



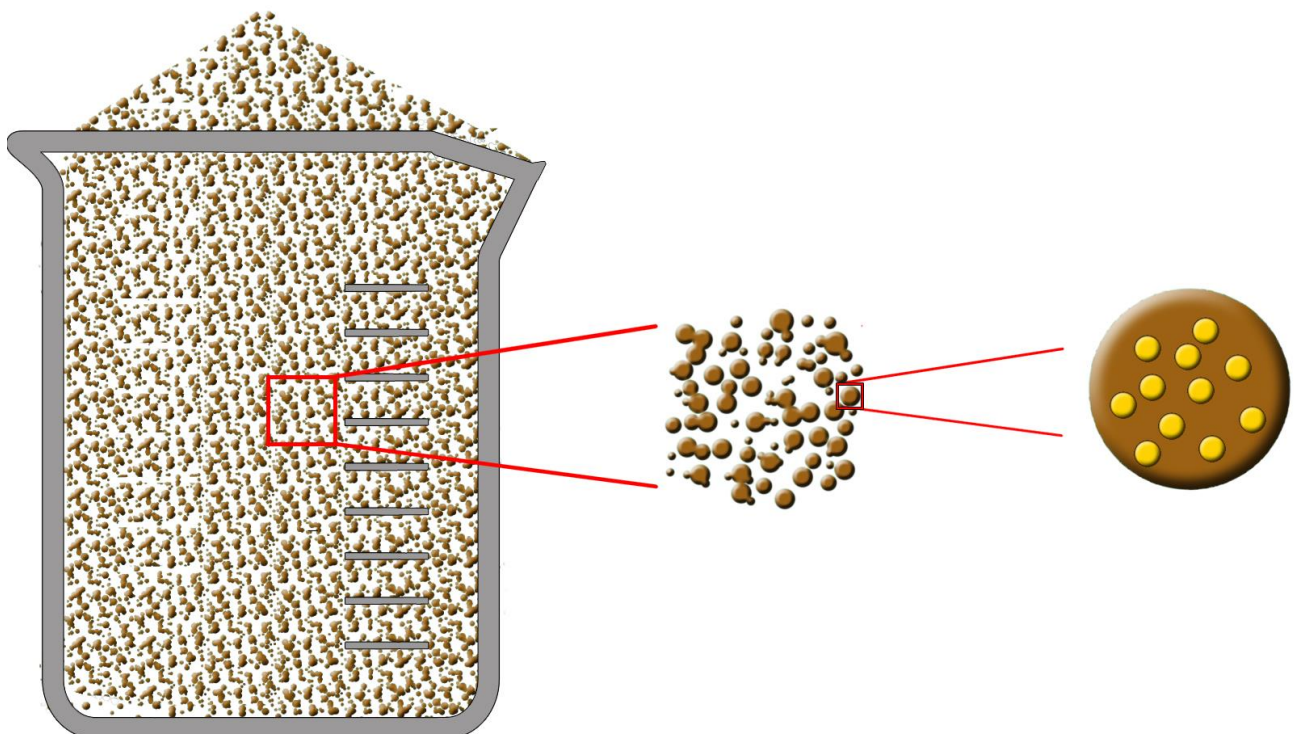
# LUND UNIVERSITY

## ENCAPSULATION AND OXIDATION OF FAT IN RECOMBINED MILK POWDER

MASTER THESIS IN FOOD TECHNOLOGY

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## **Abstract**

Fat-filled milk powders are manufactured by blending skim milk powder with vegetable oil and these powders have gained a great economic importance. Sensible selection of vegetable oil can help in satisfying both nutritional features and functional characteristics of fat for dairy application. The stability of milk powders can be affected by its composition, mainly the composition of fats which can lead to lipid oxidation. The stability of fats depends on various parameters, some of which are, the composition of the fat, crystal form of fat, the composition of powder etc. The parameter which was mainly focused in this study was the crystal form of the fat. The rate of oxidation to some extent depends directly on the fraction of unsaturated lipids and hence this parameter was also used to calculate the measurable oxidation rate. The primary goal of this thesis was to encapsulate vegetable oil in skim milk powder and determine the influence of different crystal forms of fats on the stability of milk powder. The different fats selected were based on the form in which they crystallize. These fats also varied in the fraction of unsaturation. Milk powder was encapsulated with highly unsaturated fat (liquid fat), fat with a very low fraction of unsaturation (hard fat) and in combination of both the kinds of fat, keeping in mind the objective to investigate the effect on the properties of milk powder by the addition of hard fat to liquid fat. Numerous techniques such as DSC, particle size determination, surface free-fat extraction, quantifying the extent of oxidation, X-ray crystallography and SEM were carried out. The results obtained shows that the addition of hard fat to liquid fat brings about changes in the properties and the stability of the fat-filled milk powder. With respect to oxidation, the addition of hard fat reduces the rate of oxidation compared to the rate of oxidation of just the liquid fat in milk powder. But varying the concentration of hard fat did not show a significant difference.

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## **1. Introduction**

### **1.1 Objective**

The primary goal of this thesis was to encapsulate vegetable oil in skim milk powder by the process of spray drying and determine the influence of different crystal forms of fats on the stability of milk powder. The different fats selected were based on the fraction of unsaturation of fatty acids and the form in which they crystallize. Highly unsaturated fat (liquid fat), fat with a very low fraction of unsaturation (hard fat) and in combination of both the kinds of fat were encapsulated in milk powder matrix, keeping in mind the objective to investigate the effect on the properties of milk powder by the addition of hard fat to liquid fat. The different oils used in this project were sun flower oil (SFO), palm stearin (PSt), fully hydrogenated rape seed oil (FHRSO), palm oil and hard palm oil. These fats were chosen since they crystallize in different forms. FHRSO crystallizes in  $\alpha$  form and the PSt crystallizes in  $\beta$  form. Palm stearin and fully hydrogenated oil have a very low fraction of unsaturated fatty acids and are oxidation-stable. Sunflower oil has a high fraction of unsaturated fatty acids and is oxidation-sensitive. Palm oil and hard palm oil were chosen just as a reference.

### **1.2 Hypothesis**

The hypothesis was that the crystallization process might affect the oxidation sensitivity of fat and this may vary for different fats. When hard fat is mixed with the liquid fat, the hard fat might act as a barrier and bring down the rate of oxidation of the mixture. This rate of oxidation in milk powder might be lower when compared to the rate of oxidation of milk powder with just the liquid fat.

### **1.3 Background**

#### **1.3.1 Milk and Milk Powder**

Milk can be defined as a white fluid secreted by the mammalian glands of mammals. The primary function of milk is to provide nutrition to infant mammals. Milk is used for human consumption either in its actual form or in the form of various dairy products. Due to its numerous nutritional and functional qualities, milk and milk based products are used as an ingredient in various industries (Kim, 2008; Visioli and Strata, 2014). Rapid upsurge in the collective consumption of milk by millions of people has been observed in the past few years. Even though this increase in consumption of dairy is fetching significant nutritional benefits to a large segment of populations,

many people in the developing countries are unable to afford a better quality diet accounting to their high costs (Mcmahon, Bennett and Muehlhoff, 2013).

With the increase in demand for low priced dairy products and its availability through all seasons, the dairy companies are entering a new situation which is revolutionizing the business strategies. Milk is processed into a dry form to overcome the difficulties encountered during handling, formulation and transportation of milk when it is in liquid form (Kim, 2008). Milk is a highly perishable product and converting it to milk powder enables in increasing its shelf life without significant loss in its quality, even when it is stored at an ambient temperature. Recombination is not the only application of milk powders but these dairy products can be used for various intrinsic properties, such as an ingredient in bakery, confectionaries etc. (Sharma, Jana and Chavan, 2012).

### **1.3.2 Fat-Filled Milk Powder**

Fat-filled dairy products have gained a great economic importance. The different kinds of fat-filled dairy products in the market includes the whole milk powder, whey derivatives, cream powder and milk powders enriched with different types of fats, which could be either the milk fat or vegetable oils (Vignolles *et al.*, 2009). Fat-filled milk powders are manufactured by blending skim milk powder with vegetable oil. These can be used in ice-cream, bakery, chocolate, confectionary, biscuit, bread, processed cheese, soups, sauces etc. Fat-filled milk powders are an economical substitute to the classical full-cream milk powder. When it is looked upon from a nutritional point of view, milk fat has a high proportion of non-desirable saturated fat. Comparatively, vegetable oil generally comprises of more unsaturated fatty acids and less saturated fatty acids. In addition to this, vegetable oil contains less cholesterol. Sensible selection of vegetable oil can help in satisfying both nutritional features and functional characteristics of fat for dairy application.

### **1.3.3 Spray Drying**

Milk powders are vastly produced by the process of spray drying. In this process, liquid is transformed in solid particles by the application of hot air which removes the water from the feed (Kim, 2008). Spray drying technique has been used in food industry for decades for encapsulation of food ingredients. In case of spray drying of milk with fat, the milk fat gets encapsulated within a wall or matrix which consists of lactose and proteins. Carbohydrates provides the structure of the matrix through the formation of glass and protein provides the film forming and emulsification properties (Adem *et al.*, 2007)

The properties of the spray dried powder are affected by both the composition of the feed and the operating conditions of the spray dryer. The various parameters which could be changed during the spray drying process are the inlet speed of feed, inlet temperature, outlet temperature to name a few. Increase in inlet temperature causes rapid removal of air which further leads to a low bulk density. An increase in the outlet temperature can create cracks on the powder and lead to leakage of the encapsulated fat. Lower outlet temperature aids in giving a more uniform drying of the droplets, improved bulk density and also controlled particle shrinkage (Sharma, Jana and Chavan, 2012). Spray drying can produce powder particles in size ranging from 10-50 $\mu$ m depending on the operation conditions and the composition of the feed (Adem *et al.*, 2007).

### **1.3.4 Fats**

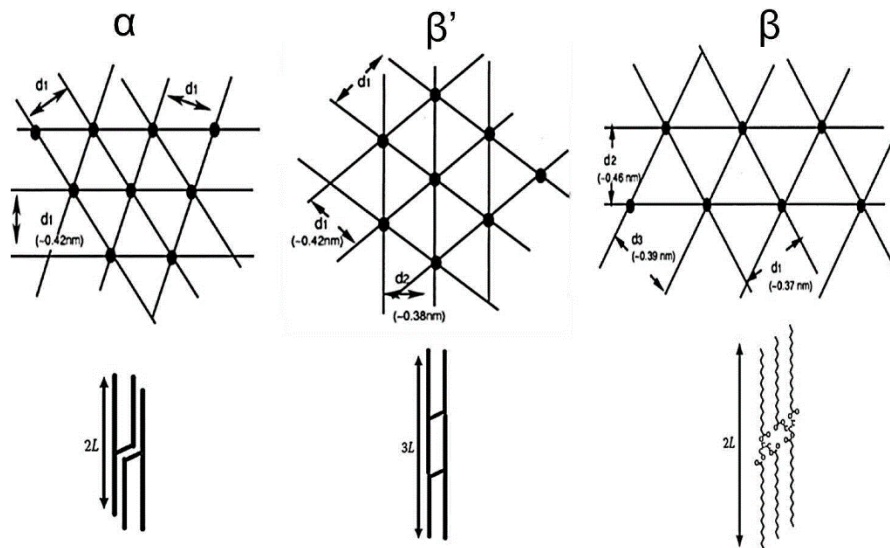
Palm oil is a vegetable fat extracted from the fruit of *Elaeis guineensis*. Palm oil showcases various brilliant properties like high productivity at a low price, high thermal oxidative stability and plasticity at room temperature (Hodate *et al.*, 1997). Plasticity is the property of fat to not melt immediately, but to soften over a range of temperature which gives them the ability to be spread or be shaped. Palm oil is refined by the process of deodorization in which odoriferous compounds and free-fatty-acids are vaporized by a steam stripping process to produce edible oil (Riyadi *et al.*, 2016). Palm oil consist of triglycerides with low and high melting points and this results in a wide range of melting temperature. This fat can be blended to different concentrations of stearin and olein to get different properties of fat. By the process of dry fractionation, palm oil can be fractionated into palm olein (liquid fraction) and palm stearin (hard fraction) (Zhang *et al.*, 2013). Palm olein can be used in the applications of dietary fat, infant formulation and frying purpose (Mohammadreza, Masoomah and Fatemeh, 2015). Palm stearin is a solid fraction of palm oil, having a high melting point at around 60°C and remains solid at room temperature (Wang, Challacombe and Marangoni, 2016). The prime fatty acid composition of palm stearin are palmitic acid (61.2%), oleic acid (27.5%), linoleic acid (6.1%) and stearic acid (4%) (Che Man *et al.*, 1999).

Depending on the specific usage of fats, certain extent of plasticity is required. Fully hydrogenated oil, sometimes also referred to as hard fat have seen applications in the field of production of margarines, shortenings, frying oil etc. These fats are also referred to as plasticizers and generally have a high melting point (Deman, Deman and Blackman, 1989). Hydrogenation of fats is a chemical process used to eliminate the double bonds in fatty acids. Coming to oils with high ratio

of polyunsaturated fatty acids to saturated fatty acids, high amount of polyunsaturated acid content is present in sunflower oil making it highly unstable (Pal *et al.*, 2015). By lowering the amount of unsaturated fatty acids and increasing the saturated fatty acids, the stability of fats can be improved with an affiliated increase in the amount of solid fat (Guinda, Dobarganes and Ruiz-mendez, 2003).

### 1.3.5 Crystalline Forms of Fat

Polymorphism is the property of a substance to exist in more than one crystalline form. Fats exhibit polymorphic behavior as they can crystallize in three major forms. These three forms of fats crystals are named as alpha ( $\alpha$ ), beta-prime ( $\beta'$ ) and beta ( $\beta$ ). The different crystal forms have different melting points and have a variation in the crystal packing. Figure 2 shows the packing of different crystal forms of fat. X-ray diffraction analysis is used in determining the polymorphic form of the fat. B crystals are the most stable while the  $\alpha$  crystals are the least stable form (Smith, 1985).



**Figure 1:** Crystal forms of fat with the spatial arrangement

### 1.3.6 Stability of Milk Powder and Lipid Oxidation

Milk powder can produce an off flavor over the storage time and these off flavors can be due to the lipid oxidation (Yanhua, Lanwei and Weijun, 2012). Hence it is vital to determine the stability of fat-filled milk powders with different vegetable oils are used. Stability of milk powders can be affected by factors like the storage conditions which includes the temperature, humidity etc.



Oxidation of encapsulated fats increases at higher humidity as the moisture can penetrate through the matrix and making the fat more prone to oxidation (Hardas *et al.*, 2002). The stability of milk powders can also be affected by its composition and in this project, the composition of fats and their effect on stability was determined. The stability of fats also depends on various parameters. One important parameter is the level of unsaturation of the fat. Fats with high proportion of saturated fatty acids have a higher stability towards oxidation but vegetable fat mixtures with high proportion of polyunsaturated fatty acids are available for dairy applications which have a high resistance towards oxidative rancidity.

To understand the stability of these powders, it is important to investigate the sample over a period of time and observe the change in their properties. The most important factor to determine the stability is to investigate the oxidation of samples. Milk powders have lipids in them and lipid can undergo oxidation and hence become rancid. This oxidative rancidity is one of the major reasons behind deterioration of food. Oxidation of lipids typically involves a primary oxidative reaction which is then followed by a secondary oxidation reaction. Considering the lipids, the major cause of these oxidation is due to the unsaturation in lipid. The rate of oxidation to some extent depends directly on the fraction of unsaturated lipids (Gray, 1978). Among the samples used for the thesis project, PSt and FHRSO have a very low unsaturation and hence are oxidation stable while the sunflower oil has high unsaturation and is oxidation sensitive. Sunflower oil was used as a model oil and was also used in combination with PSt and FHRSO in different proportions to determine the effects of the hard fat on the oxidation sensitive sunflower oil.

#### **1.4 Experimental Setup**

The products of primary oxidation are hydroperoxides which are also referred to as peroxides (Gray, 1978). Therefore, the concentration of peroxide was determined to examine the extent of primary oxidation. Ferric-thiocyanate method was used for this analysis. The principle behind this method is that when a portion of the sample is dissolved in a mixture of methanol/1-decanol/hexane and upon addition of iron(II) chloride and ammonium thiocyanate, iron(II) gets oxidized to iron(III) complex with ammonium thiocyanate by the peroxides and imparts red colour. Photometric determination of this solution quantifies the amount of hydroperoxides (ISO, 2006)

Thiobarbituric acid test (TBAR) was used to determine the secondary oxidation of samples. This protocol works on the principle of formation of a pink complex due to the reaction between TBA

and malonaldehyde and other reacting secondary oxidation products. This pink complex has a strong absorbance in the range of 530-535nm (Semb, 2012)

Spray dried powders have become a very important ingredient in various applications and knowing the surface composition of the powder would aid in understanding the quality of the powder and further in the development of new products (Kim, 2008). Since the focus is on the effect of fat on milk powder, determination of the amount of surface free-fat was carried out to check how this value varies over the storage period. The surface morphology of powder particles was also observed under scanning electron microscope to draw a clearer conclusion of the results.

Since the samples contain both the liquid fat and the solid fat, it was not very clear about how this would affect the crystalline state of the fat over the storage period. X-Ray crystallography was done on the fats and milk powder to determine the crystalline state of the sample. Differential scanning calorimeter was also done over the storage period to know the melting enthalpy and the melting point of the samples.

## **2. Materials and Methods**

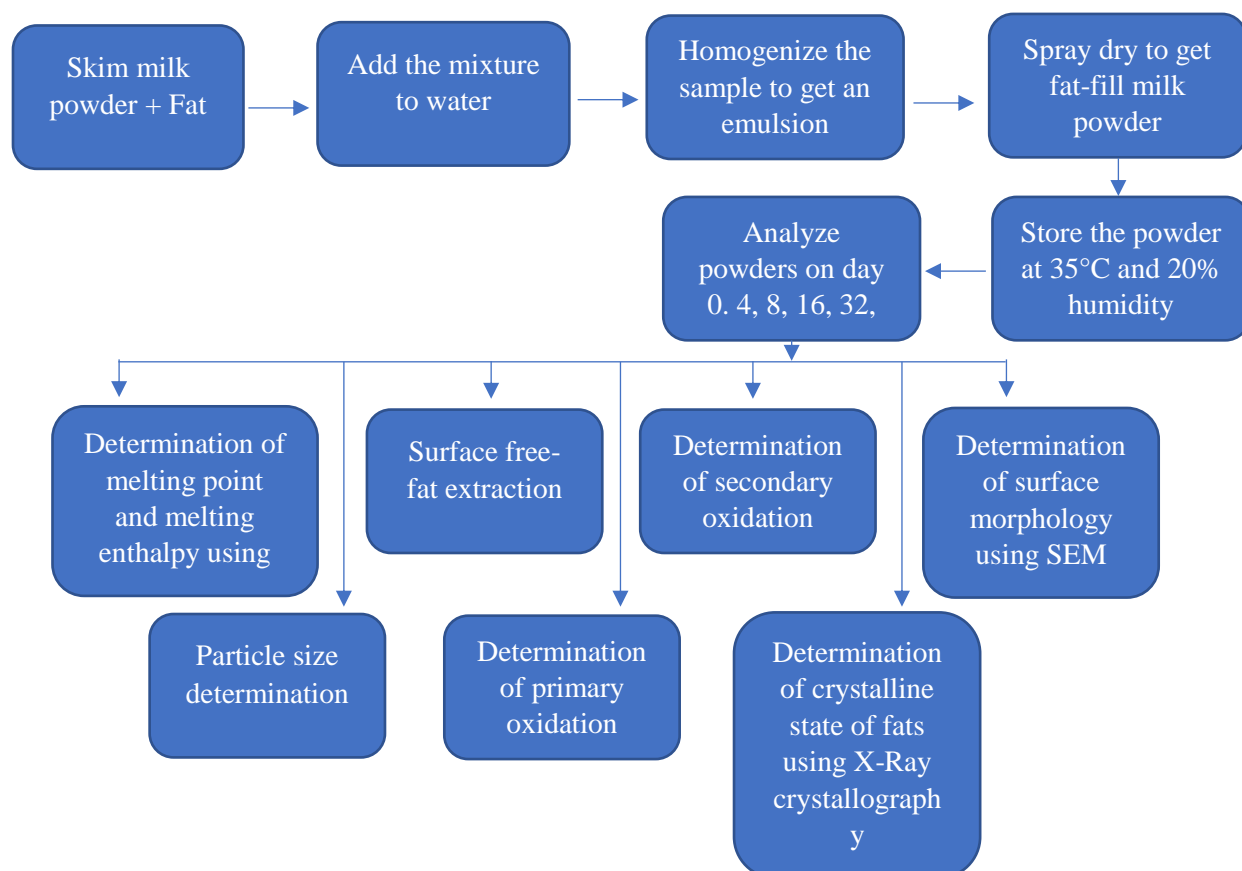
### **2.1 Experimental Outline**

Different fats and their mixtures were used to produce fat-filled milk powders. These milk powders contained 50% of skimmed milk powder and 50% of fat on dry basis. SFO was used as the reference sample. PSt and FHRSO were used separately and also in combination with SFO. Powders were also produced with palm oil and hard palm oil. Table 1 shows the composition of different powders produced for the project. SFO was used as the reference sample since it is highly sensitive to oxidation and thus, the oxidation of the hard fats can be compared in terms of oxidation of SFO.

**Table 1:** Composition of fat-filled milk powder produced; SFO: Sun flower oil, PSt: Palm stearin, FHRSO: Fully hydrogenated rapeseed oil

Sample	Composition
Sample 1	Skimmed milk powder + SFO (100%)
Sample 2	Skimmed milk powder + PSt (100%)
Sample 3	Skimmed milk powder + [PSt (70%) + SFO (30%)]
Sample 4	Skimmed milk powder + [PSt (30%) + SFO (70%)]
Sample 5	Skimmed milk powder + FHRSO (100%)
Sample 6	Skimmed milk powder + [FHRSO (70%) + SFO (30%)]
Sample 7	Skimmed milk powder + [FHRSO (30%) + SFO (70%)]
Sample 8	Skimmed milk powder + Hard palm oil (100%)
Sample 9	Skimmed milk powder + Deodorized palm oil (100%)

Figure 1, project overview demonstrates the outline of the process involved in the production of these milk powders and then the experiments carried out on these powders.



**Figure 2:** Overview of the production of fat-filled milk powder and the analysis done on the powders

## **2.2 Materials**

Various chemicals, glassware and instruments were used during the project. Potassium acetate (Scharlab chemicals, Germany) was used to maintain humidity of 20% when the samples were stored. For the determination of primary oxidation, the chemicals used were methanol (Honeywell Specialty Chemicals, Germany), 1-decanol (Fluka Chemie, Germany), n-hexane (VWR Chemicals, BDH Prolabo, France), HCl (VWR Chemicals, BDH Prolabo, France), ammonium thiocyanate (Merck, Germany), hydrogen peroxide (Acros organics, Belgium) and iron powder (Fluka, Germany). The chemicals used for the determination of secondary oxidation are Trichloroacetic acid (Merck, Germany), thiobarbituric acid (SIGMA-ALDRICH, Germany), tetramethoxypropane.

## **2.3 Sample Preparation**

Fat samples and skim milk powder used were obtained from AAK, Karlshamn, Sweden. An emulsion of oil-in-water was made with 20% solid content. The 20% solid content had 50% of skimmed milk powder and 50% of fat. To prepare the emulsion, 60g of skimmed milk powder was weighed and dissolved in 600ml of water. To this, 60g of fats was added. Since some of the fats used were solid at room temperature, they were melted at 10°C above its melting point before it was weighed and added to skimmed milk powder. To this mixture, water was added and the volume was made up to 600ml. This was then homogenized using Panda Plus 2000 laboratory homogenizer at 200 bar. The inlet chamber of the homogenizer was connected to a hot-water bath running at 75°C which helped in keeping the samples at an elevated temperature during the homogenization process. This entire process was carried out for all the 9 samples to get an emulsion. Around 500ml of emulsion was obtained from the 600ml solution.

To get the final powder, the emulsions were subjected to spray drying. Spray drying was carried out in Buchi mini spray dryer, B-290 done with an inlet temperature of 180°C and outlet temperature of 80°C. The aspirator was set at 100% and the feed flow was set between 70%-80% to get the desired outlet temperature. The samples were heated to 60°C before it entered the inlet valve to avoid fat crystallization in the inlet tube, which could lead to a blockage. The temperature around the atomizer was also kept at 60°C using a hot water bath. An average of 40g of milk powder was obtained from 500ml of emulsion.

These powders were put into beakers and the beakers were placed inside a plastic box with a lid. The plastic box was covered with aluminum foil to avoid the light entering the box. The base of

the box was filled with potassium acetate to maintain a humidity of 20%. The box was placed inside a heating cabinet (Termaks) with its temperature set to 35°C. The samples were taken out during its analysis and were then placed back in the heating cabinet.

#### **2.4 Differential Scanning Colorimetry (DSC)**

DSC was carried out using Exstar 6000 (Seiko instruments, Exstar 6000) just for the pure fat samples and also for the fat-filled milk powders produced from these fat samples. About 5mg of sample was weighed in an aluminum pan, sealed and placed inside the colorimeter. Empty sealed aluminum pans were used as the reference. Before the pans were placed into the colorimeter, the temperature of the cabinet was brought down to 0°C. Once the samples were placed with the reference pan, the temperature of the cabinet was increased from 0°C to 100°C at a rate of 5°C per minute to obtain the melting thermogram. The thermogram was analyzed to obtain the melting point and the melting enthalpy of the sample.

#### **2.5 Particle Size Determination**

Particle size determination was carried out using the Malvern Mastersizer 2000 (United Kingdom) by the technique of laser light diffraction and the surface weighted diameter  $D[3,2]$  was determined. 0.5g of powder was dispersed in 1ml of water and this dispersion was run in the mastersizer with a motor speed of 1200 rpm. The obscuration was aimed at 9% The refractive index set for palm oil which is 1.455. The results obtained were in form of a graph which showed a peak which indicated the particle size.

#### **2.6 Surface Free Fat Extraction**

To extract the surface free fat, 300mg of sample was weighed and transferred on to a Whatman No. 4 filter paper with 25µm pore size and washed 2 times with 5ml of n-hexane. The filtrate solution which contained the extracted fat was collected in a 200ml conical flask. This solution was allowed to evaporate until a constant weight of the extract was obtained. The weight of the conical flask with the extracted fat was subtracted from the weight of the empty flask to get the amount of surface free fat. This value was converted to get the surface free fat in terms of grams of surface free fat/g fresh powder (Kim, 2008).

## 2.7 Determination of Primary Oxidation by Ferric-Thiocyanate Method

The products of primary oxidation are hydroperoxides which are also referred to as peroxides (Gray, 1978). Therefore, the concentration of peroxide was determined to examine the extent of primary oxidation. Ferric-thiocyanate method in accordance to the ISO 3976:2006(E) (2006) was used for this analysis. The principle behind this method is that when a portion of the sample is dissolved in a mixture of methanol/1-decanol/hexane and upon addition of iron(II) chloride and ammonium thiocyanate, iron(II) gets oxidized to iron(III) complex with ammonium thiocyanate by the peroxides and imparts red colour. Photometric determination of this solution quantifies the amount of hydroperoxides (ISO, 2006)

### Reagents:

Methanol/1-decanol/n-hexane mixture: 2 volume parts of 1-decanol was mixed with 1 volume part of n-hexane. To this solution, 3 volume parts of methanol was added and mixed.

Iron(II) chloride solution: 0.35g of iron(II) chloride tetrahydrate was mixed in about 100ml water. To this, 2ml of hydrochloric acid (10mol/l) was added and mixed.

Ammonium thiocyanate solution: 30g of ammonium thiocyanate was dissolved in 100ml of water.

Iron(III) chloride standard solution: 0.5g of iron powder was dissolved in 50ml of HCL in a 500ml volumetric flask. 2ml of hydrogen peroxide solution was added and excess hydrogen peroxide was removed by boiling for 5 minutes. The solution was cooled down to room temperature and then diluted to 500ml mark.

### Procedure:

Reagent blank: 9.9ml of methanol/1-decanol/n-hexane mixture was added to a test tube. To this, 0.05ml of ammonium thiocyanate solution was added and mixed. Reagent blank was transferred to a photometer cell and extinction  $E_1$  was measured against the methanol/1-decanol/n-hexane blank.

### Test Sample Blank

1mg of sample was weighed and 9.6ml methanol/1-decanol/n-hexane was added to it. The mixture was mixed gently to dissolve the fat in the sample. 0.05ml of ammonium thiocyanate was added

and mixed again. This mixture was transferred to a photometer cell and extinction  $E'_0$  of blank was measured against methanol/1-decanol/n-hexane mixture.

#### Test Portion

1mg of the sample was weighed into a test tube and 9.6ml of methanol/1-decanol/n-hexane was added to it. 0.05ml of ammonium thiocyanate was added to the mixture and mixed. To this, 0.05 ml of iron(II) chloride solution was added. Extinction  $E_2$  was measured against methanol/1-decanol/n-hexane.

#### Extinction coefficient of the red iron(III) complex

0.5ml, 1ml, 1.5ml and 2ml of iron(III) chloride standard was taken in four different test tubes to obtain  $5\mu\text{g}$ ,  $10\mu\text{g}$ ,  $15\mu\text{g}$  and  $20\mu\text{g}$  of  $\text{Fe}^{3+}$  respectively. To these test tubes, 9.4ml, 8.9ml, 8.4ml and 7.9ml of methanol/1-decanol/n-hexane mixture was added respectively. 0.05ml of ammonium thiocyanate solution and 0.05ml hydrochloric acid (0.2mol/L) was added to each tube and mixed. Measure the extinction ( $E_{\text{Fe}}$ ) of each sample against methanol/1-decanol/n-hexane. Using the obtained data, a graph was plotted to obtain a line equation.

$$Y = a + bX$$

Where,

Y is the extinction value  $E_{\text{Fe}}$

X is the mass of  $\text{Fe}^{3+}$

a is the intercept of the regression

b is the extinction coefficient of iron(III) complex, which was used for further calculations

#### Calculation of extinction

Extinction E attributed to iron(III) complex was calculated by using the equation:

$$E = E_2 - (E_0 + E_1)$$

Calculation of mass of Fe<sup>3+</sup>

The mass of Fe<sup>3+</sup>, m<sub>c</sub> was calculated using the following formula and was expressed in micrograms.

$$m_c = E/b$$

Calculation of peroxide value (PV)

PV was calculated and expressed in terms of millimoles of oxygen per kilogram by using the following equation:

$$PV = 0.5m_c / 55.84 m$$

Where, 55.84 is the atomic mass of Fe<sup>3+</sup>

(ISO, 2006)

## **2.8 Determination of Secondary Oxidation by TBARS Method**

Determination of secondary oxidation by TBARS method was carried out using the protocol obtained from (Takeungwongtrakul and Benjakul, 2013).

15 g of Trichloroacetic acid (TCA) was weighed into a beaker and 50ml 0.25M HCl was added to dissolve the TCA. 0.75g of thiobarbituric acid was added to the TCA solution and was thereafter transferred to a 100ml Volumetric flask.

To obtain a standard curve, 164.5mg of 1,1,3,3-tetramethoxypropane (TMP) was dissolved in 100ml of Milli-Q water. 1ml of this solution was taken and diluted with 100ml of milli-Q water. To get a standard curve, solution B and milli-Q water were added in proportion as shown in appendix 7.

From each of the standard concentrations made, 0.5ml of it was taken and mixed with 2.5ml of TBAR solution in a 15ml tube with screw caps. The solution was heated at 90°C for 10 minutes and were allowed to cool down to room temperature. These standard solutions were then centrifuged at 3600g for 20 minutes using Beckman Coulter, allegra X-15R centrifuge and absorbance was measured using Varian, 50 Bio UV-visible spectrophotometer at 532nm.

To obtain the spectrophotometric readings of the samples, 0.125g of powder was weighed and dispersed in 0.5ml of water. To this, 2.5ml of TBAR solution was added. The samples were then



heated at 90°C for 10 minutes and centrifuged at 3600g for 20 minutes. The absorbance was taken at 532nm.

Calculation:

$$\text{TBARS } (\mu\text{M TMP/g}) = [(A - B) - m \text{ value}] / (k \text{ value} * w)$$

Where m and k value were obtained from the standard curve equation given by:  $y = kx + m$

A is the absorbance of the sample

B is the absorbance of blank

W is the weight of the sample

(Takeungwongtrakul and Benjakul, 2013)

## 2.9 X-Ray Crystallography

X-Ray crystallography was carried out at AAK, Karlshamn using Siemens XRD D5000. The protocol used was according to the standard protocol used at AAK. This analysis was done both on fats and powders. The fats were melted and then stored at 35°C. From the time when these fats were melted, the analysis was done at 30 minutes and after 1 week to check if they change their crystalline state over the storage period. The powders were analyzed only once during the week 4 from the day of storage. During the analysis of fat crystals by X-ray diffraction, two kinds of spacing can be observed. One is called the long spacing while the other is called the short spacing. To characterize the polymorphic form of fat, short spacing was used. The short spacing of the most common polymorphs are:  $\alpha$  with a single spacing at 4.15Å (21.39° 2-theta),  $\beta'$  with two spacings at 3.8Å (23.38° 2-theta) and 4.2Å (21.13° 2-theta) or with three spacings at 4.27Å (20.88° 2-theta), 3.97Å (22.48° 2-theta) and 3.71Å (24.02° 2-theta)). The short spacing for  $\beta$  is observed at 4.6Å (19.27° 2-theta) and one more generally at 3.8Å (23.38° 2-theta) (Deman, 1992).

The percentage of  $\beta$  and ( $\alpha + \beta'$ ) in the samples were calculated. To calculate the percentage of  $\beta$  crystals in the samples, the intensity of  $\beta$  was divided by the total intensity of  $\beta$  and ( $\alpha + \beta'$ ) put together. Hence,

$$\% \beta = \text{Intensity of } \beta / \text{Intensity of } \beta + (\alpha + \beta')$$

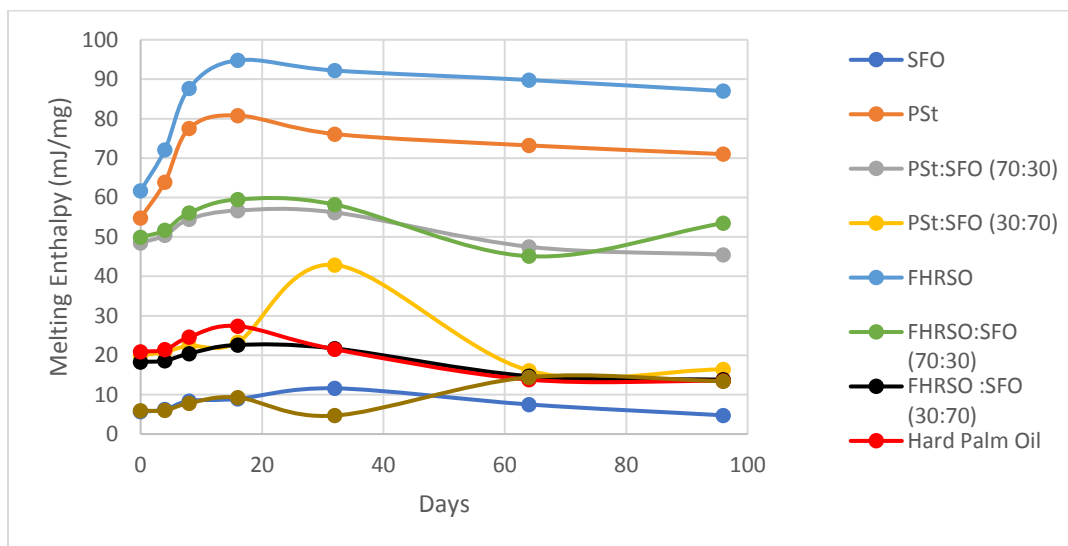
## 2.10 Scanning Electron Microscopy (SEM)

The morphology of the spray-dried particles was examined by using a JEOL JSM 6700F NT Scanning Electron Microscope (SEM) (Tokyo, Japan). The analyzed powder particles were mounted on aluminum stubs and sputter-coated with a mixture of gold and palladium (ratio 80/20). The coating time was set to 180 s resulting in a layer thickness of 15 nm. The SEM images were obtained under high vacuum using 10 kV accelerating voltage and a below-the-lens secondary electron imaging (SEI) detector.

## 3. Results

### 3.1 Differential Scanning Colorimetry

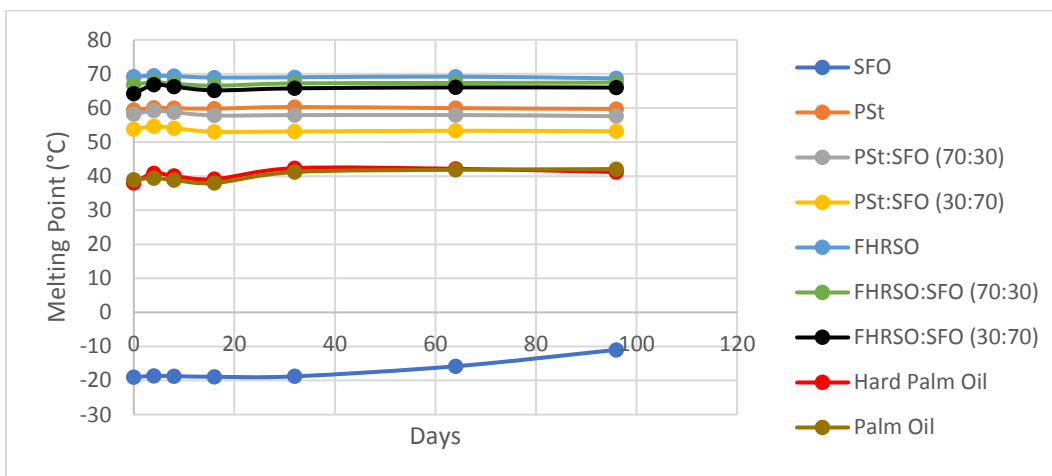
DSC was carried out for fat samples and the powders which are mentioned in table 1. For the fat samples, DSC was done only once and in duplicates. The fats were melted at 10°C above the melting point before they were run in DSC. For the powders, the analysis was done on day 0, 4, 8, 16, 32, 64 and 96. Melting enthalpy and melting points were obtained from this analysis and the results are shown in appendix 1 and 2, respectively. Using these results, graphs were plotted with days on x-axis and melting enthalpy and melting point on y-axis in separate graphs. Figure 3 and 4 demonstrates the melting enthalpy and melting temperature of powder.



**Figure 3:** Melting enthalpies of all powder samples measured at different points over storage time

From the melting enthalpy curve, it can be seen that there is an increase in the melting enthalpy in the first 16 days for all the samples. From day 16 till day 96 there is a slight decrease in the melting

enthalpy. The trend observed here is very uniform and the decrease in enthalpy is not significant. The hard-fat samples have a higher melting enthalpy and also the increase in melting enthalpy in the first 16 days is much higher than the rest of the samples. It can also be noted that, with the addition of SFO to the hard fat samples, the melting enthalpy decreases proportionally. FHRSO shows the highest melting enthalpy while the palm oil and SFO show a very low melting enthalpy. The result of day 32 for PSt:SFO (30:70) show a sudden increase in the value of melting enthalpy and this could be due to an experimental error. The initial increase appears quite significant while the decrease after long storage leads to some uncertainty in relation to the interpretation. It may be noted that the results show the extent of crystallization. The crystallization enthalpy of the FHRSO and PSt could be assumed to correspond to more or less 100% crystallization. Thus the 70/30 samples are close to 70% while the 30/70 samples appear a bit lower around 25%.



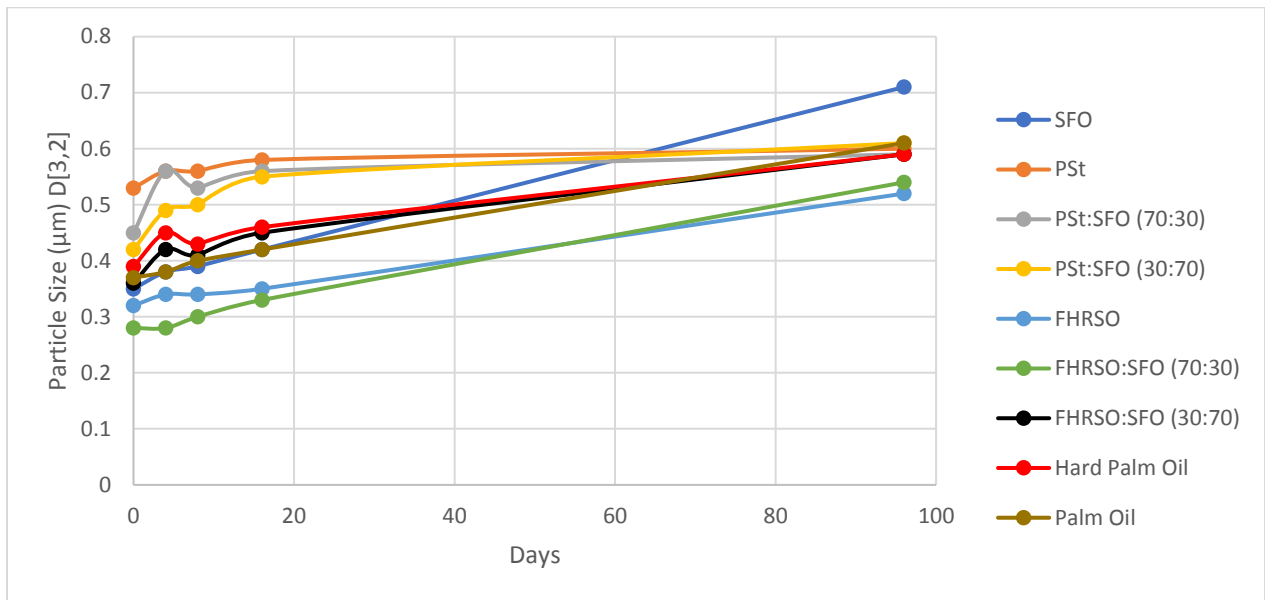
**Figure 4:** Melting temperature of all powder samples measured at different points over storage time

At the first, the obtained data looks realistic and the results were reproducible. The values obtained were also in accordance with the data available in literature. The melting point of the samples are relatively constant throughout the study period. There are slight changes in the melting points in the first 16 days but they are not significant. FHRSO shows the highest melting point with a value around 70°C and the SFO shows the lowest melting point.

### 3.2 Particle Size Determination

The size of emulsion droplets was analyzed as soon as the emulsions were prepared. The emulsion droplet size refers to the size of the emulsion formed after re-dissolving the powder in water. The

emulsion size determination was done on the samples on day 0, 4, 8, 16, 32, 64 and 96. The protocol was changed during the analysis on day 32 and day 64 and this gave values which were very different from the values obtained during the analysis on the other days. Due to this reason, the values from day 32 and 64 have been excluded from the results. Appendix 3 shows the results obtained from the particle size analysis and figure 5 is a plot of particle size vs days.

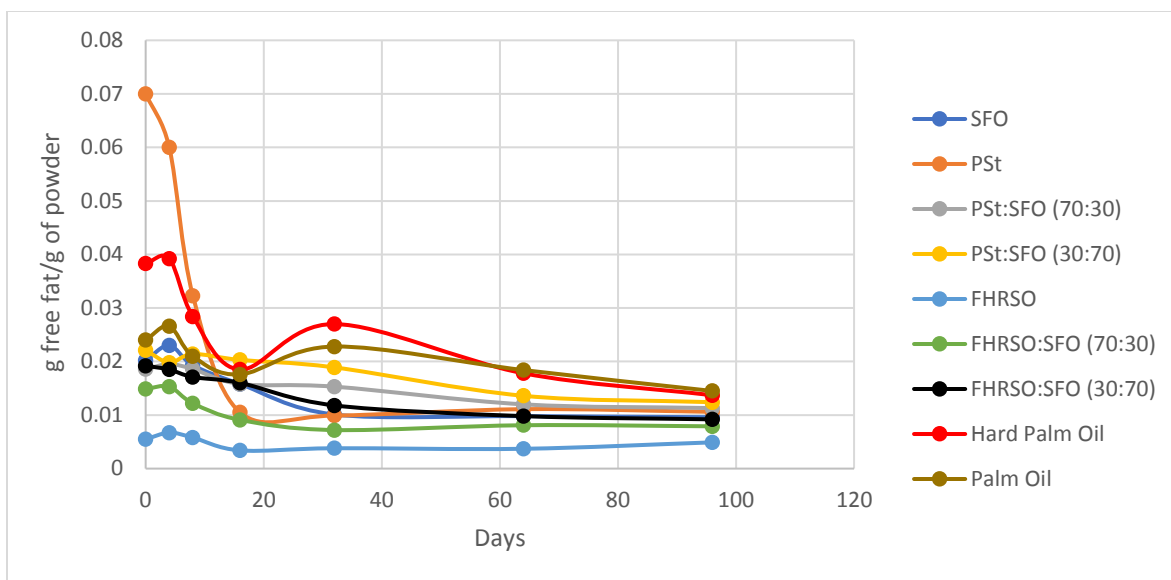


**Figure 5:** Emulsion droplet size of recombined milk powder

From the graph, it can be seen that the particle size increases over time. PSt and its mixtures do not show a significant increase in the particle size, whereas in the rest of the samples, the particle size is much higher on day 96 than on the day 0. In case of SFO, the particle size has almost doubled.

### 3.3 Surface Free Fat Extraction

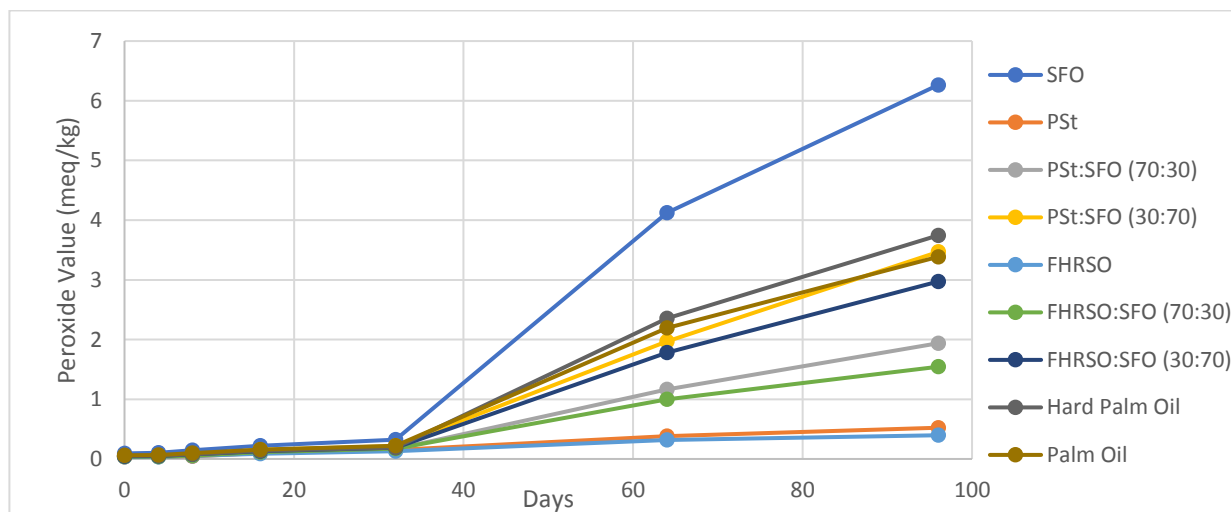
Surface free fat extraction was done on powders on day 0, 4, 8, 16, 32, 64 and 96. The results obtained are shown in appendix 4. These results were plotted in a graph with amount of surface free fat extracted against the days (Figure 6). From the graph, it can be see that the values obtained are very consistent. The amount of surface free fat decreases for most of the samples and this variation is observed drastically only during the starting 16 days. Further from day 16, the decrease in free fat is very gradual except for Hard palm and palm oil where the surface free fat slightly increases during day 32 and the starts to decrease.



**Figure 6:** Amount of extracted surface free fat from the powders at different intervals

### 3.4 Peroxide Value by Ferric Thiocyanate method

Peroxide value was determined on the powder samples to investigate the extent of primary oxidation in the samples. The analysis was carried out on day 0, 4, 8, 16, 32, 64 and 96. The results obtained are as shown in appendix 5 and a graph was plotted was all the samples with days on the x-axis and the peroxide value on the y-axis. Figure 7 shows the change in the peroxide value as a function of time.



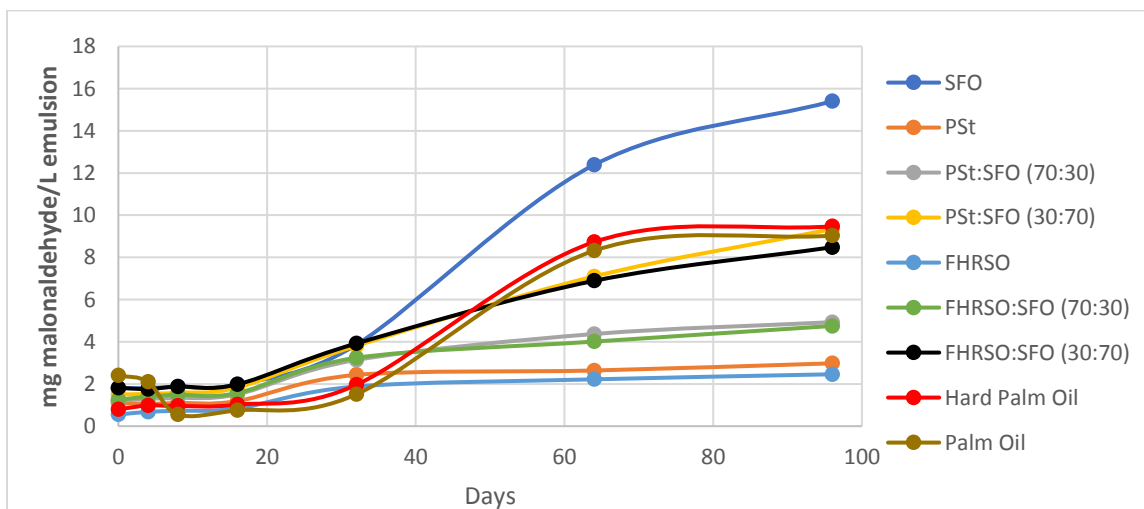
**Figure 7:** Peroxide value as a function of time

The curve shows an initiation phase, an acceleration phase and the 3<sup>rd</sup> phase where the slope of the curve is lower than that of the acceleration phase. The initiation phase is till the day 32 where there is a slight oxidation happening. The acceleration phase can be seen from day 32 till day 64 where

there is a rapid increase in the peroxide value. From the day 64 till day 96, the increase is not as rapid as it was during the period between day 32 and 64. It can be observed that the extent of oxidation varies from sample to sample. Except for the PSt and FHRSO, a common thing which can be noticed is that the oxidation of rest of the sample starts to accelerate from the day 32. SFO has the highest oxidation value and PSt and FHRSO have a very low oxidation value throughout the study period. With the addition of the SFO to the hard fats, it can be seen that the oxidation of those samples was more than that of just the pure hard fat.

### 3.5 Secondary Oxidation by TBARS Method

The extent of secondary oxidation was determined on the powders on day 0, 4, 8, 16, 32, 64 and 96. The results obtained are shown in appendix 6. A graph was plotted using the obtained results with days on x-axis and the secondary oxidation value on y-axis. Figure 8 shows the change in the secondary oxidation as a function of time.



**Figure 8:** Secondary oxidation as a function of time

From the graphs, it can be observed that there is secondary oxidation taking place in all the sample to an extent. It is again seen that SFO has the highest secondary oxidation while the hard fats have a very low secondary oxidation value. In SFO, hard palm oil and palm oil, it can be observed that there is an initiation phase, an accelerated phase and a constant phase. It can also be noticed that, with the addition of SFO to the hard fat (PSt and FHRSO), the extent of secondary oxidation increases. This trend was observed even with the primary oxidation.

### 3.6 X-ray crystallography

The results obtained from the X-ray crystallography were graphs representing the peak count against the angle of diffraction (2-theta). The number of  $\alpha$ ,  $\beta'$  and  $\beta$  crystals were calculated using the intensity of the peak corresponding to the polymorphic form of the observed crystal. Table 2 shows the results obtained from the fat samples and table 3 shows the results obtained from the powders.

**Table 3: Crystal forms obtained from fat samples**

	30 minutes		1 week	
	% $\beta$	% ( $\alpha + \beta'$ )	% $\beta$	% ( $\alpha + \beta'$ )
PSt	43%	57%	22%	78%
PSt:SFO (70:30)	85%	15%	78%	22%
PSt:SFO (30:70)	85%	15%	88%	12%
FHRSO	0%	100%	0%	100%
FHRSO:SFO (70:30)	97%	3%	98%	2%
FHRSO:SFO (30:70)	96%	4%	97%	3%
Hard Palm Oil	43%	57%	39%	61%
Palm Oil	45%	55%	22%	78%

**Table 4: Crystal forms obtained from powder samples**

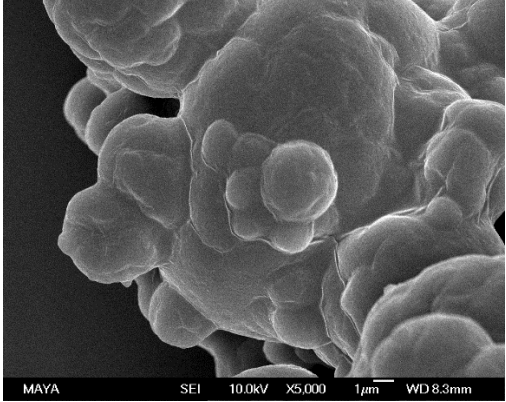
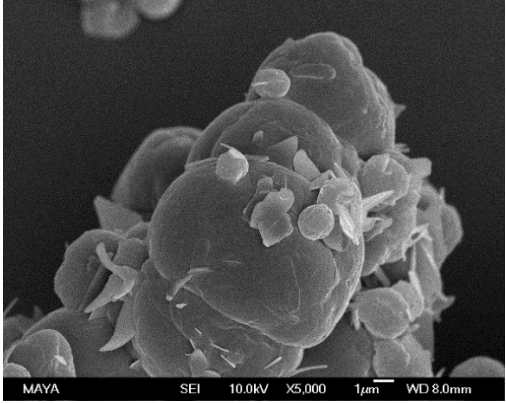
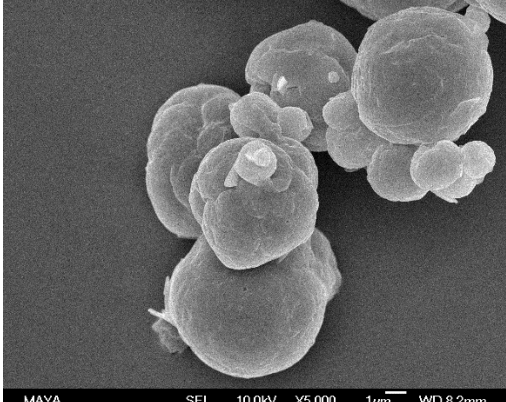
	% $\beta$	% ( $\alpha + \beta'$ )
PSt	100%	0%
PSt:SFO (70:30)	100%	0%
PSt:SFO (30:70)	100%	0%
FHRSO	65%	35%
FHRSO:SFO (70:30)	93%	7%
FHRSO:SFO (30:70)	100%	0%
Hard Palm Oil	68%	32%
Palm Oil	43%	57%

Table 3 and 4 shows the change in the crystal state of the samples after they were stored for 1 week. PSt stabilizes in the  $\beta'$  form and the FHRSO stabilizes in the  $\alpha$  form. But with the addition of SFO to these two samples, both the samples shift their configuration to stabilize in  $\beta$  form. Addition of liquid fat is leading to a system crystallizing in  $\beta$  form. Palm oil is seen to be stabilizing the  $\beta'$  form while hard palm oil has a  $\beta$  configuration. In the results obtained from the powder samples, it can be observed that except for the palm oil, rest of the samples have converted to  $\beta$  form.

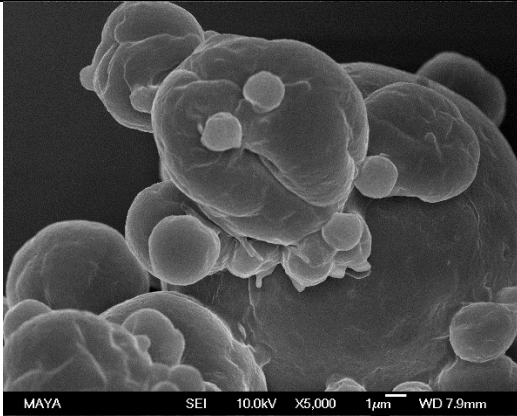
### 3.7 Scanning Electron Microscopy

The images obtained from the SEM analysis are shown in the table 5. Different samples showed different surface morphology. The samples with just the hard fat showed fat crystals formed on the surface of the powder particles while. With the addition of the SFO to these hard fats, there was a reduction in these crystals on the surface. Most of the particles showed a spherical structure of the powders with size varying from sample to sample.

**Table 5:** SEM images of fat-filled milk powder with different fat samples

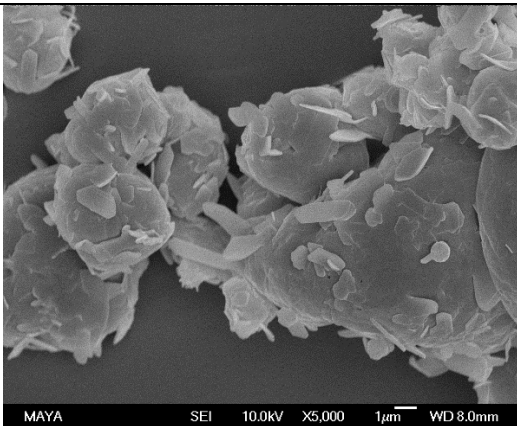
 <p>MAYA SEI 10.0kV X5,000 1µm WD 8.3mm</p>	<p><b>Sunflower oil</b></p> <p>The image shows agglomerated particles which are relatively smooth on the outer surface. There are no visible cracks on the particle.</p>
 <p>MAYA SEI 10.0kV X5,000 1µm WD 8.0mm</p>	<p><b>Palm oil stearin</b></p> <p>The particles observed here are more spherical and smooth compared to SFO. Flakes of fat crystals are observed on the powder particles.</p>
 <p>MAYA SEI 10.0kV X5,000 1µm WD 8.2mm</p>	<p><b>Palm oil stearin:Sunflower oil (70:30)</b></p> <p>Spherical powder particles are observed with fewer fat crystals on the surface.</p>





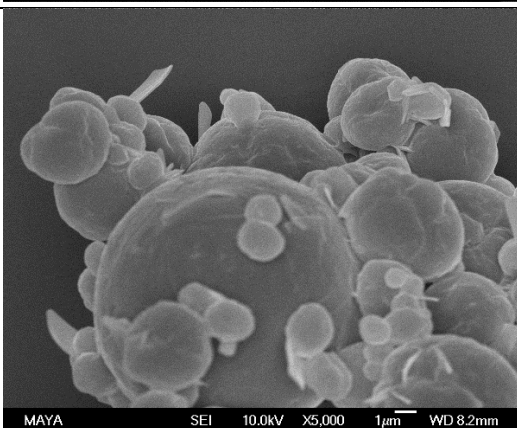
### **Palm oil stearin:Sunflower oil (30:70)**

With the higher amount of liquid fat in the sample, there are almost no fat crystals on the surface of the particles. Some dents can be observed on the powder particles as well.



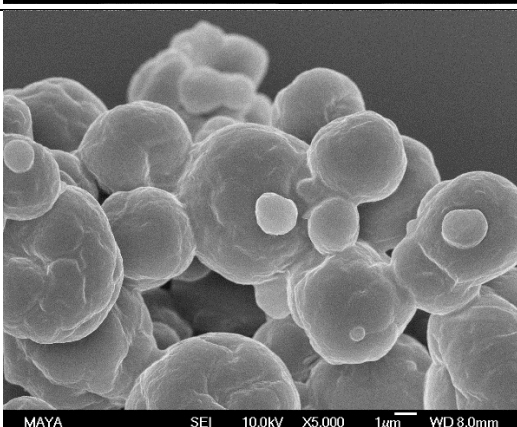
### **Fully hydrogenated rapeseed oil**

Compared to PSt, FHRSO shows higher amount of fat crystals on the surface of the particle. Some of the crystals are sticking out of the surface while some are seen to be embedded to the powder particle.



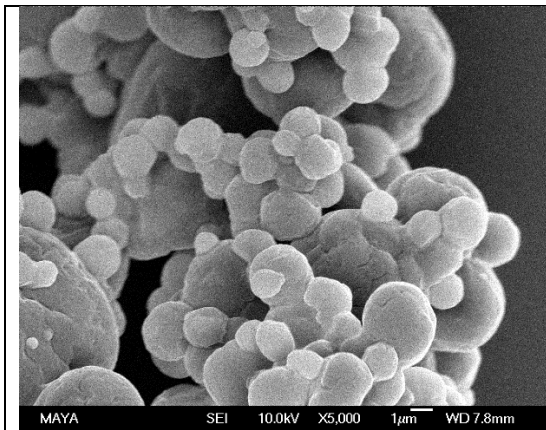
### **FHRSO:SFO (70:30)**

With the addition of liquid fat to the hard fat, the amount of fat crystals has reduced. The particles appear to be spherical with variation in size.



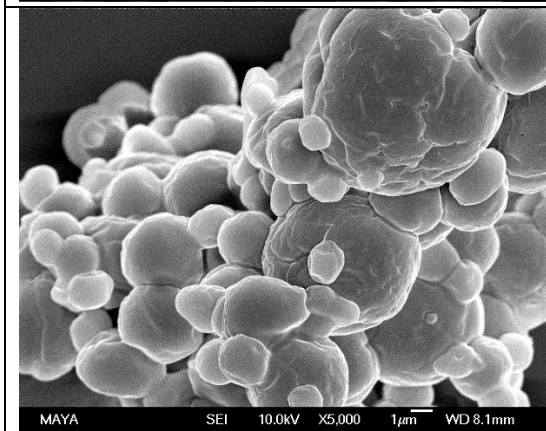
### **FHRSO:SFO (30:70)**

With 70% of liquid fat in combination with FHRSO, there are almost no fat crystals observed on these particles. The particles are spherical with some valleys created on the surface of the particle.



#### **Hard palm oil**

powder particles are agglomerated with particle size being much smaller compared to the rest of the samples.



#### **Palm oil**

These are big agglomerates of the particles with variation in the size. The smaller particles appear to be much smoother compared to the larger particles where some valleys have been created.

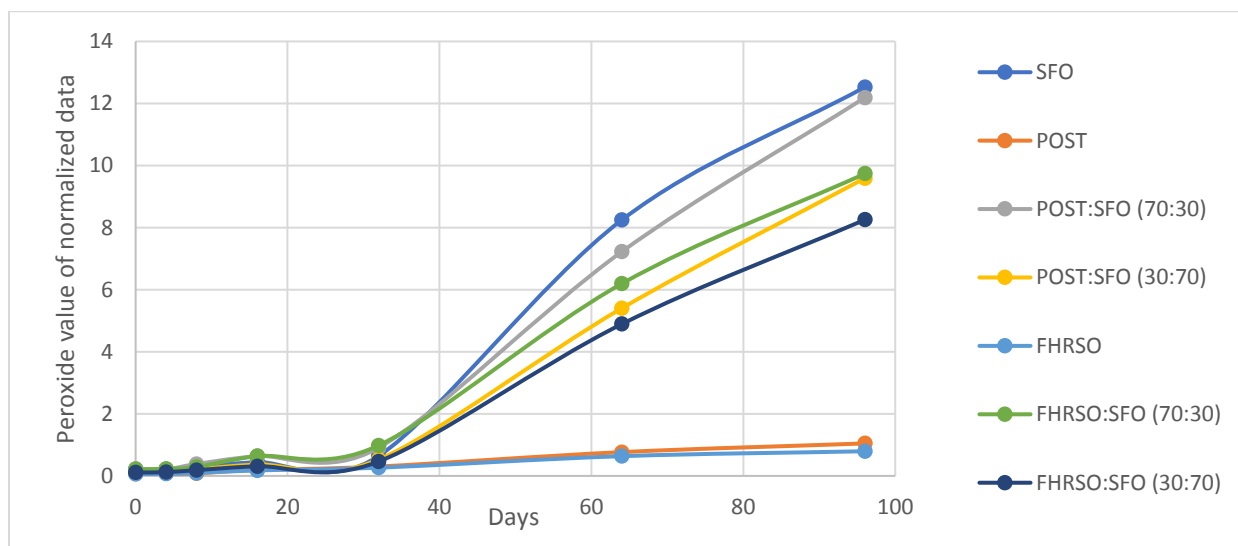
## **4. Discussion**

The study incorporated encapsulation of different fats and their mixtures in milk powder. In order to determine the stability of these samples, numerous techniques such as DSC, particle size determination, surface free-fat extraction, quantifying the extent of oxidation, X-ray crystallography and SEM were carried out.

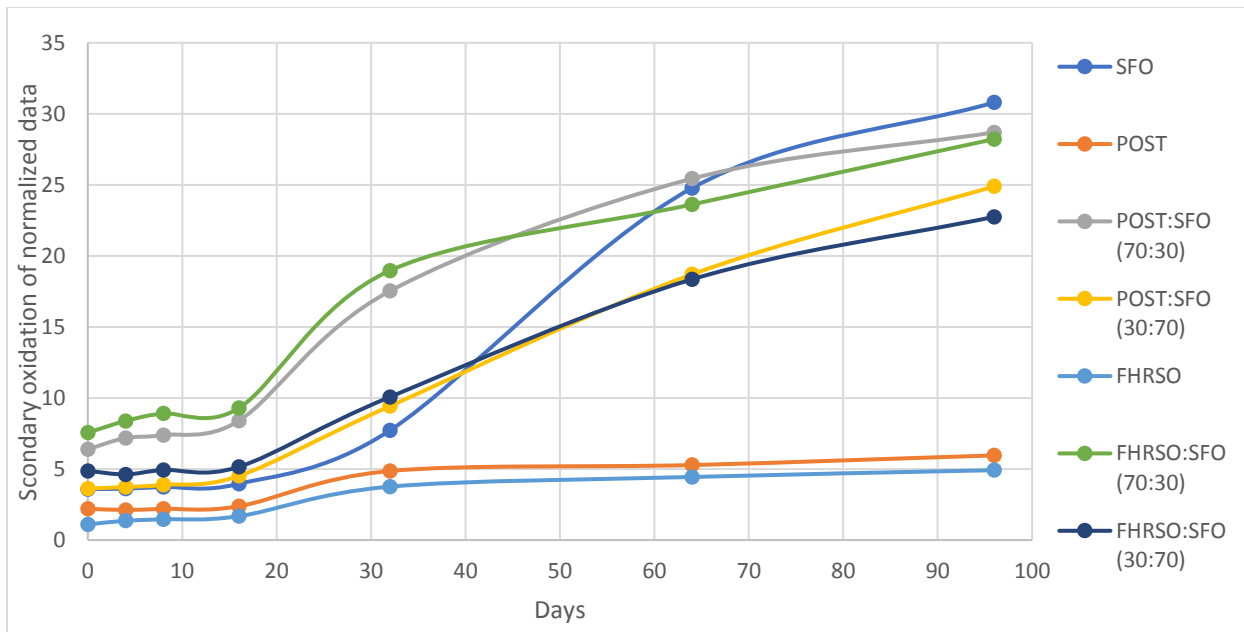
The melting enthalpy curve depicted a narrow increase in melting enthalpy for all the samples during the first 16 days. This increase could have been a might be due to the changes in the crystalline state of the fat in the samples. The melting enthalpy was low when the fats were not in their stable configuration, but as they crystallize into their stable form, the energy required to melt them also increases. The change is the crystalline state of the fat also affects the melting point of the sample. This is what was observed in the first 16 days of the melting point curve as well (figure 4). With the change is the crystalline state of the fat and as they tend to configure in their stable state, the melting enthalpy increases and thus the melting point as well. After 16 days, it was

observed that both the melting enthalpy and melting points did not deviate significantly. This could be due to the stable configuration of the fat after the first 16 days.

The results obtained from the determination of extent of oxidation shows an increase in the amount of both primary and secondary oxidation (figure 7 and 8). SFO had the highest level of oxidation while the hard fats showed the least. Palm oil oxidized less compared to SFO. This is mainly because SFO has higher amount of unsaturation compared to the palm oil fractions. It was important to determine the effect of addition of hard fat with SFO. To conclude the effect of addition of hard fats, the data was normalized and the extent of oxidation was expressed in terms of SFO content. The data was normalized only for the samples with the mixture of hard fat and SFO. To normalize the data, the obtained oxidation value was divided by a fraction of 0.5. This was done because only 50% of the solid content was fat. This obtained value was again divided by the fraction of the SFO to get the final oxidation value in terms of SFO. Figure 9 and 10 shows the graph of normalized values of primary and secondary oxidation against days respectively.

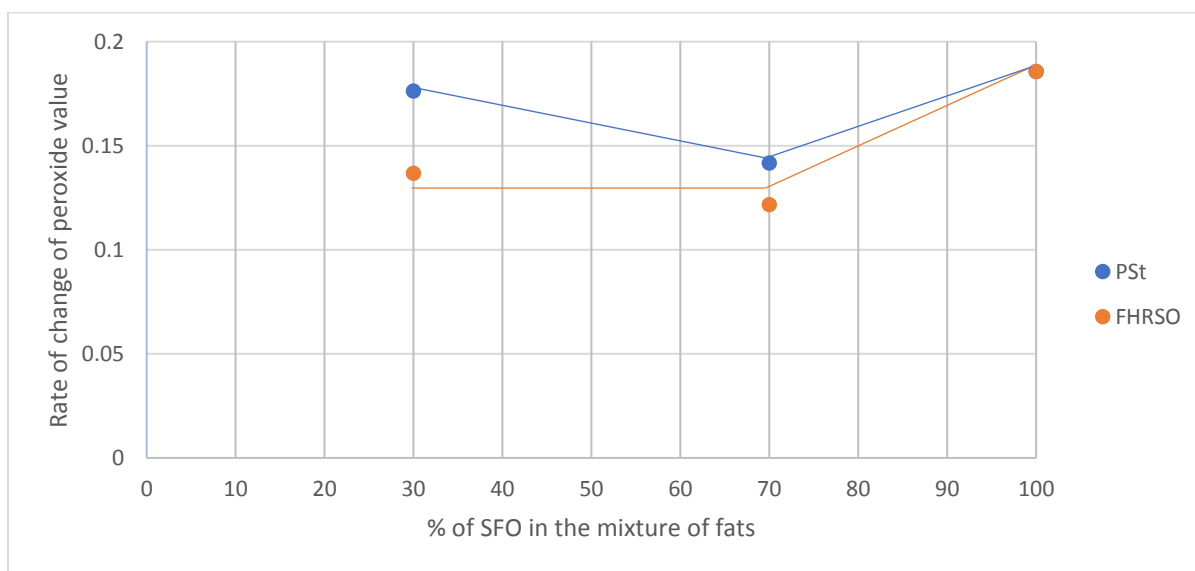


**Figure 9:** Peroxide Value of normalized data

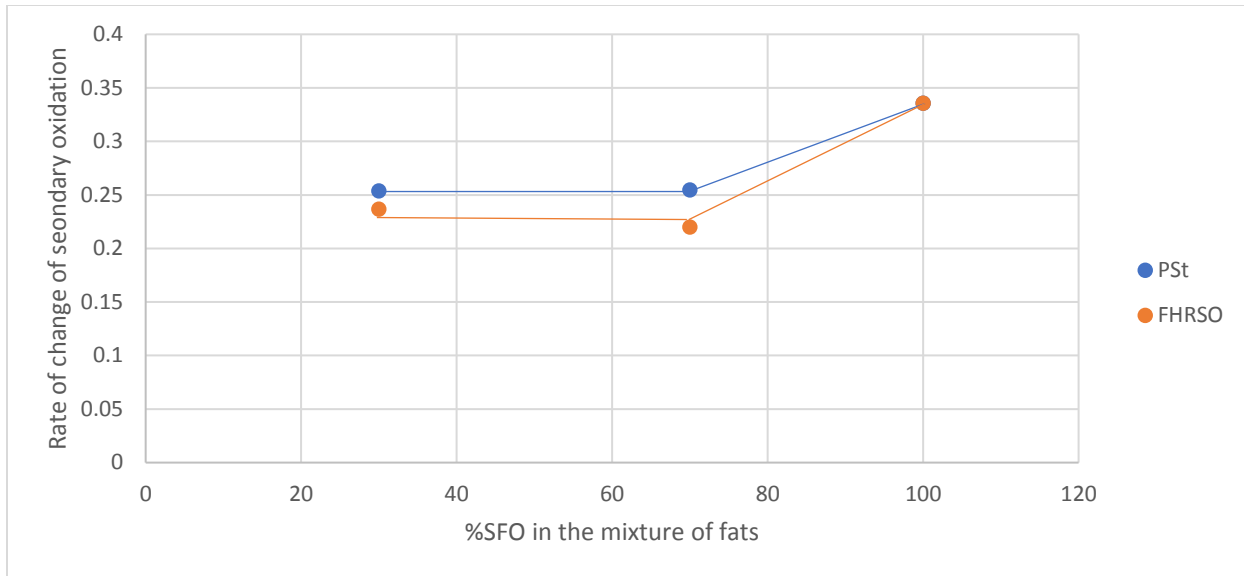


**Figure 10:** Secondary oxidation of normalized data

The graphs obtained from the normalized data shows the extent of oxidation but still does not give a clear picture of the effect of addition of hard fat with SFO. For a better understanding of this, the rate of oxidation was calculated using the slopes. The main intention was to study oxidation stability. The key parameter is thus the oxidation rate as it describes the dynamics of the samples. This rate of oxidation was plotted against the amount of SFO in the mixture. Figure 11 and 12 shows the graph of rate of oxidation against the amount of SFO during the primary and secondary oxidation respectively.



**Figure 11:** Rate of change of peroxide value against amount of SFO



**Figure 12:** Rate of change of secondary oxidation against amount of SFO

The graphs of rate of oxidation against the amount of SFO shows two curves. The blue line represents the mixture of PSt and SFO and the orange line represents the mixture of FHRSO and SFO. In the graph for the primary oxidation, it can be seen that the rate of oxidation is highest when the SFO is 100%. In case of PSt, a minimum is observed when 70% PSt is mixed with SFO and the rate of oxidation rises again when 30% of PSt is mixed with SFO. But in case of FHRSO, a minimum is not clearly observed. There is no significant difference in the rate of oxidation when 30% or 70% FHRSO is mixed with SFO. One clear observation which could be made is that the addition of hard fat reduced the rate of oxidation of the unsaturated fat fraction.

A similar observation can be made with the graph of rate of secondary oxidation against the amount of SFO. But in this case, there is no minima observed for neither of the samples. But again, it is very evident that the addition of hard fat has reduced the rate of secondary oxidation.

The particles size determination showed a constant increase in the size of the fat globules. The highest increase was seen in the liquid fats while the particle size of hard fats did not increase as much as for the liquid fat. The increase in particles size could be because of some fat globules which may come together to form a single fat droplet. Since the powders were stored at 35°C, it would have been easier for the liquid fat to migrate and coalesce with the other fat droplets and hence they have rather rapid increase in the particle size.

From the SEM results, it was seen that hard fats formed crystal structures on the surface of the powder particles. This may be due to improper encapsulation of fat in the milk powder and hence they are present on the surface of the powder particles. With the addition of liquid fat to the hard fat, the encapsulation was much better and hence there are fewer fat crystals seen on the surface. Powder particles in case of SFO showed bulges on the surface. This might be the powder particles which are getting embedded with the other powder particles over the storage time. The high agglomeration of the powder particles might be because of high fat content of the powder which makes them sticky and attach to each other.

## **5. Conclusion**

The results obtained shows that the addition of hard fat to liquid fat brings about changes in the properties and the stability of the fat-filled milk powder. With respect to oxidation, the addition of hard fat reduces the rate of oxidation compared to the rate of oxidation of just the liquid fat in milk powder. But varying the concentration of hard fat did not show a significant difference. From the hypothesis, it was expected the crystallizing fat will make the powder more sensitive to oxidation, but it was observed that it rather had a stabilizing effect on the powder.

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## 7. Appendix

### Appendix 1

Melting enthalpies of fats and powders at different points over the storage period

	Fats (mJ/mg)	Milk Powders (mJ/mg)						
		Day 0	Day 4	Day 8	Day 16	Day 32	Day 64	Day 96
<b>SFO</b>	26.7	5.6	6.2	8.4	8.9	11.6	7.5	4.7
<b>PSt</b>	145	54.8	63.9	77.5	80.8	76.1	73.2	71.0
<b>PSt:SFO (70:30)</b>	110	48.5	50.4	54.5	56.7	56.2	47.5	45.5
<b>PSt:SFO (30:70)</b>	36.3	20	20.8	22.4	23.3	42.9	17.1	16.4
<b>FHRSO</b>	187	61.7	72.1	87.7	94.8	92.2	89.8	87.0
<b>FHRSO:SFO (70:30)</b>	133	49.9	51.7	56.1	59.5	58.2	54.4	53.5
<b>FHRSO:SFO (30:70)</b>	57.5	18.3	18.6	20.4	22.6	21.7	14.7	13.8
<b>Hard Palm oil</b>	28.9	20.9	21.4	24.6	27.4	21.5	13.8	13.5
<b>Palm oil</b>	19.5	5.9	6	7.8	9.2	4.6	14.4	13.4

### Appendix 2

Melting temperature of fats and powders at different points over the storage period

	Fats (°C)	Milk Powders (°C)						
		Day 0	Day 4	Day 8	Day 16	Day 32	Day 64	Day 96
<b>SFO</b>	-26	-19	-18.6	-18.7	-18.9	-18.8	-15.8	-11.9
<b>PSt</b>	60.5	59.4	60	59.9	59.8	60.3	59.9	59.7
<b>PSt:SFO (70:30)</b>	58.6	58.2	59.2	58.7	57.8	57.9	57.9	57.6
<b>PSt:SFO (30:70)</b>	52.6	53.7	54.7	54.1	53	53	53.3	53.2
<b>FHRSO</b>	69.2	69.2	69.5	69.3	68.9	69	69.2	68.7
<b>FHRSO:SFO (70:30)</b>	68.4	66.9	67.6	67.1	66.5	67.2	67.3	67.5
<b>FHRSO:SFO (30:70)</b>	62.7	64.2	66.8	66.3	65.2	65.8	66	65.9
<b>Hard Palm oil</b>	41.5	38	40.8	40	39.1	42.3	42.1	41.2
<b>Palm oil</b>	39.9	38.9	39.4	38.9	37.9	41.2	41.8	42

### Appendix 3

Emulsion size of homogenized milk and reconstituted milk powder

	Homogenized Milk ( $\mu\text{m}$ )	Reconstituted Powder ( $\mu\text{m}$ ) D[3,2]				
		Day 0	Day 4	Day 8	Day 16	Day 96
<b>SFO</b>	0.42	0.35	0.38	0.39	0.42	0.71
<b>PST</b>	0.48	0.53	0.56	0.56	0.58	0.6
<b>PSt:SFO (70:30)</b>	0.45	0.45	0.56	0.53	0.56	0.59
<b>PSt:SFO (30:70)</b>	0.49	0.42	0.49	0.5	0.55	0.61
<b>FHRSO</b>	0.45	0.32	0.34	0.34	0.35	0.52
<b>FHRSO:SFO (70:30)</b>	0.45	0.28	0.28	0.30	0.33	0.54
<b>FHRSO:SFO (30:70)</b>	0.41	0.36	0.42	0.41	0.45	0.59
<b>Hard Palm oil</b>	0.44	0.39	0.45	0.43	0.46	0.59
<b>Palm oil</b>	0.49	0.37	0.38	0.40	0.42	0.61

### Appendix 4

Extracted amount of surface free fat

	Day 0 (g free fat/g powder)	Day 4 (g free fat/g powder)	Day 8 (g free fat/g powder)	Day 16 (g free fat/g powder)	Day 32 (g free fat/g powder)	Day 64 (g free fat/g powder)	Day 96 (g free fat/g powder)
<b>Sunflower Oil</b>	0.0202	0.023	0.0193	0.0158	0.0101	0.0098	0.0096
<b>PSt</b>	0.07	0.06	0.0323	0.0105	0.0099	0.0111	0.0106
<b>PSt:SFO (70:30)</b>	0.0186	0.0195	0.0186	0.0158	0.0153	0.0120	0.0113
<b>PSt:SFO (30:70)</b>	0.0221	0.0198	0.0214	0.0203	0.0189	0.0136	0.0124
<b>FHRSO</b>	0.0055	0.0067	0.0058	0.0034	0.0038	0.0037	0.0049
<b>FHRSO:SFO (70:30)</b>	0.0149	0.0153	0.0122	0.0091	0.0072	0.0081	0.0079
<b>FHRSO:SFO (30:70)</b>	0.0192	0.0185	0.0171	0.016	0.0118	0.0098	0.0092
<b>Hard Palm oil</b>	0.0383	0.0392	0.0284	0.0185	0.027	0.0178	0.0137
<b>Palm oil</b>	0.024	0.0266	0.021	0.0176	0.0228	0.0184	0.0145

## Appendix 5

Peroxide value of powder samples at different points over the storage period

	Day0	Day 4	Day 8	Day 16	Day 32	Day 64	Day 96
	<b>(meq/Kg)</b>						
<b>SFO</b>	0.093	0.102	0.146	0.223	0.321	4.123	6.264
<b>PSt</b>	0.036	0.038	0.042	0.097	0.151	0.383	0.523
<b>PSt:SFO (70:30)</b>	0.042	0.040	0.066	0.116	0.168	1.164	1.938
<b>PSt:SFO (30:70)</b>	0.049	0.052	0.084	0.149	0.216	1.971	3.467
<b>FHRSO</b>	0.031	0.033	0.047	0.089	0.132	0.318	0.397
<b>FHRSO:SFO (70:30)</b>	0.038	0.041	0.053	0.114	0.175	0.996	1.544
<b>FHRSO:SFO (30:70)</b>	0.045	0.049	0.075	0.128	0.19	1.779	2.972
<b>Hard Palm oil</b>	0.053	0.055	0.074	0.135	0.187	2.357	3.745
<b>Palm oil</b>	0.061	0.064	0.098	0.156	0.223	2.194	3.386

## Appendix 6

Secondary oxidation value of powder samples at different points over the storage period

	Day 0	Day 4	Day 8	Day 16	Day 32	Day 64	Day 96
	<b>(mg malonaldehyde/L emulsion)</b>						
<b>SFO</b>	1.79	1.81	1.87	1.98	3.86	12.39	15.4
<b>PSt</b>	1.1	1.06	1.1	1.19	2.43	2.64	2.98
<b>PSt:SFO (70:30)</b>	1.19	1.30	1.34	1.51	3.14	4.37	4.93
<b>PSt:SFO (30:70)</b>	1.5	1.53	1.59	1.83	3.81	7.10	9.34
<b>FHRSO</b>	0.55	0.68	0.73	0.84	1.88	2.22	2.46
<b>FHRSO:SFO (70:30)</b>	1.25	1.4	1.49	1.57	3.24	4.01	4.75
<b>FHRSO:SFO (30:70)</b>	1.82	1.76	1.88	1.98	3.92	6.89	8.48
<b>Hard Palm oil</b>	0.8	0.98	0.96	1.02	1.96	8.73	9.47
<b>Palm oil</b>	2.4	2.1	0.56	0.75	1.51	8.32	9.03

## Appendix 7

Composition of TMP solution and milli-Q water to obtain a standard curve

	<b>0<math>\mu</math>M TMP</b>	<b>8<math>\mu</math>M TMP</b>	<b>16<math>\mu</math>M TMP</b>	<b>24<math>\mu</math>M TMP</b>	<b>32<math>\mu</math>M TMP</b>
<b>Milli-Q Water (ml)</b>	50	46	42	38	34
<b>Solution B (ml)</b>	0	4	8	12	16
<b>Corresponding ppm</b>	0	1.31	2.62	3.94	5.2