liquid. There is a difference of 7% between the final corrected ST fraction at 0.1 °C/min and the ones collected at the two higher cooling rates, with the final ST fraction collected at 0.1 °C/min showing higher ST crystal content. This supports the earlier remark that an increase in cooling rate during palm oil crystallisation will result in an increase in the entrainment levels in the final ST cake. Smaller crystals are usually generated at higher cooling rates, causing difficulty in filtering the crystallising slurry and resulting in more liquid being retained on the filter paper, within and between crystal agglomerates. This then increases the amount of SUU and UUU TAGs in the ST fraction.
5.5.2 Corrected ST composition

The ST compositions at the various cooling rates studied were corrected for entrainment using the same method as for the isothermal studies. Figure 5-25 illustrates the corrected ST composition quantified at a cooling rate of 0.1 °C/min (see Appendix C.6 for the corrected ST compositions at 0.3 °C/min and 0.5 °C/min). Only the compositions of the 6 major SSS and SUS TAGs were plotted for comparison purposes as all other TAGs showed concentrations of less than 3%.

In general, the SSS TAGs are still seen to decrease in the ST while SUS TAGs increase as the crystallisation progresses at all cooling rates. The reduction in the amount of PPP in the corrected ST composition observed at 0.1 °C/min and 0.3 °C/min can be attributed to its depletion from the OL while the amount of POP keeps rising as it has not depleted in the OL (see Figure 5-12 for the OL composition at 0.1 °C/min). At 0.1 °C/min, the increasing concentration of POP surpassed that of PPP at the end of the experiment, as depicted in Figure 5-25. When the cooling rate was increased to 0.3 °C/min and 0.5 °C/min, this behaviour was no longer observed for
these two TAGs and the separation between them became larger at higher cooling rates. When higher cooling rates are employed, the larger degree of supercooling most probably had not allowed sufficient time for the SSS TAGs to crystallise out completely from the OL and more SSS TAGs are likely to be retained in the OL, as discussed earlier in Section 5.3.2.

The most prominent effect the cooling rate had on the corrected ST composition is the increased separation between TAGs with concentrations more than 5% at higher cooling rates. Conversely, there is less separation between TAGs with concentrations less than 5% which are seen to group together more closely as the cooling rate increases. Only the latter observation agrees with Himawan et al. (2007) who reported that increasing the cooling rate decreases the extent of separation of TAGs in a mixture. The reason for the former contradicting observation is unclear.

5.5.3 Partition coefficient of TAGs based on corrected ST composition

The partition coefficient of palm oil TAGs based on the corrected ST composition were determined similar to the method used for the uncorrected data. Figure 5-26 illustrates the $K_d$ values of major SSS and SUS TAGs in palm oil calculated based on the corrected ST composition at a cooling rate of 0.1 °C/min (see Appendix C.7 for similar plots at cooling rates of 0.3 °C/min and 0.5 °C/min). Only $K_d$ values of the TAGs that predominantly crystallise into the ST phase with concentrations above 2% are reported here.
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Figure 5-26 Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at 0.1 °C/min (based on corrected ST composition)

From these results, it can be seen that the same behaviour observed with the uncorrected ST data is observed here whereby all SSS TAGs showed $K_d$ values approaching zero at all cooling rates in study, indicating that they strongly partitioned into the ST phase regardless of the cooling rate employed. Figure 5-26 shows that the majority of SUS TAGs, i.e. POP, POS and SOS (except for PLP) showed preferential partitioning into the ST at lower cooling rates. PLP showed a higher affinity for the OL phase at all cooling rates as depicted by its $K_d$ values which were greater than 1 in all cases. This contradicts with the study reported by Deroanne et al. (1976) which suggested that PLP crystallises preferentially compared to POP due to the greater linearity (presence of two double bonds) in the linoleic acid chain. The reason for its contradicting behaviour in this study is unclear.
5.5.4 Crystallisation rates of TAGs based on corrected ST composition

The crystallisation rates of TAGs based on the corrected ST composition was calculated according to the method previously applied on the uncorrected ST composition. The percentage of conversion of each TAG into the ST phase, \( m_i^{ST} \), expressed as the mass of TAG in ST per 100 g crystallising palm oil as a function of time for 0.1 °C/min and 0.5 °C/min are illustrated in Figure 5-27 and Figure 5-28 respectively for TAGs with concentrations above 3% (mainly SSS and SUS TAGs) (see Appendix C.8 for a similar plot at 0.3 °C/min).

These results indicate that when the entrainment factor was incorporated into the calculation of the crystallisation rates of TAGs, the percentage of conversion of TAGs into the ST phase is dominated by PPP followed by POP, PPS, PLP, POS and MPP. This was primarily the main difference between the corrected and uncorrected ST composition whereby crystallisation rates from the uncorrected ST composition showed POP having the highest conversion instead. This observation is similar to that obtained in the previous isothermal studies in Chapter 4. At 0.1 °C/min, the crystallisation rate of PPP decreases to below that of POP at the end of the experiment, as shown in Figure 5-27, indicating ceasing driving force of PPP and its complete depletion from the liquid phase. This same behaviour however, was not observed when cooling rates of 0.3 °C/min and 0.5 °C/min were applied.

At lower cooling rates, for example, 0.1 °C/min (Figure 5-27), the conversion of TAGs into the ST phase is seen to occur more gradually compared to the much faster conversion of TAGs into the ST phase at higher cooling rates, i.e. 0.5 °C/min (Figure 5-28). This is evident when comparing the steepness of the slopes of curves at these two different cooling rates. The total counts curves as detected by FBRM (Figure 5-1 and Figure 5-2) also correlate well with this observation. Hence, the employment of higher cooling rates during the non-isothermal crystallisation of palm oil promotes faster transformation of TAGs from the OL phase to the ST phase.
Figure 5-27  Mass of TAG in ST per 100 g palm oil at 0.1 °C/min (based on corrected ST composition)

Figure 5-28  Mass of TAG in ST per 100 g palm oil at 0.5 °C/min (based on corrected ST composition)
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**Figure 5-29**  Mass of TAG in OL per 100 g palm oil at 0.1 °C/min (based on corrected OL fraction and corrected ST composition)

**Figure 5-30**  Mass of TAG in OL per 100 g palm oil at 0.1 °C/min – TAGs <5% (based on corrected OL fraction and corrected ST composition)
To further corroborate the assumption made for the entrainment calculation that UUU TAGs do not crystallise, the percentage of conversion of TAGs into the OL phase was determined at each cooling rate and plotted against time. Figure 5-29 and Figure 5-30 shows this trend for TAGs below and above 5% respectively when a cooling rate of 0.1 °C/min was applied (see Appendix C.8 for the same trends at 0.3 °C/min). It can be seen that the UUU TAGs showed fairly constant values throughout the constant cooling run at both cooling rates, suggesting that these TAGs do not crystallise into the ST phase but rather remain uncrystallised in the OL phase. The same behaviour is observed for SUU TAGs. This supports the earlier assumption in the proposed entrainment calculation method that the UUU TAGs (as well as the SUU) do not convert into the solid phase and that only the SSS and SUS TAGs crystallise at the conditions of the experiment.

5.5.5 Crystallisation driving forces of TAGs based on corrected ST composition

The crystallisation driving forces, $\Delta \mu$ of the TAGs that were confirmed to crystallise, namely SSS and SUS TAGs, were determined based on the corrected ST composition via the same method that was employed for the uncorrected data and were plotted against temperature. Figure 5-31 and Figure 5-32 shows the evolution of $\Delta \mu$ of these TAGs based on the corrected ST compositions at 0.1 °C/min and 0.5 °C/min respectively (see Appendix C.9 for the plot of the $\Delta \mu$ of TAGs based on the corrected ST composition at 0.3 °C/min).

At all cooling rates, the $\Delta \mu$ of SSS TAGs generally show decreasing trends as the temperature decreases. The $\Delta \mu$ of PPS is seen to decrease to zero with decreasing temperature regardless of the cooling rate employed while the $\Delta \mu$ of MPP similarly shows the same behaviour except at 0.5 °C/min. The $\Delta \mu$ of PPP only decreases to zero when a cooling rate of 0.1 °C/min was studied. This observation suggests that as the cooling rate increases, the exhaustion of the $\Delta \mu$ of SSS TAGs from the OL phase proceeds according to decreasing $T_m$. 

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Figure 5-31  Crystallisation driving force versus temperature for palm oil TAGs at 0.1 °C/min (based on corrected ST composition)

Figure 5-32  Crystallisation driving force versus temperature for palm oil TAGs at 0.5 °C/min (based on corrected ST composition)
It can also be observed that the decreasing $\Delta \mu$ of SSS TAGs are subsequently replaced by the $\Delta \mu$ of SUS TAGs that in general show a steady increase from the first filtration point until the end of the experiments. The SUS TAG bearing the highest $T_m$ will have the highest $\Delta \mu$ among this TAG group. In the previous section, it was shown that the crystallisation rates of PPP dropped below that of POP towards the end of experiments (e.g. Figure 5-27). This crossover matches well with the decrease in $\Delta \mu$ for PPP depicted in Figure 5-31. These results depict that quantification of the $\Delta \mu$ of TAGs using the corrected ST composition (composition which was corrected for entrainment) agrees well with the theoretical basis of thermodynamic $\Delta \mu$.

5.5.6 Conclusion from proposed entrainment calculation

Results from the previous section have shown that when the entrainment factor was used in correcting the ST fraction, the resultant corrected ST compositions depicted PPP having the highest composition compared to POP in uncorrected ST data, a similar observation with respect to isothermal studies. It was also shown that the overall amount of UUU and SUU TAGs within the OL remained constant throughout the experiment, providing an indication that they should always remain uncrystallised in the OL phase. When the crystallisation driving forces of TAGs were quantified using the corrected ST composition, it was found that the trend of SSS TAGs showed reducing driving force as the temperature decreases, which is in good agreement with the true thermodynamic behaviour of TAGs. Hence, this study substantiates the need to consider the entrainment factor when dealing with the TAG composition of the ST fractions obtained from the non-isothermal crystallisation of palm oil.
5.6 CONCLUSIONS

The influence of cooling rate variation on the onset of nucleation, crystallisation rates of TAGs and on the crystallisation driving force of TAGs during non-isothermal palm oil crystallisation has been studied. An increase in the cooling rate resulted in a decrease in the temperature of the onset of nucleation. The crystal chord sizes were generally lower at higher cooling rates due to the higher supercooling employed. Low cooling rates were seen to promote crystal growth relative to nucleation due to the lower degree of supercooling. However, subsequent attrition of these TAG crystals were thought to have occurred due to the high viscosity in the crystallising slurry which enhances crystal-crystal and crystal-agitator collision frequency, further causing breakage of crystal fragments and resulting in the birth of secondary nuclei. At higher cooling rates (>0.1 °C/min), it was shown that agglomeration of crystals was enhanced which, from the FBRM data, was indicated by a decrease in the fine particles with a simultaneous increase in the coarse particles.

A compositional analysis on the filtered OL and ST fractions obtained from this study showed that the SSS TAGs depleted entirely from the OL at lower cooling rates. The reason more SSS TAGs were retained in the OL at higher cooling rates was thought to be either due to the much smaller crystals produced at higher cooling rates which prohibits their retention on the filter paper during filtration, thus causing them to pass through the filter paper, or that the crystallisation process was progressing too rapidly for all the SSS TAGs to be efficiently crystallised out of the OL.

The crystallisation rates of individual TAGs in the OL and ST have been evaluated and determined. A study on the crystallisation rates of TAGs showed that SUS TAGs (i.e. POP) showed the highest crystallisation rate into the ST followed by POO, similar to results obtained from the previous isothermal study. In general, increasing the cooling rate concomitantly increased the crystallisation rates of TAGs as evident by the steeper concentration curves observed at higher cooling rates. Conversely, the crystallisation process was seen to take place more gradually at lower cooling rates due to the lower rate of increasing supercooling applied.
An estimation of the crystallisation driving forces of individual TAGs in their β' forms have shown that the variation of cooling rate on palm oil crystallisation had no significant effect on the sequence of the Δμ at different cooling rates and that the overall Δμ of TAGs decreased with decreasing T_m. This implies that the Δμ for each TAG during non-isothermal palm oil crystallisation is highly influenced by the degree of supercooling, ΔT and thus the T_m, which is generally the difference between the operating T and the T_m of the individual TAGs. A look at the effect of cooling rate on the crystallisation Δμ of TAGs have shown that the of SSS TAGs experienced a decrease towards the end while the Δμ of all other TAGs increased steadily from the start at all cooling rates. At some point in time, the Δμ of SSS TAGs, namely PPS and MPP are overtaken by the Δμ of POS and SOS, suggesting the depletion of PPS and MPP from the liquid phase in the crystallising slurry.

An estimation of the entrainment levels in the samples obtained from the non-isothermal crystallisation of palm oil indicated that the entrainment level increases with increasing cooling rate. At a lower cooling rate, i.e. 0.1 °C/min, it was found that there was no significant difference between the old ST fraction and corrected ST fraction calculated from the proposed entrainment calculation method. This implies that the larger crystals generated at lower cooling rates reduced the amount of liquid entrainment by enhancing the filterability of crystals which, at higher cooling rates caused much of the liquid to be retained within the ST cake.

A further examination of the corrected ST composition determined using the calculated entrainment levels interestingly showed that the SSS TAGs primarily had the highest concentration in the ST followed by the SUS TAGs. The SUU and UUU TAGs showed unchanging concentration in the OL fraction, further supporting the assumption that these TAGs do not crystallise into the ST fraction at all and that only the SSS and SUS TAGs converted into the solid phase. It has also been shown that the separation between TAGs with concentrations higher than 5% prevails at higher cooling rates and vice versa for TAGs with concentrations less than 5%.

The quantification of the crystallisation Δμ of SSS and SUS TAGs based on their corrected ST composition revealed that the Δμ of all SSS TAGs showed decreasing Δμ which reduced to zero at the end of experiments at lower cooling rates (0.1...
°C/min), which corresponds to their complete depletion from the OL. The reduction of the $\Delta \mu$ of SSS TAGs is seen to occur according to decreasing $T_m$ as the cooling rate increases. At lower cooling rates (0.1 °C/min and 0.3 °C/min), as the $\Delta \mu$ of SSS TAGs decreases to zero, their $\Delta \mu$ are completely succeeded by all the SUS TAGs. This behaviour follows the expected thermodynamic behaviour of TAGs with respect to their crystallisation driving force, further affirming that the entrainment factor is a highly important factor that needs to be considered when studying the true behaviour of TAGs during crystallisation of palm oil.
6 REMELTING STUDIES ON PALM OIL

6.1 INTRODUCTION

This chapter shall present the results from a study on the post-crystallisation stepwise remelting of palm oil. This study was conducted in order to investigate the melting and partitioning behaviour of palm oil TAGs when palm oil is subjected to successive remelting steps following the isothermal crystallisation at a predetermined temperature. This chapter shall start with the presentation of the effect of the stepwise remelting process on the particle population and mean particle size obtained from the FBRM monitoring device. The change in the filtration product yields, TAG composition (partitioning behaviour) by HPLC and TAG crystallisation rates shall be discussed in detail thereafter followed by the assessment of the level of entrainment in the collected samples and its effect on the sample filterability.

6.2 EXPERIMENT PERFORMED

The experimental methods for conducting this study were previously described in Chapter 3. Sample preparation was carried out using the same procedure as with the isothermal and non-isothermal study, according to subsection 3.2.1. The post-crystallisation stepwise remelting experiment was conducted according to the method described in Section 3.2.5. This was essentially an isothermal hold at 24 °C followed by stepwise increases of 2 °C every 30 minutes. The particle population and size as well as multiple events occurring within the system were monitored using the FBRM. Filtered samples were weighed and subjected to TAG compositional analysis by HPLC as described in Section 3.4.
6.3 EXPERIMENTAL RESULTS AND DISCUSSION

6.3.1 FBRM response

Figure 6-1 illustrates the oil temperature \( T_{oil} \), setpoint temperature \( T_{sp} \) and total particle counts profile as a function of time obtained from the experiment on the post-crystallisation stepwise remelting study of palm oil. When the palm oil was initially crystallised at an isothermal temperature \( (T_i) \) of 24 °C, the total particle counts profile showed a similar trend comparable to the trend observed earlier in the isothermal experiments (see Figure 4-1 in Chapter 4), indicating the reproducibility of the FBRM response. As the \( T_0 \); \( t \) is increased in a stepwise manner from 24 °C to 30 °C, it can be seen that the total particle counts gradually started to decrease in number. A further comparison between the fine and coarse particle counts, as depicted in Figure 6-2 shows a steady decrease in the fine particles with a corresponding increase in the coarse particles, suggesting the likelihood of crystal agglomeration (or possibly ripening) slowly taking place as the temperature is raised. The square-weighted mean chord length plot further corroborates this, showing a slight increase in the particle size during this period, as shown in Figure 6-3.

An interesting observation when the \( T_1 \) was further increased successively from 30 °C to 40 °C is a repeating trend involving a decrease and subsequent increase in the total particle counts trend with every 2 °C increase in \( T_1 \), indicated by a sudden dip during the temperature increase followed by a steady rise during the isothermal hold (Figure 6-1). This probably suggests the melting or dissolution of certain components within the slurry followed by a nucleation of crystals taking place. An examination of the fine and coarse particle counts profile (Figure 6-2) when the \( T_1 \) is raised shows the effect is more pronounced with the fine particles. The trend of the coarse particle counts is steadily increasing up to 34 °C. An examination of the square-weighted mean chord length of crystals plotted together with the fine and coarse particle counts profiles shows that the dips in the particle counts correspond to an increase in the mean chord length of particles, depicted by the peaks observed at these particular moments in time. These observations imply that a recurring agglomeration-segregation process was taking place within the oil bulk in this temperature range.
Chapter 6  Remelting studies on palm oil

Figure 6-1  Temperature and total counts profile for the post-crystallisation remelting experiment on palm oil

Figure 6-2  Fine and coarse particle counts as a function of time during the post-crystallisation stepwise remelting study on palm oil
Chapter 6 Remelting studies on palm oil

Figure 6-3 Square-weighted mean chord length, coarse and fine particle counts profile as a function of time during the post-crystallisation remelting experiment on palm oil

Figure 6-4 Yield (%) of filtration products obtained throughout the experiment
A possible explanation for this is that as $T_i$ increases to 40 °C, a deagglomeration of the crystals formed earlier may have taken place. This may be attributed to the melting of the crystalline bridge that binds crystals together in the agglomerates as the temperature is successively raised, giving a rise to the number of fines with a corresponding drop in the number of coarse particles. There may also be a sudden melting of fines superimposed on top of this when the temperature is increased. An examination of the filtration product yields in Figure 6-4 reveals that the yield of the ST fraction starts to increase from 34 °C, reaching a maximum at 38 °C where there was more ST than OL collected at this temperature. This suggests that there was a possibility that the fine crystals may have clogged up the filter paper, resulting in more liquid being retained within the ST cake and further increasing the yield of the ST fraction.

The difference between the yields at 34 °C and 38 °C is primarily due to the increasing entrainment of the liquid within the solid crystals, whereby the filtration stage became difficult starting at 36 °C due to the presence of very fine crystals in thick slurry. A wet ST was produced from the filtration at 36 °C, with the OL fraction composed of a mixture of uncrystallised liquid and ST crystals that had seeped through the filter paper. The same difficulty in filtration was also encountered at 38 °C where the sample consisted of thick slurry of undistinguishable tiny crystals. It is also worth noting that the filtration stage may also be greatly affected by the room temperature, as this is much lower than the temperature of the slurry in the crystalliser. Hence, there may have also been some recrystallisation of the TAG components occurring as the samples were vacuum filtered at room temperature.

The fluctuating trend in the total counts profile mentioned earlier is observable until the $T_i$ reaches 40 °C, the temperature at which a sudden decrease in the total counts occurs followed by a very sharp increase to about 60000 counts when the temperature was raised to 42 °C. An immediate decrease subsequently follows this pinnacle, where the total counts then plummet to around 26000 counts. A further inspection of the fine and coarse particle counts profiles depicts that a simultaneous sharp decrease in the coarse particles is seen to occur concomitantly with the sharp increase in fine particles, as shown in Figure 6-2. This is also confirmed by the square-weighted mean chord length of particles in Figure 6-3 which show a simultaneous drop in the crystal
mean chord length from 300 µm to approximately 120 µm. This strongly points to a breaking apart of the coarse crystals into small crystal fragments from the melting of bridges between these elements. At 40 °C, most of the filtered slurry seeped through the filter paper as OL, leaving a semi-dry ST as the retentate and further yielding nearly 85% of OL, as depicted in Figure 6-4.

From Figure 6-1, it can be seen that as \( T \) is increased further from 42 °C to 44 °C, the number of particles continues to decrease to 17600 counts before abruptly increasing again to slightly over 28500 counts. The fine particle counts also depict this sudden rise at 44 °C, as shown in Figure 6-2. A possible reason for the sudden increase in the total counts and fine crystals at 44 °C could be due to a deagglomeration of crystals occurring at this stage.

At 42 °C and 44 °C, the majority of crystals passed through the filter paper, indicating the diminutive size of particles within the slurry at these two temperatures, bringing the ST fraction yield down further to between 13% and 21%. When a temperature of 46 °C was reached, the total particle counts finally plunged to a minimum (near zero) (see Figure 6-1), serving as an indication that the oil had melted thoroughly at this temperature and that the tiniest of particulates have finally dissolved into the liquid phase. Filtration of the oil at 46 °C resulted in the entire sample passing into the filter paper and no retentate being collected. This is confirmed by the yield of the OL and ST collected at this final temperature, with the yield of the former reaching almost 100% and vice versa. The 2% yield portrayed as the ST fraction was actually the amount of OL absorbed by the filter paper and no crystals were retained by the filter paper at this temperature.

Although it can be postulated that the very low total particle counts at 46 °C may signify that there were no longer any crystal matter present in the oil bulk, a visual observation of the oil bulk showed a small degree of cloudiness throughout. These are thought to be very small crystals (probably SSS) remaining in the liquid which are below the detection limit of the FBRM (0.8 µm). Raising the temperature to 70 °C removed this cloudiness resulting in a fully melted sample.
A repeat of this experiment also showed fairly consistent trends in the FBRM response with characteristic dips and increases in the fines and coarse particle counts resulting from each step increase in temperature.

### 6.3.2 Measured HPLC composition

The composition of TAG components in the filtered OL and ST fractions collected at each temperature were analysed using HPLC according to the method outlined in subsection 3.4.3 (Chapter 3). Analyses of ST fractions were performed only on samples up to 44 °C as no ST fraction was collected at 46 °C (as no crystals were retained). Figure 6-5 illustrates the composition of all TAGs in the OL fraction from samples obtained during the post-crystallisation stepwise remelting study of palm oil while Figure 6-6 shows the same for TAGs with concentrations below 5%. The most significant TAG behaviour observed is the enrichment of the SSS TAGs in the OL fraction as the temperature is increased, with PPP experiencing the most substantial increase from 0.4 wt% at 24 °C to about 4.5 wt% at a temperature of 44 °C, as shown in Figure 6-6. There was not much variation in the concentration of the SUS TAGs in the OL as the temperature is increased, while there was a very slight decrease in the concentrations of all SUU and UUU TAGs. It is also noteworthy that a comparison between the ST composition obtained from the isothermal experiment at 24 °C (Figure 4-6) in Chapter 4 and those obtained from this remelting experiment at 24 °C show broadly similar values, indicating the reproducibility of the experiments.

It can be assumed that the enrichment of SSS TAGs in the OL fraction as the system temperature increases may have been due to their gradual melting with every 2 °C increase in $T_i$ since a temperature increase would definitely trigger the melting of some TAGs. However, it is uncertain whether this may be ruled out for the SSS TAGs as their higher melting temperatures would actually have allowed them to remain in a crystallised form at the temperatures in study (refer to Table 2-9 in subsection 2.6.1 of Chapter 2). A look at the concentration of these TAGs in the ST fraction as depicted in Figure 6-7 and Figure 6-8 revealed that there were no significant changes in their concentrations which showed a very slight decrease from 24 °C to 34 °C. Possible reasons for this behaviour could be due to: (i) decreasing size of ST crystals as the
temperature is increased, which is due to the melting of the outer layer of crystals consisting of SUS TAGs (melting temperatures between 30 °C and 40 °C) causing the SSS TAGs, which form the inner structure of crystals, to pass through the filter paper together with uncrystallised TAGs in the OL, (ii) increasing amount of SUU and UUU TAGs in the ST due to entrainment or (iii) the intersolubility effect of higher melting TAGs in lower melting TAGs whereby the lower melting TAGs may have melted first as the temperature increased and this would probably have solubilised the higher melting TAGs, since lower melting TAGs can act as a solvent to higher melting TAGs (Zhou & Hartel, 2006; Himawan et al., 2006).

From the plots of the TAG compositions in the ST fraction in Figure 6-7 and Figure 6-8, it is clear that SUS TAGs are generally seen to decrease gradually whereas the concentrations of SUU and UUU TAGs do not change much throughout the experiment, only slightly increasing at 36 °C. Correspondingly, there is a sudden decrease in the SSS TAGs in the ST fraction at 36 °C, owing to the fact that the OL fraction collected at this point was composed of a mixture of liquid and ST crystals that had seeped through the filter paper and that most probably a large portion of the SSS TAGs had also passed through together with the OL phase (see discussion in previous section). Another possible explanation for this is the higher entrainment of OL observed at 36 °C as depicted in Figure 6-4. This may have caused the dip in SSS TAGs at this temperature from the dilution of these TAGs.

A further increase in the temperature of the system from 36 °C to 46 °C resulted in the concentration of the SSS TAGs in the ST to increase, with PPP experiencing the highest increase in concentration to around 20 wt%, surpassing the concentration of POO at 42 °C and decreasing slightly at 44 °C, as depicted in Figure 6-7. PPS also showed the same trend in increasing concentrations which, towards the end, were similar to those of POS, as shown in Figure 6-8. MPP did not show any significant increase and its concentration more or less showed a final value that was the same as its initial value at the start of the experiment. The amount of SUS TAGs, namely POS however, continued decreasing in amount in the ST towards the end of the experiment and this could mainly be attributed to the gradual melting of this TAG group as the temperature increases.
Figure 6-5  Raw TAG composition of OL fraction as a function of temperature during the post-crystallisation stepwise remelting of palm oil

Figure 6-6  Raw TAG composition of OL fraction as a function of temperature during the post-crystallisation stepwise remelting of palm oil (TAGs<5%)
Figure 6-7  Raw TAG composition of ST fraction as a function of temperature during the post-crystallisation stepwise remelting of palm oil

Figure 6-8  Raw TAG composition of ST fraction as a function of temperature during the post-crystallisation stepwise remelting of palm oil (TAGs<5%)
An interesting further observation is the sudden decrease in the SSS TAGs in the OL fraction at 38 °C and 44 °C shown in Figure 6-6. This coincides with an increase in the total counts and fines at these two temperatures (although not the dramatic increase at 40 °C) as depicted earlier in Figure 6-1 and Figure 6-2. The yields of the ST fraction at these two temperatures also showed a corresponding increase. This may further support our earlier assumption that a nucleation and/or deagglomeration event was likely to have taken place during these two periods. Shi et al (2005) reported that fat crystal networks consisted of high-melting lipid crystalline particles linked by lower-melting lipid bridges. Hence, it could be that during this period, the lower melting TAGs which presumably formed bridges connecting the higher melting TAG crystals together in palm oil agglomerates were slowly melting as the temperature was raised.

An explanation for the event that took place at 38 °C can be explained as follows. As the temperature approached 38 °C, most of the SUS TAGs will have already progressively melted as their melting points lie between 30 °C and 38 °C (see Table 2-9 in subsection 2.6.1 of Chapter 2). This melting event would eventually have reduced the size of the existing crystals due to deagglomeration caused by the melting of the bridges (lower melting TAGs, i.e. SUS) of the crystal agglomerates, further increasing the number of fine particles at 38 °C (Figure 6-2). Since SSS TAGs would presumably be among the first TAG components to crystallise in a palm oil system due to their higher $T_m$, further melting of the outer layer of these crystals which presumably consisted of SUS TAGs would have left behind the internal structure (nucleus) of the crystals which presumably consisted of SSS TAGs. The fact that the filtration stage was performed at room temperature may have also caused these TAGs to nucleate again and recrystallise. Coupling these two events resulted in a mass production of very fine particles, which clogged up the filter paper and subsequently increased the ST yield (Figure 6-4). The same explanation can be applied at 44 °C, although emphasis should be made on the recrystallisation of the SSS TAGs as the yield of ST prior to this point was already very low (~13 to 15%).

A convenient way to study the behaviour of TAGs during the post-crystallisation stepwise remelting of palm oil is to follow the changes in the cumulative masses of each TAG in the ST with change in temperature. These were calculated in a similar
method to that used in the isothermal and non-isothermal crystallisation studies on palm oil, i.e. using the method described in Section 3.5.2. The cumulative masses of TAGs in the ST fraction, expressed in grams of TAG in ST per grams of total TAG are plotted against temperature in Figure 6-9.

![Figure 6-9 Cumulative mass of TAGs in the ST fraction during the post-crystallisation stepwise remelting of palm oil](image)

A general and notable observation from Figure 6-9 is that the proportion of SSS TAGs in the ST is higher (>75%) than the other TAGs (<35%). The large separation between these two TAG groups could be ascribed to the higher melting points of the SSS TAGs which cause them to crystallise preferentially and the rest of the TAGs being grouped together at lower concentrations indicate their co-crystallisation behaviour. At the beginning of the experiment at 24 °C, it is clear that nearly all of the SSS TAGs are accumulated in the ST as depicted by their higher concentrations ranging between 0.75 and 0.94. As the temperature increases from 24 °C to 34 °C, the amount of these TAGs in the ST began to decrease gradually while all other TAGs showed broadly unchanging behaviour. The decline of the SSS TAGs from the ST may either be due to the decreasing size of crystals which cause the crystals to pass
through the filter media or the solubilisation of these TAGs into the OL phase by the lower melting TAGs (a Hildebrand effect) (Zhou et al., 2006).

A further increase in temperature to 36 °C resulted in the cumulative amount of SSS TAGs still decreasing while the other TAGs showed increasing accumulation in the ST, with both TAG groups increasing suddenly at 38 °C. This is mainly due to the high level of entrainment in the retentate collected at this temperature as a result of the large amount of tiny crystals produced (see similar discussion in Section 6.3.1), producing a high amount of ST collected (Figure 6-4). These tiny crystals may have clogged the filter paper, further complicating the filtration process and entrapping most of the OL and thus lower melting TAGs, within the ST crystals.

Above 38 °C, all TAGs showed decreasing amounts in the ST until around 42 °C, the temperature where all TAGs showed a sudden increase again in the ST, reaching another peak at 44 °C. Here, the earlier separation between the SSS TAGs with the rest of the other TAGs is apparent again. Again, the possible explanation for this could be due to the recrystallisation of the TAGs during the filtration stage at room temperature and clogging of the filter paper. Even though every attempt was made to keep the time between sampling and filtration step to a minimum, the difficulty in filtering the slurry had eventually prolonged the filtration time. Since the room temperature was below the temperature of the slurry at the time of sampling, this may have further caused some of the TAGs that were already melted to recrystallise into the ST fraction again. When the crystalliser temperature was finally increased to 46 °C, no ST fraction was collected and the entire sampled solution passed through the filter paper as OL. This is reflected in the cumulative masses of all TAGs which drop to zero at the end of the experiment (Figure 6-9).
6.4 FURTHER ANALYSIS

6.4.1 Cumulative crystallisation masses of TAGs

The rate of conversion of TAGs into the solid and liquid phases can be monitored by following the cumulative crystallisation masses of TAG in either phase as the crystallisation process progresses. In this study, this value was calculated using the same method employed in the previous two chapters using the method described in section 3.5.2 (see Chapter 3). Figure 6-10 and Figure 6-11 depicts the conversion of TAGs into the ST and OL phases, expressed in grams of TAG in ST or OL per 100g starting palm oil, respectively.

It can be seen from Figure 6-10 that in general, all TAGs show fairly the same trend throughout the experiment, bearing a similar curve shape to the yield of the ST (see Figure 6-4), with POP having a higher conversion in the solid phase followed by POO, PPP, POL, PLP and the rest of the other TAGs. The amount of all TAGs in the ST fraction between 24 °C to 34 °C does not change much, except for the slight decrease in SSS and SUS TAGs. The only significant behaviour observed is the sudden increase in the crystallisation masses of TAGs which is apparent at 38 °C and this is mainly due to the higher entrainment and thus higher yield of ST fraction collected at this point.

The cumulative masses of TAGs in the OL fraction show the reverse trend of the ST fraction, as depicted by Figure 6-11. In theory, adding the two sets of curves should give constant values corresponding to the overall composition of palm oil. There is also a substantial decrease in the cumulative crystallised mass of the TAGs in the OL fraction at 38 °C which corresponds well with their increase in the ST fraction at this point. Above this temperature, all masses of TAGs generally increase in the OL fraction due to their gradual melting from the ST phase. At the end of the experiment at 46 °C, it was found that the cumulative mass of TAGs in the OL phase is comparable to that of the starting palm oil (results not reported here).
Figure 6-10  Mass of TAG in ST per 100g palm oil during the post-crystallisation stepwise remelting of palm oil

Figure 6-11  Mass of TAG in OL per 100g palm oil during the post-crystallisation stepwise remelting of palm oil
6.5 CORRECTION CALCULATION FOR ENTRAINMENT

6.5.1 Entrainment level calculation

The entrainment correction calculations for samples obtained from the post-crystallisation stepwise remelting of palm oil was performed using the method applied to the isothermal and non-isothermal crystallisation of palm oil. Entrainment levels were calculated according to the method described earlier in section 3.5.7 (see Chapter 3) based on the assumption that UUU TAGs do not crystallise at the temperatures used in this study. Figure 6-12 depicts the calculated entrainment levels, uncorrected ST fraction, uncorrected OL fraction and the corrected ST fraction (after entrainment correction) of the collected samples as a function of temperature. Entrainment levels are expressed as the percentage of the ratio of the entrained OL in the ST cake (EN/ST).

![Figure 6-12 Entrainment level, uncorrected ST, uncorrected OL and corrected ST fraction as a function of temperature during the post-crystallisation stepwise remelting of palm oil](image_url)

Partitioning of triacylglycerols in the fractional crystallisation of palm oil
From Figure 6-12, it was found that the entrainment levels in the ST cake between 24 °C and 30 °C were rather similar to the yield of the uncorrected OL fraction, accounting to about 70% of the weight of the ST cake. These results show very similar values to the entrainment levels calculated in the isothermal crystallisation study of palm oil which was discussed earlier in Chapter 4 (see section 4.5.2), demonstrating the reproducibility of the crystallisation of palm oil at these temperatures. The important observation on the equal amount of entrainment levels and yields of the uncorrected OL fraction between 24 °C and 30 °C implies that about half of the liquid fraction (if entrainment had not occurred) was retained as entrapped liquid within the ST cake. A comparison between the uncorrected ST fraction and the corrected ST fraction shows a difference of about 20% between them. This suggests that only 11% of the palm oil is crystallising and the remaining 20% is contributed by the amount of entrained liquid within the ST.

It can be seen also from Figure 6-12 that the entrainment level gradually increased as the system temperature was increased further from 30 °C to 36 °C, reaching a maximum of 85% entrainment at 36 °C. This was probably due to the decreasing crystals size observed at these temperatures which prevented efficient separation of the liquid phase from the solid phase of the slurry as discussed earlier in the previous section. The increasing entrainment level here is also supported by the trend of the uncorrected ST yield which is shown to gradually increase as well, signifying that more liquid was being retained in the ST cake.

Above 38 °C, the entrainment level is seen to decline steadily to about 70% until a temperature of 42 °C is reached. This reduction could be due to the fact that large portions of the sampled slurry, mainly composed of very fine crystals, were beginning to pass through the filter paper, thereby increasing the original yield of OL again as depicted by the solid black line with non-filled circles. At 44 °C, the entrainment level in the ST cake increased briefly by as much as 5% and as discussed earlier in the previous section, this increase may be due to the recrystallisation of some of the already melted slurry during the filtration stage. When the oil temperature reached 46 °C, the entrainment level dropped to zero and this was the result of the entire sample of the palm oil slurry permeating through the filter paper, indicating that the majority of the oil bulk had melted.
6.5.2 Corrected ST composition

The corrected ST composition of samples obtained from the post-crystallisation stepwise remelting study on palm oil was quantified using the same method as for the isothermal and non-isothermal crystallisation studies on palm oil. This was performed by incorporating the entrainment levels calculated in the previous subsection into equation (3-18) in subsection 3.5.7 (see Chapter 3). Figure 6-13 illustrates the corrected ST composition for the TAGs with concentrations above 2% in the ST as a function of temperature for the entire duration of the experiment. All other TAGs showed concentrations less than 2% and are thus not reported here.

Figure 6-13 Corrected ST composition as a function of temperature during the post-crystallisation stepwise remelting of palm oil

A comparison between the corrected ST composition and the uncorrected ST composition (see Figure 6-7 and Figure 6-8) shows that the most noticeable difference is that the TAG with the highest concentration in the former is PPP followed by POP, PPS and the rest of the other TAGs whereas in the latter, the order of TAGs was POP, POO, PPP followed by the concentrations of all other TAGs in decreasing concentrations. These results indicate that if the entrainment factor was taken into
account when dealing with the uncorrected ST composition, results would always show the TAG with the higher \( T_m \), i.e. PPP crystallising in higher amounts compared to POP and POO. Another noteworthy observation is that the percentages of SSS TAGs in the corrected ST fraction trebled compared to the uncorrected data reported earlier in subsection 6.3.2 (see Figure 6-7 and Figure 6-8).

From Figure 6-13, the concentrations of SSS TAGs in the corrected ST fraction are seen to remain constant within the temperature range of 24 °C to 30 °C. However as the temperature was increased further to 34 °C, their concentrations in the corrected ST fraction rose to more than five times the amount in the uncorrected data, with PPP forming up to about 80% of the total corrected ST composition. POP and POO showed a simultaneous decrease during this period, dropping to concentrations below zero at 34 °C. This observation suggests that the calculation method for entrainment is not entirely perfect as negative concentrations are not physical. Hence, further improvements in the entrainment correction procedure may be required to fix this problem.

At temperatures higher than 36 °C, the concentrations of PPP and POP are seen to increase with the rise in temperature, suggesting the possibility of enriching these TAGs in the ST fraction further at higher temperatures. PPS, MPP and PLP did not show any significant change in their concentrations while POS and POO showed concentrations dropping to below zero at 44 °C. An unusual behaviour is the presence of POO in the corrected ST fraction at all temperatures except 34 °C in significant amounts. The fact that POO’s \( T_m \) of 19 °C lies way below all the temperatures used in this study contradicts the behaviour shown in Figure 6-13 as this would mean that it ought to have remained largely uncrystallised in the OL. Its existence in the ST fraction is most likely due to an intersolubility effect with certain TAGs where co-crystallisation occurs and allows POO to be retained in the ST as well. This can be confirmed from Figure 2-22 (see Section 2.6.2 in Chapter 2) where solid PPP takes about 85% of POO into solid solution (Wesdorp et al., 2005).
6.5.3 Cumulative crystallised masses of TAGs based on corrected ST composition

The cumulative crystallised masses of TAGs in the corrected OL and ST fractions based on the corrected ST composition were calculated in the same manner as for the uncorrected data reported earlier in subsection 6.4.1. Figure 6-14 illustrates this for TAGs with concentrations above 2 wt% in the uncorrected ST fraction, as a function of temperature while Figure 6-15 and Figure 6-16 illustrates the same for all TAGs and TAGs with concentrations less than 2% in the corrected OL fraction, respectively. The corrected OL and ST fractions in this case refer to the OL and ST fractions that have been corrected for entrainment, hence giving the pure fractions of both.

![Figure 6-14](image_url)  
**Figure 6-14**  Mass of TAG in ST per 100g palm oil (based on corrected ST composition) during the post-crystallisation stepwise remelting of palm oil
Figure 6-15  Mass of TAG in OL per 100g palm oil (based on corrected ST composition) during the post-crystallisation stepwise remelting of palm oil

Figure 6-16  Mass of TAG in OL per 100g palm oil – TAGs <5% (based on corrected ST composition) during the post-crystallisation stepwise remelting of palm oil
Based on the results in Figure 6-14, it can be seen that PPP showed the highest conversion into the solid phase followed by POP, PPS, PLP, POS and MPP. These results differ greatly from the results obtained when the uncorrected ST composition was used where POP showed the highest conversion followed by POO, PPP and all other TAGs as illustrated earlier in Figure 6-10. The conversion of SSS TAGs into the ST phase is seen to decrease gradually with increasing temperature, only briefly increasing at 38 °C and 44 °C. Although the ST composition was already corrected for entrainment, this trend shows some similarity with the trend observed from the uncorrected data (Figure 6-14). This probably indicates that the entrainment correction method is not perfect and requires more improvement.

On the other hand, the cumulative masses of TAGs in the OL based on the corrected ST composition as shown in Figure 6-15 reveals that the trends of the SUU and UUU TAGs remain fairly constant throughout the experiment, suggesting that these TAGs barely crystallise at all at the conditions of the experiment and remain uncrystallised in the OL. This behaviour was not observed in the uncorrected data (Figure 6-11) which showed that the trends in the cumulative masses of TAGs followed the same trend as the OL yield (Figure 6-4). Apart from that, only the SSS and certain SUS TAGs, i.e. POP and POS show a substantial conversion or melt into the OL phase as the temperature increases as shown in Figure 6-16. This in a way indicates that the majority of the ST fraction would actually only consist of these TAGs.

6.6 CONCLUSIONS

A study on the post-crystallisation stepwise remelting of palm oil was conducted in order to gain insight into the state of palm oil crystals after an isothermal hold at 24 °C was performed. A variety of data are available to aid in the interpretation of the results from this study i.e. the focused beam reflectance measurement (FBRM) response, high performance liquid chromatography (HPLC) composition of OL and ST and the yields of the filtration products. Of these, only the OL composition data is unlikely to be affected by entrainment which previous chapters have indicated to be a
large effect. The data can be corrected for entrainment by assuming that no UUU are present in the solid, as carried out in previous chapters.

Results from this study have shown that various complexities (different processes) were possibly occurring. That more than one process is occurring at any one time makes the interpretation of the data difficult. Some of the complexities observed were: (i) the straight melting of crystals which reduces the crystal mass but may not necessarily impart upon crystal size in a direct manner if melting occurs "within" a crystal, (ii) deagglomeration of crystals to form small crystals was also observed to occur, which was assumed to be caused by the melting of liquid bridges that bind together the individual crystals within agglomerates, (iii) secondary nucleation as a result from agitator effects, (iv) agglomeration of crystals (as suggested by FBRM), (v) crystal ripening, (vi) variations in filtration efficiency from small crystals blocking the filter paper and (vii) the possibility that small crystals can pass through the filter paper. Interpretations of the results can also be made by looking at the background theory of phase behaviour of fats.

The OL composition data shows unchanging behaviour from all TAGs except SSS which all increase in OL significantly over the temperature range (Figure 6-6). There is also a reduction of the SSS in the ST (Figure 6-9). Both of these observations suggest that the melting of the SSS TAGs is the major process occurring within the oil bulk. The entrainment calculation mutually supports this by showing that the solid fraction is actually very small (11% and decreasing) but is dominated (~50% or greater) by PPP and PPS so that they are the only compounds that melting significantly affects. POP has been shown to be the only other TAGs to crystallise to any significant degree. However it is already present in large quantities in the OL, hence when the temperature is raised, the melting effect on OL is not large. PPP and PPS do gradually dissolve into the OL phase as the temperature is raised following the Hildebrand behaviour of TAGs.

There are also various blips that are seen within the data which are directly a contribution of experimental error from the study. Some negative concentration values observed in the corrected ST composition at 34 °C suggest that the correction
method has its flaws and is not entirely perfect. The fact that there are variations in the filtration efficiency further complicates this matter.

Data from the FBRM have shown that this is not a complete match with the HPLC and yield data. This might be due to the fact that melting can occur from within a crystal which may not impart much variation on the mean chord sizes of crystals. The increases in temperature causes an initial reduction in the total particle counts (melting) and this is followed by a restoration process (deagglomeration or secondary nucleation) which is more apparent for the fine particles. The increase in temperature to 42 °C causes a whole scale reduction in the coarse particle counts accompanied initially by an increase in the fine particles. This suggests that the melting process occurs by large crystals falling apart as the "glue" melts away to produce small crystals. Many of these smaller crystals however then rapidly disappear. It was also observed that melting of palm oil is largely complete by 46 °C but the oil bulk still showed some cloudiness, indicating the presence of small seed crystals which are presumably dominated by SSS TAGs.
7 CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

7.1 CONCLUSIONS

The crystallisation process plays a pivotal role in the fractionation of oils and fats since proper control of the crystallisation stage is crucial to obtain end products with desired physical and chemical characteristics. Palm oil has been established as the most extensively industrially fractionated vegetable oil and the resultant separated liquid olein (OL) and solid stearin (ST) fractions have long served as major feedstock for the production of a variety of food and non-food produce alike. A crucial understanding of the crystallisation behaviour of the components that constitute the bulk of this oil, i.e. triacylglycerols (TAGs) is therefore imperative in order to facilitate better control of the crystallisation conditions in the operation of palm oil fractionation. The fractionation process in palm oil has been widely researched and theoretical concepts in the thermodynamics and kinetics of fat crystallisation in general have long been established, however a review of current literature in this field (Chapter 2) suggested that there are limited studies hitherto pertaining to the fundamental thermodynamic driving force (Δμ) for crystallisation. Thus, the principal aim of this research was generally to investigate the partitioning behaviour of TAGs during the crystallisation stage of palm oil fractionation and how this relates to their thermodynamic driving force for crystallisation based upon the differences in chemical potential. These aims were also coupled with an investigation into the application of the FBRM technique in monitoring the evolution of palm oil crystallisation which is unprecedented in the study of crystallising fat systems.

A study on the partitioning behaviour of palm oil TAGs during the isothermal crystallisation of palm oil under shear was conducted (Chapter 4). TAG compositional results obtained from HPLC analyses showed that the concentration of trisaturated (SSS) TAGs in the ST fraction increased with increasing isothermal temperature (T).
This suggested that there was a possibility that these TAGs could be enriched further at higher temperatures due to their survival at these conditions. However, there was still an appreciable amount of monosaturated (SUU) and triunsaturated (UUU) TAGs present within the ST cake at all $T_i$, albeit the pure component melting temperatures ($T_m$) of these TAGs were much lower than the temperatures in study (24 °C to 32 °C).

Previous literature studies have shown that the presence of a substantial amount of SUU and UUU TAGs within the ST fraction was largely a contribution of entrainment, i.e. the occurrence of entrapped OL within and between ST crystals in the filter cake. Hence, a novel method was devised to estimate the level of entrainment within the filter cake samples and to quantify the 'correct' ST composition of crystals consolidating the effect of entrainment within the calculation. The basis for this correction is the assumption that no UUU TAGs should crystallise to any significant extent. Following this assumption, any UUU components detected in the ST should only be due to the presence of entrained OL. The level of entrainment can be deduced (via a mass balance) from the relative levels of UUU components in the OL (which can be considered to be always a true value) and the measured ST samples. The details of the correction method are described in Chapter 3. The correction was performed in order to examine to what extent the correction for entrainment had on the TAG composition of the filter cake, crystallisation rates of TAGs and crystallisation driving forces of TAGs within palm oil.

In the isothermal crystallisation study (Chapter 4), it was shown that the entrainment levels in the filter cake calculated from the proposed method were very much comparable to those reported in the literature by previous workers. The amount of OL entrained within the filter cake during the initial stages of each experiment was found to be rather high but gradually decreased as crystallisation progressed towards the end. This was thought to be due to the differences in the crystallisation stages and crystal sizes at these two time periods. The calculated entrainment values were then incorporated into the method proposed for correcting the ST composition, based on the mass balance of the collected cake and filtrate. Results from the corrected ST composition revealed a key finding where PPP showed a much higher composition into the ST followed by POP, PPS and the rest of the other TAGs compared to the uncorrected ST composition which showed the TAG with the highest composition in
the ST was POP followed by POO, PPP and other TAGs. The results from the corrected ST composition are in good agreement with the thermal characteristics of these TAGs which in an ideal system would show the TAG with the highest $T_m$ crystallising in higher amounts compared to TAGs with much lower $T_m$. A comparison of the cumulative crystallised mass of TAGs between uncorrected and corrected ST composition also showed similar trends with regards to the TAG with the higher conversion into the ST phase. The corresponding cumulative crystallised mass of TAGs in the corrected OL fraction based on the corrected ST composition supported the earlier assumption that the UUU TAGs had not crystallised at all $T_i$ in study.

It can be concluded that the proposed entrainment calculation in this thesis has been shown to be a novel method to predict reasonable entrainment values in the filter cake which were very much comparable to the values reported in the literature studies using other techniques. Whilst this method "forces" the values of UUU TAGs, it does not force the values of SUU, SUS and SSS TAGs, which makes the corrected data appear more realistic compared to the uncorrected data. For example the SUU TAGs are not seen to crystallise to any appreciable extent either, which is in line with the fact that the majority of SUU and UUU TAGs must predominantly crystallise at lower crystallisation temperatures as suggested by their lower pure component $T_m$'s tabulated in Chapter 2 and as evident from the DSC analysis of palm oil (Chapter 3). However, the proposed method for estimating entrainment has shown that it is not without minor flaws. Results from Chapter 6 on the post-crystallisation stepwise remelting study of palm oil showed several negative TAG concentration values in the corrected ST composition arising from the proposed entrainment calculation method, suggesting that the proposed calculation method is not entirely perfect. It is worth noting that the entrainment correction is only useful in studying the crystallisation process alone and does not take into consideration of the effects of filtration. This study was not intended to provide a practical solution to the entrainment problem occurring within the fractionation industry but merely attempts to unravel the underlying 'true' crystallisation behaviour of TAGs if the entrainment levels were assumed to be largely contributed by the presence of UUU TAGs within the ST cake. One still needs to address the issue of entrainment in practice.
A further unprecedented attempt to determine the chemical potential driving force of individual TAGs during the isothermal crystallisation of palm oil (Chapter 4) revealed that there was a major difference between the uncorrected ST composition and the corrected ST composition. The driving forces calculated using the corrected data showed $\Delta \mu$ of SSS TAGs approaching zero towards the end of each study whereas this was not observed from the uncorrected data which mainly showed no significant change in the $\Delta \mu$ of TAGs. This new finding shows that the ST composition corrected for entrainment allows palm oil TAGs to follow the expected thermodynamic behaviour of TAGs regarding their crystallisation driving force, highlighting the great influence of entrainment levels within the ST cake when studying the true crystallisation behaviour of TAGs in a palm oil system.

In all the studies conducted within this thesis, i.e. isothermal crystallisation (Chapter 4), non-isothermal crystallisation (Chapter 5) and post-crystallisation stepwise remelting (Chapter 6) of palm oil, it has been clearly demonstrated that the FBRM technique was capable of providing useful and reproducible information regarding the in-situ, real-time particle population and size during the crystallisation and melting of a palm oil system. FBRM was also successful in detecting the occurrence of multiple crystallisation events such as primary nucleation, crystal growth, secondary nucleation events, agglomeration as well as deagglomeration of crystal entities taking place within the palm oil system. The only drawback found when using FBRM was the slight delay in the detection of the clouding of palm oil which indicates the nucleation event. This was thought to be due its lack of sensitivity in detecting particulate matter below the size of 0.8 $\mu$m and visual observation is still required when attempting to detect the first signs of nucleation. Nevertheless, the results presented from the FBRM study proved reliable and thus provide a key contribution in the development of particulate characterisation techniques within the oils and fats industry and particularly in palm oil systems.

Post-crystallisation stepwise remelting studies on palm oil were also studied in this work (Chapter 6). Key results from the HPLC analyses of TAGs revealed that the major melting process occurring as the $T_i$ is increased were dominated by the melting of the SSS TAGs. This was supported by their gradual increase in the OL and reduction in the ST. Entrainment calculations equally supported this by the depiction
of a small amount of true solid phase (11%) which more than 50% was dominated by SSS TAGs. FBRM showed that the melting process was also accompanied by the deagglomeration and falling apart of crystal entities due to the melting of the bridges that link individual crystals together in agglomerates. This was confirmed by the observed increase in the population of fine crystals and simultaneous decrease in coarse crystals. The results from FBRM also indicated that the melting process of palm oil was largely complete by 46 °C although a small degree of cloudiness was observed throughout the oil bulk. These findings have generated new insight into the mechanism and behaviour of particles during the melting of isothermally crystallised palm oil which contributes significantly to the current knowledge on the melting characteristics of palm oil.

A study on the more complicated non-isothermal crystallisation of palm oil under shear showed that the partitioning behaviour of TAGs was highly influenced by differences in the cooling rates studied (Chapter 5). The depletion of SSS TAGs from the OL decreased with increasing cooling rate. This was thought to be linked to either the smaller crystal size produced at higher cooling rates or the crystallisation process progressing too rapidly for all the SSS TAGs to be efficiently crystallised out from the OL. An estimation of the entrainment levels in the filtered fractions using the proposed entrainment correction method revealed that the entrainment level increases with increasing cooling rate due to the size of crystals produced which varied with cooling rate as suggested by FBRM. A direct result of the entrainment correction procedure on the ∆µ was the decrease ∆µ of SSS TAGs to zero with the ∆µ of SUS TAGs succeeding those of the SSS TAGs at the end of experiments at lower cooling rates. This further affirms the need to consider the entrainment factor when studying the true behaviour of TAGs during the crystallisation of palm oil.

To summarise, the work presented in this thesis has provided some key discoveries and new insights relating to the partitioning behaviour of TAGs during the crystallisation of palm oil. Entrainment has been shown to have a huge effect on the experimental concentration data obtained for the ST samples. A new calculation method to predict the level of entrainment within the filter cake has been proposed, which has shown to yield very similar levels of entrainment to those published by earlier researchers. This has resulted in "corrected" compositions which vary in a
manner that is consistent with fundamental thermodynamic driving force considerations. The FBRM has been proven to be a powerful tool in monitoring the evolution of crystallisation and characterisation of crystals in terms of population and size within a palm oil system. All these findings have extended previously published work and have advanced the current knowledge in the area of fats crystallisation and in particular, in the study of the crystallisation of palm oil.

7.2 SUGGESTIONS FOR FUTURE WORK

The findings from the work presented in this thesis can be extended in future works which are suggested as follows:

i. The proposed entrainment calculation method could be validated against the actual solid fat content measurements which can be obtained (in principle) via the use of pulsed nuclear magnetic resonance (pNMR) in crystallising fat systems. This will provide ways to improve the proposed method in predicting entrainment within filtration products from fat fractionation processes.

ii. A relationship can be developed to correlate and transform the chord length distribution (CLD) obtained from FBRM to the actual particle size distribution (PSD) of crystals within fat crystallisation systems and in particular in the crystallisation process of palm oil. This will provide valuable information of the PSD of crystals which is crucial to obtain high separation efficiency during the subsequent filtration process.

iii. This study confirms that entrainment is a key problem in fractionation processes. Further studies can examine the downstream solid-liquid separation process in greater depth and the effect, for example, of crystal size and shape on separation efficiency.
REFERENCES


References


*Partitioning of triacylglycerols in the fractional crystallisation of palm oil* 238
A.1 Proof of mean chord length calculations

Unweighted Mean Chord, $\overline{C_u}$

$$\overline{C_u} = \frac{\sum_{i=1}^{k} Y_{i,u} M_i}{\sum_{i=1}^{k} Y_{i,u}} = \frac{\sum_{i=1}^{k} \left( \frac{n_i}{\sum_{i=1}^{k} n_i} \right) M_i}{\sum_{i=1}^{k} n_i M_i^0} = \frac{\sum_{i=1}^{k} n_i M_i}{\sum_{i=1}^{k} n_i M_i^0}$$

Length Weight Mean Chord, $\overline{C_l}$

$$\overline{C_l} = \frac{\sum_{i=1}^{k} Y_{i,l} M_i}{\sum_{i=1}^{k} Y_{i,l}} = \frac{\sum_{i=1}^{k} \left( \frac{n_i M_i}{\sum_{i=1}^{k} M_i} \right) M_i}{\sum_{i=1}^{k} \frac{n_i M_i}{\sum_{i=1}^{k} n_i M_i}} = \frac{\sum_{i=1}^{k} n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2}$$

Length Square Weight Mean Chord, $\overline{C_s}$

$$\overline{C_s} = \frac{\sum_{i=1}^{k} Y_{i,s} M_i}{\sum_{i=1}^{k} Y_{i,s}} = \frac{\sum_{i=1}^{k} \left( \frac{n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2} \right) M_i}{\sum_{i=1}^{k} \frac{n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2}} = \frac{\sum_{i=1}^{k} n_i M_i^2}{\sum_{i=1}^{k} n_i M_i^2}$$
Length Cube Weight Mean Chord, $\bar{C}_c$

$$\bar{C}_c = \frac{\sum_{i=1}^{k} Y_{i,c} M_i}{\sum_{i=1}^{k} Y_{i,c}} = \frac{\sum_{i=1}^{k} \left[ \left( \frac{n_i M_i^3}{\sum_{i=1}^{k} n_i M_i^3} \right) M_i \right]}{\sum_{i=1}^{k} \left( \frac{n_i M_i^3}{\sum_{i=1}^{k} n_i M_i^3} \right)} = \frac{\sum_{i=1}^{k} n_i M_i^4}{\sum_{i=1}^{k} n_i M_i^3}$$

- $n_i$: Counts in an individual measurement channel
- $M_i$: Midpoint of an individual channel
- $Y_i$: Percentage (%) per channel
- $\bar{C}$: Average chord
- $k$: Upper channel # ($2 \leq k \leq 38$)
- $u$: Unweighted
- $l$: Length weight
- $s$: Length square weight
- $c$: Length cube weight
APPENDIX B ISOTHERMAL CRYSTALLISATION OF PALM OIL UNDER SHEAR

B.1 Measured HPLC concentrations of TAGs at different isothermal crystallisation temperatures

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Figure B1-1 Raw TAG composition of OL fraction at 26 °C
Figure B1-2  Raw TAG composition of ST fraction at 26 °C

Figure B1-3  Raw TAG composition of OL fraction at 28 °C
Figure B1-4  Raw TAG composition of ST fraction at 28 °C

Figure B1-5  Raw TAG composition of OL fraction at 30 °C
Figure B1-6  Raw TAG composition of ST fraction at 30 °C
8.2 Partition coefficient ($K_d$) values of palm oil TAGs

Figure B2-1  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 26 °C

Figure B2-2  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 28 °C
Figure B2-3  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 30 °C

Figure B2-4  Partition coefficient ($K_d$) values of palm oil TAGs as a function of time at 32 °C
B.3 Total mass of TAGs in palm oil based on adding together the calculated quantities for each phase

Figure B3-1 Total mass of TAG in palm oil at 26 °C based on adding together the calculated quantities for each phase
Figure B3-2  Total mass of TAG in palm oil at 28 °C based on adding together the calculated quantities for each phase

Figure B3-3  Total mass of TAG in palm oil at 30 °C based on adding together the calculated quantities for each phase
Appendix B

Figure B3-4  Total mass of TAG in palm oil at 32 °C based on adding together the calculated quantities for each phase

B.4  Cumulative crystallised mass of TAGs in ST fraction

Figure B4-1  Mass of TAG in ST per 100g palm oil at 26 °C
Figure B4-2  Mass of TAG in ST per 100g palm oil at 28 °C

Figure B4-3  Mass of TAG in ST per 100g palm oil at 30 °C

Partitioning of triacylglycerols in the fractional crystallisation of palm oil
B.5 Crystallisation driving forces of palm oil TAGs

Figure B5-1 Crystallisation driving force versus time of palm oil TAGs at 26 °C

Figure B5-2 Crystallisation driving force versus time of palm oil TAGs at 28 °C
Figure B5-3  Crystallisation driving force versus time of palm oil TAGs at 30 °C
APPENDIX C NON-ISOTHERMAL CRYSTALLISATION OF PALM OIL UNDER SHEAR

C.1 Measured HPLC concentrations of TAGs at different cooling rates

Figure C1-1 Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.3 °C/min
Figure C1-2  Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.3 °C/min (TAGs<6%)

Figure C1-3  Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.3 °C/min
Figure C1-4  Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.3 °C/min (TAGs <5%)

Figure C1-5  Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.5 °C/min
Figure C1-6 Raw TAG composition of OL fraction from palm oil crystallised at a cooling rate of 0.5 °C/min (TAGs<5%)

Figure C1-7 Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.5 °C/min
Figure C1-8  Raw TAG composition of ST fraction from palm oil crystallised at a cooling rate of 0.5 °C/min (TAGs<5%)
C.2 Partition coefficient ($K_d$) values of palm oil TAGs

![Partitioning graph](image)

**Figure C2-1** Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at $0.3 \, ^\circ\text{C/min}$
C.3 Cumulative concentration of TAGs in the ST fraction

Figure C3-1 Cumulative concentration of palm oil TAGs in the ST fraction at 0.3 °C/min
C.4 Crystallisation rates of palm oil TAGs

![Crystallisation rates of palm oil TAGs diagram](image)

**Figure C4-1** Mass of TAG in ST per 100g palm oil at 0.3 °C/min
C.5 Crystallisation driving forces of palm oil TAGs

Figure C5-1 Crystallisation driving force of palm oil TAGs in the \( \beta' \) form versus temperature at 0.3 °C/min

Figure C5-2 Crystallisation driving force of palm oil TAGs in the \( \beta' \) form versus temperature at 0.5 °C/min
C.6 Corrected ST composition

Figure C6-1 Corrected ST composition at 0.3 °C/min using entrainment calculation based on UUU

Figure C6-2 Corrected ST composition at 0.5 °C/min using entrainment calculation based on UUU
C.7 Partition coefficient of TAGs based on corrected ST composition

Figure C7-1 Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at 0.3 °C/min (based on corrected ST composition)

Figure C7-2 Partition coefficient ($K_d$) values of palm oil TAGs as a function of temperature at 0.5 °C/min (based on corrected ST composition)
C.8 Crystallisation rates of TAGs based on corrected ST composition

Figure C8-1 Mass of TAG in ST per 100 g palm oil at 0.3 °C/min (based on corrected ST composition)

Figure C8-2 Mass of TAG in OL per 100 g palm oil at 0.3 °C/min (based on corrected OL fraction and corrected ST composition)
Figure C8-3  Mass of TAG in OL per 100 g palm oil at 0.3 °C/min – TAGs <5% (based on corrected OL fraction and corrected ST composition)
C.9 Crystallisation driving forces of palm oil TAGs based on corrected ST composition

Figure C9-1 Crystallisation driving force versus temperature for palm oil TAGs at 0.3 °C/min (based on corrected ST composition)
APPENDIX D

List of Publications

Conference proceedings
