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Effect of High Intensity Ultrasound on the Crystallization Behavior of Interesterified Fats

Jeta Vijay Kadamne
Utah State University

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EFFECT OF HIGH INTENSITY ULTRASOUND ON THE CRYSTALLIZATION

BEHAVIOR OF INTERESTERIFIED FATS

by

Jeta Vijay Kadamne

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

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UTAH STATE UNIVERSITY
Logan, Utah

2018
ABSTRACT

Effect of high intensity ultrasound on the crystallization behavior of interesterified fats

by

Jeta Kadamne, Doctor of Philosophy

Utah State University, 2018

Major Professor: Dr. Silvana Martini
Department: Nutrition, Dietetics, and Food Sciences

This study evaluates the effect of high intensity ultrasound (HIU) on the crystallization behavior of interesterified fats (IE) with saturated fatty acids at the sn-2 position. Also, the combined effect of agitation, temperature, and HIU on crystallization behavior of a commercial IE was studied in a separate study. Results show that at lower temperatures, HIU was most effective when agitation was stopped just prior to sonication versus agitation after sonication. The study on the IE fats involved IE samples with palmitic and stearic acid at the sn-2 position along with their physical blends. The amount of these fatty acids at the sn-2 position was either 20 or 30%. The effect of HIU was compared based on their microstructure, solid fat content, melting behavior, and rheological properties. Research showed that HIU was effective at forming small crystals and at increasing solid fat content, rheological properties. Data showed that increased saturation improved the effectiveness of HIU in palmitic based samples. The presence and crystallization of higher melting triacylglycerols (TAG) in stearic based samples induced superior rheological properties compared to the stearic samples. HIU also
induced crystallization of lower melting TAG and this effect was observed all the crystallization temperatures.

The IE sample with 30% stearic acid at the sn-2 position sample had approximately 2% tristearin. The crystallization of the tristearin free fractioned sample showed that HIU was effective at inducing crystallization and developing small crystals in the sample even in the absence of high melting TAG.

The descriptive study on flavor perception of 2-butanone and 2-nonanone from these fats showed that flavor perception was higher from solid IE samples compared to the liquid samples (p<0.05). Results indicated that for the use of sonicated samples in food products, adjustments must be done to optimize the flavor profile of the food product for the best sensory appeal. The findings from this dissertation showed that HIU is effective at inducing harder texture in samples with low saturated non-trans IE-fats by inducing smaller crystals and crystallization of lower melting TAG. The effect of HIU varies and is more effective in fats with higher melting TAG.

(309 Pages)
PUBLIC ABSTRACT

Effect of high intensity ultrasound on the crystallization behavior of interesterified fats

by

Jeta Kadamne

The process of partial hydrogenation produces \textit{trans} fats and the fats that undergo this process are called partially hydrogenated fats (PHF). Clinical studies have shown a strong association between PHF and coronary heart diseases. In 2015 The U.S. Food and Drug Administration removed the \textit{Generally recognized as safe} or “GRAS” status of PHF. These fats were used in confectionary, margarines, shortenings, doughnuts, cookies, cakes, etc. The PHF serve a function in foods by providing a higher shelf life and a desired harder structure due to their higher melting point. Hence, the food industry is currently looking for PHF alternatives which serve the function but have no harmful health effects. One of the alternatives to replace PHF is to use interesterified fats that have a low level of saturation that makes them healthier. However, these new fats are too soft with restricted use in many food applications. In this study, we explored the use of high intensity ultrasound (HIU) to improve the functional properties of interesterified fats and make them harder. Our study showed that HIU formed small crystals in these fats and increased their viscosity. The results from this study on the flavor release from the interesterified fats showed that the physical structure and hence the amount of solid fat in the sample affected its flavor perception. The solid fats had higher flavor perception than the liquid fat samples. The goal of this study is to improve the functionality of the
interesterified fats using HIU and understand the flavor release from these fats to make substitution in food products easier.
This dissertation is dedicated to my husband
DR. RAHUL PATIL
ACKNOWLEDGMENTS

I would like to thank Dr. Silvana Martini for accepting me into her research group and her continued support, guidance, mentorship towards my degree and research throughout these years. I would also like to thank my committee members Dr. Marie Walsh, Dr. Donald McMahon, Dr. Jerrad Legako and Dr. David Britt for their research suggestions and assistance throughout my Ph.D. I give special thanks to Dr. Roberta Claro da Silva, Dr. Ashwini Wagh, Dr. Ying Lu, Xiaoxi Wang, Dr. Sarbojeet Jana, Ms. Jiwon Lee, Ms. Juhee Lee, Ms. Arkopriya Chail, Dr. Yubin Ye, Tara Johnson and Alana Ward for their help and support over these years.

I would like to thank my mother, Jayashri V. Kadamne for instilling in me the importance of education and to always strive for the best and to my father, Late Vijay V. Kadamne for his unconditional love. I would also like to thank my grandparents, Dr. V.B. Kadamne, Mrs. Vimal V. Kadamne, Late (Adv.) B.T. Kate and Late Mrs. Kumudini B. Kate for their love, support and encouragement throughout my life.

A big thank you to my husband, Dr. Rahul Patil for his support and understanding with this journey and for being there always.

Jeta Vijay Kadamne
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<tr>
<td>AMF</td>
<td>Anhydrous milk fat</td>
</tr>
<tr>
<td>AOCS</td>
<td>American Oil Chemists' Society</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>ECN</td>
<td>equivalent number of carbons</td>
</tr>
<tr>
<td>EFA</td>
<td>Essential fatty acids</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acids</td>
</tr>
<tr>
<td>G'</td>
<td>storage modulus</td>
</tr>
<tr>
<td>G''</td>
<td>elastic modulus</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>GM</td>
<td>Genetic modification</td>
</tr>
<tr>
<td>GMP</td>
<td>Genetically modified oil</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HDL-C, C</td>
<td>High density lipoprotein cholesterol</td>
</tr>
<tr>
<td>IE</td>
<td>Interestified fats</td>
</tr>
<tr>
<td>IESBO</td>
<td>Interestified soybean oil</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LERO</td>
<td>Low erucic rapeseed oil</td>
</tr>
<tr>
<td>LOO</td>
<td>triolein</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectroscopy</td>
</tr>
<tr>
<td>MUFA</td>
<td>monounsaturated fatty acids</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>No HIU-</td>
<td>no HIU</td>
</tr>
<tr>
<td>No HIU- CA</td>
<td>no HIU with continued agitation</td>
</tr>
<tr>
<td>HIU@10-A10</td>
<td>HIU at 10 min with agitation stop at 10 min</td>
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<tr>
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<tr>
<td>HIU@10-A10</td>
<td>HIU at 10 min with continued agitation</td>
</tr>
<tr>
<td>HIU@10-CM</td>
<td>HIU at 10 min with continued agitation</td>
</tr>
<tr>
<td>LOO</td>
<td>1-linoleoyl-2-oleyl-3-oleyl-snglycerol</td>
</tr>
<tr>
<td>LPO</td>
<td>1-linoleoyl-2-palmitoyl-3-oleyl-sn</td>
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<td>no HIU with agitation stop at 10 min</td>
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<td>HIU@10-A10</td>
<td>HIU at 10 min with agitation stop at 10 min</td>
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CHAPTER 1
INTRODUCTION

Lipids in foods provide a significant source of energy and essential fatty acids [1], they provide structure and mouthfeel to foods [2] and they also act as a delivery media for lipid-soluble vitamins and flavors [1]. In food applications, the ideal lipid should have a broad range of physical and functional properties. Shortenings are lipids that occur as: 
(a) semisolids, (b) liquids, or (c) pallets or powders [3]. Semisolid shortenings are used for all-purpose applications where their physical properties are tailored to specific uses such as cakes, icings, whipped toppings and frying [3, 4]. These physical properties can be optimized by choosing shortenings of different chemical compositions [5] or through processing conditions [6, 7]. Liquid shortenings are mainly used in the production of yeast-raised and chemically leavened baked goods [8]. The advantages of liquid shortenings are easy transportation and pourability. Pallets and powdered shortenings are hard stock shortenings produced through hydrogenation and are commonly used in puff pastry or when a very sharp melting profile is needed [3, 9]. The advantage of this type of shortening is that it is easy to handle and it re-melts easily [10]. *Trans* fatty acid (TFA) based shortenings are ideal for food applications since, depending on the processing conditions used and the amount of TFA in the shortening, they can be used as plasticized semisolids, liquids, or flakes. That is, TFA based shortenings can be formulated with a wide range of functional properties that make them ideal for any food application [11].

Since 2015, partially hydrogenated oils (PHO) do not have a *GRAS* status [12] and hence the food industry is directed towards replacing the PHO’s in food systems. Industrially generated PHO are the main source of artificially formed TFA. The healthier
PHO alternatives would be fats free of trans fats and low in saturated fatty acids. The major problem faced by food producers when replacing TFA in foods with healthier lipid is achieving desired functional and physical properties of the bulk lipid [13]. TFA are produced through hydrogenation of different types of oils. As stated earlier, the type of oil used and the specific processing conditions used to hydrogenate those oils can generate TFA-based fats with a wide range of customizable functional and physical properties for use in different applications by the food industry. The versatility of TFA-based fats is difficult to achieve with healthier TFA-free fat sources that have a lower content of saturated fatty acids (SFA) and higher contents of mono- and polyunsaturated fatty acids [13]. When low-fat foods are produced or when healthier fats are used, this multifunctionality is lost and the physical and sensorial quality of the reformulated product changes [13, 14].

Over the years, food producers have successfully substituted fats with high content of TFA with palm-based fats [4], fat blends [15], or interesterified fats [IE] [16]. Palm-based fats provide a partial solution to the TFA problem, but they are far from ideal since they can have high amounts of saturated fats which increase the incidence of cardiovascular diseases [17] compared to low saturated vegetable oils. Therefore, food producers are constantly searching for novel technologies or fats that can produce lipids with low SFA content and enhanced nutritional properties without compromising physicochemical and sensory quality.

Apart from zero trans fats, customers are looking for healthier lipids with a lower content of SFA and a higher content of cis-, mono-, and -polyunsaturated fatty acids. Many spreads have been reformulated with fats containing these fatty acids and these
include Bertolli (blend of rapeseed, palm, sunflower and olive oil) [Unilever, UK, London, UK], Smart Balance (blend of canola, palm, fish, flax, olive and soybean oils) [Pinnacle Foods, Inc., Parsippany, NJ, USA], Earth balance (blends of palm fruit, canola, flax, algal oils and olive oil) [Pinnacle Foods, Inc., Parsippany, NJ, USA], and I can’t believe it’s not Butter! (olive oil) [Unilever, Englewood Cliffs, NJ, USA]. Even though TFA have largely been eliminated from food products in the USA, food companies are constantly looking for healthier lipids that possess desirable functional and physical properties for use in food formulations. The nutritional and functional properties of fats strongly depend on their fatty acid and TAG composition.

Fats primarily consists of TAG which have a glycerol backbone. A glycerol molecule has a three-carbon chain with a hydroxyl functional group on each of the carbon atoms. The Fischer projection of the glycerol molecule is shown in Figure 1. TAG are formed when each of the hydroxyl functional groups on the glycerol molecule are esterified to three fatty acids (Figure 1). The positional isomers of TAG are differentiated from each other based by stereospecific numbering of the carbon atoms on the glycerol backbone. In the Fischer projection of the glycerol molecule, the second hydroxyl group denoted to the left represents the sn-2 position, while the hydroxyl groups above and below it represents the sn-1 and sn-2 position, respectively.

By using the process of interesterification (enzymatic or chemical), fats can be tailor-made using desired fatty acids and provide a viable and healthy solution for reducing TFA in many food formulations [16, 18-20]. Recent research has shown that TAG with saturated fatty acids at the sn-2 position are a healthy lipid source as they lower postprandial lipemia [21, 22]. The first group of the IE fats developed for this study
used a blend of fats rich in triolein (OOO) and tripalmitin (PPP) as the starting material. The blend was enzymatically interesterified until all the PPP was consumed in the reaction to develop IE rich in TAG with palmitic acid at the sn-2 position. The second group of IE fats were developed with starting blend of fats/oils rich in OOO and tristearin (SSS) and enzymatically interesterified until all the SSS was consumed [23]. The goal was to develop an IE with TAG rich with stearic acid at the sn-2 position.

The current study focuses on two problems:

1. TFA has a harder texture at room texture due to the denser packing of the molecules which imparts a higher melting point. In contrast, fats containing TAG with SFAs at the sn-2 have a softer texture and hence lack the physical properties of TFA. This limits their use to only a few food applications. Therefore, if these healthy fat sources are to be used in the food industry, new processing technologies need to be developed to improve their functional properties. Several studies have shown that high intensity ultrasound (HIU) can be used to induce crystallization of fats and significantly improve their functional
properties, including texture, viscoelasticity, and melting profiles [24-27]. Therefore ultrasound-induced crystallization could be used in IE fats tailored with SFAs at the sn-2 position to improve their functional properties and allow the formulation of healthier foods.

Although the effect of HIU has been studied in different lipid systems [24-26, 28], the effectiveness in low saturated fats (20-30% saturated fatty acids) have not been investigated so far. Also, comparison of the effects of HIU on fats with similar fatty acid but different TAG structure has not been done either. The gap in knowledge regarding the effect of HIU on low saturated fats and changes in TAG and fatty acids need to be addressed.

2. Reformulation of foods with the change in the type and the amount of fat causes changes in the flavor of foods. Also, almost nothing is known of the role of lipid structure and chemical composition on flavor perception in bulk. Therefore, sensory studies aimed to understand flavor perception in bulk lipids can provide insight into product reformulation with changes to lipid type.

**Hypothesis**

The crystallization behavior and functional properties of IE with saturated fatty acids at the sn-2 position can be changed by the application of HIU. In addition, we hypothesize that the chemical composition and crystalline structure of the fats affect flavor release.

**Objectives**

1. To understand the effect of the application of HIU at different agitation conditions and crystallization temperatures on the crystallization behavior of a
commercial IE soybean oil. The effects of HIU on the induction period of crystallization, crystal microstructure, solid fat content, melting characteristics, rheology and X-ray will be evaluated.

2. Determine the effect of high intensity ultrasound at different supercooling levels on the crystallization behavior of IE with 20 and 30% palmitic acid at the sn-2 position. Evaluate and compare the functional properties of the non-sonicated and sonicated IE with their non-sonicated physical blends.

3. Evaluate the effect of high intensity ultrasound at different supercooling levels on the crystallization behavior of IE with 20 and 30% stearic acid at the sn-2 position.
   a) Quantify and compare the functional properties of non-sonicated and sonicated IE and their physical blends at the same supercooling level
   b) Quantify and compare the functional properties of non-sonicated and sonicated IE at lower supercooling levels
   c) Draw conclusions on the effect of HIU on functional properties of IE with stearic and palmitic at the sn-2 position from the previous study.

4. Fractionate the IE with 30% stearic acid at the sn-2 position to remove the residual tristearin and to evaluate if sonication affects the crystallization behavior or functional properties of the fractionated sample

5. Perform a descriptive sensory analysis to evaluate if there is a change in flavor intensity perception with the change in the fat type (liquid IE and physical blends containing stearic and palmitic acid) or upon sonication (crystallized IE samples containing both stearic and palmitic acid)
The study presented here will focus on the use of HIU to change the bulk structure of IE fats which will improve their functional properties making them suitable for TFA substitution in food products. Due to their softer texture, the IE provide an ideal experimental system to understand the extent of change induced by HIU. By comparison of the flavor perception in sonicated and non-sonicated samples, IE fats will also provide an excellent system with different crystalline structure to understand the link between the physicochemical properties of lipids and their flavor perception.

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CHAPTER 2

LITERATURE REVIEW

**Nutritional properties of lipids**

Lipids provide 9 kcal/g of energy [1] and the American Heart Association recommends a lipid intake of < 30% of the total daily calorie intake [2]. Edible lipids consist mainly of triacylglycerols (TAG) which are composed of three fatty acids esterified to a glycerol backbone. The properties of edible lipids depend on the type of fatty acids such as saturated, monounsaturated, and polyunsaturated along with their position on the glycerol backbone. Edible lipids are obtained either from meat, milk, or plant sources. The American Heart Association recommends consuming lipids from nuts, vegetable oils, or fish as they contain mono- and polyunsaturated fatty acids. Lipids also play an important role as delivery media for lipid-soluble vitamins and flavor and are sources of essential fatty acids (EFA) such as alpha linoleic acid (\(\omega-3\) C18:2) and linoleic acid (\(\omega-6\) C18:2). [3]. The body requires EFA for cell growth and nourishment, brain development, regulation of water loss from the skin, blood pressure regulation and for the development of the placenta and mammary glands in pregnant women [4]. A higher intake of EFA than average is recommended for pregnant women to compensate for the requirements of fetal growth [4]. Conjugated linoleic acid, an EFA has anti-cancer, anti-obesity and anti-inflammatory properties and is also known to reduce blood pressure and improve bone health [5].

A meta-analysis by Mensink and Katan [6] showed that upon substitution of carbohydrates by unsaturated fats at the same calorie level, there was reduction in the serum low density lipoprotein cholesterol (LDL-C) levels, however the effect was lower
for monounsaturated fatty acids (MUFA) than polyunsaturated fatty acids (PUFA). The most common monounsaturated fatty acid is oleic acid and it is found in olive oil and high oleic oils produced by genetic modification. Substitution of carbohydrates from the diet by unsaturated fats decreased the risk of coronary heart disease (CHD). Similar to the effect on the LDL-C, the CHD lowering effect was higher for PUFA than MUFA. Keys et al. [7] also showed that CHD risk was lower in populations where diet was rich in olive oil which is a source of MUFA. Shai et al. [8] conducted a trial to compare the effects of a low fat Mediterranean diet and a low carbohydrate diet on weight loss, diabetes biomarkers, cholesterol, and TAGs. The Mediterranean diet was fixed on calories (1500 kcal for women/day and 1800 kcal for men/day), was heavy on vegetables, rich in MUFA, and 35% of calories were obtained from fats and oils. The data collected over two years showed that the MUFA rich diet induced weight loss and was effective in diabetes regulation by significantly reducing the fasting glucose and insulin levels compared to the other diets. The diet also showed to increase high density lipoprotein cholesterol (HDL-C) levels, decrease TAG and lower LDL-C levels.

SFA containing lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) are used in food applications including cookies, pie crusts, cakes, frosting, spreads, etc. These fats are solid at room temperature and they are usually obtained from animal or vegetable sources. SFA were found to increase the LDL-C concentration while also increasing the HDL-C concentrations [9]. Raised LDL-C levels are strongly correlated to progress of CHD. Several studies [10-12] concluded a higher risk of CHD with the consumption of saturated fats.
The dietary guidelines of 2015-2020 for an average healthy adult for fats and oils consumption suggest a total avoidance of \textit{trans} fats with <10\% calories of daily calories from saturated fats and an increase in the consumption of mono- and polyunsaturated fats at the expense of saturated fats \cite{13}. These guidelines come with a recommendation of a regular physical activity for well-being. These can be achieved by reading the food labels and choosing the right types of lipids for cooking.

\textit{Trans} fatty acids or TFA are found either naturally in ruminant meat and milk fat or in industrially manufactured PHO \cite{14, 15}. Partial hydrogenation process is used to saturate some of the unsaturated fatty acids by adding hydrogen atoms across them to increase its oxidative stability and also the solid fat content in the oil which converts a the oil from liquid to semisolid texture \cite{14}. The hydrogenation reaction of linolenic acid is shown in Figure 2-1. Linolenic acid has 3 double bonds which upon addition of H$_2$ in presence of a catalyst saturates a double bond to produce linoleic acid. The double bonds in the linoleic acid could be \textit{cis/cis}, \textit{cis/trans} or \textit{trans/trans}, due to geometric isomerization \cite{16}. The molecules may also undergo positional isomerization and the positions of the double bonds on the fatty acid chain may change \cite{16}. The isomerization occurs when the fatty acids are desorbed from the catalyst surface \cite{16}. With addition of H$_2$, the linoleic acid gets saturated until it is converted to stearic acid with no double bonds \cite{16}. The double bond in the oleic acid also could be either \textit{cis} or \textit{trans} with change in the positions of these double bonds.

The adverse effects of consumption of TFA generated by partial hydrogenation on the risk of developing cardiovascular diseases has been shown by several authors. In a meta-analysis by Mensink and Katan \cite{9} it was found that substitution of carbohydrates
by TFA formed by partial hydrogenation at 1% energy levels increased the LDL-C levels and decreased the HDL-C levels which poses as a risk factor for CVD. There was a significant increase in the Total:HDL-C cholesterol ratio compared to other classes of fatty acids – saturated, cis-monounsaturated and cis-polyunsaturated [9]. Koba et al. [18] in 2002 showed strong correlation between smaller and denser LDL-C and coronary artery disease and a subsequent study in 2003 by Maugher et al. [19] showed that TFA (created by partial hydrogenation) consumption reduced the LDL particle size.

Apart from changes in LDL-C and HDL-C levels, industrially generated TFA were also shown to raise the serum TAG and Lp(a) lipoprotein which are risk factors for cardiovascular diseases (CVD) [20] and increased the (Apo)B and decreased the ApoA-I levels which are CHD risk factors [21]. TFA consumption has also been shown to reduce
insulin sensitivity [21] and to induce pro-inflammatory effects by raising the TNF-α and IL-6 levels [22].

The high TFA levels in industrially produced PHO is the cause of concern for the various health effects of TFA and hence these industrially generated PHO should be removed from food products. The health and clinical studies show that substitution of TFA by healthier MUFA and PUFA will provide added health benefit over negative effects of TFA.

The role of fats and oils in food products

Fats and oils provide mouthfeel, flavor, and appearance to foods and serve as a heat exchange medium for frying applications [23]. In food products, these are used as bulk fats as in chocolates and frying, in oil in water emulsions such as milk, coffee creamers, or chocolate milk, and water in oil emulsions such as butter and spreads. Fats provide creaminess to ice creams, yoghurts, cream cheese and mayonnaise, or thickness to an emulsion beverage, snap and gloss to chocolates and spreadability to butter, margarines, and shortenings. These properties are a result of the composition of the fat and the polymorphic form of the fat crystals and crystalline network of the bulk fat. For example, the β form of cocoa butter imparts the snap and glossiness to the chocolate, β’ imparts plasticity to the shortenings while β form gives pourability to the shortening [24]. Hence palmitic rich fats are favored in bakery applications as they mainly crystallize in the β’ polymorph which provides the desired plasticity [25].

Lipids with high levels of saturated fatty acids are solid at room temperature and are called fats; while lipids with high levels of unsaturated fatty acids are liquid at room temperature and are called oils. Choice of lipids in food products are based on the texture
they provide, their fatty acid composition, flavor, stability in food products, market needs, and nutritional properties. In order to improve spreadability at refrigerated conditions, lower the levels of saturated fat and to include unsaturated fatty acids in spreads, blend of oils with the required characteristics may be used. Poor selection of fats or processing conditions can affect the quality of the final product. For example, the use of highly unsaturated fats in frying operations can induce rancid notes in the final products [26] or poor tempering and storage conditions of confectionery products can change the polymorphic form of cocoa butter and thus induce bloom in chocolates [27]. Use of saturated fats for salad dressing may be inappropriate as it may crystallize and restrict spreadability and hence reduce the overall appeal. Also, change in the cooling temperature, cooling rate, agitation, etc., may change the microstructure of the fat which may eventually change the strength of the crystalline network leading to changes in rheological properties of the fat and/or the food network [28].

Thus, fats and oils are fundamental to the final flavor and texture of foods. Processing of fats and oils demands thorough considerations to keep the properties of the food product intact and constant.

Alternate processing techniques to develop trans-free fats

(i) Fractionation

Fractionation processes have been extensively used in the food industry to separate high and low melting fractions of fats [29]. This technique entails separation of the fractions based on physical process of separation with no changes to the basic molecular structure of the fatty acids in the TAG molecule. Dry fractionation involves melting the fat followed by partial and controlled crystallization to induce nucleation and
eventually grow larger crystals which are then subsequently filtered [30]. The separated liquid portion is termed as olein fraction while the solid fraction is called the stearin fraction. Crystallization is performed at precise temperatures to separate fat fractions with precise solid fat curves and melting points [31]. Hence, it is a very important step for the quality of the fractions separated and the yield of the process. This process employs either static or stirred crystallizers and vacuum or membrane filters. Alternatives to dry fractionation include solvent fractionation or detergent fractionation where solvent or detergent are added respectively to the oil for separation purposes. Fractionation is also employed during edible oil refining (termed as winterization) to separate the high melting fat fractions or waxes from oils such as soybean, sunflower, cottonseed, and peanut to develop crystal free clear oil at room temperature [25]. Palm oil (PO) (melting point 35 ºC) is commonly fractionated into several fractions of different iodine value based on their melting points. These fractions include palm super olein (melting point 10 ºC), palm olein (melting point 20 ºC), soft palm mid fraction (melting point 25-30 ºC), palm mid stearin (melting point 30 ºC) and palm stearin (melting point 48 ºC) [29, 32]. The removal of the lower melting unsaturated TAG fractions imparts oxidative stability to the stearin fraction. Also, the stearin fractions such as palm stearin have a hard texture similar to the one observed in partially hydrogenated fats and hence can find applications as trans-free fat.

(ii) Genetic modification

Genetic modification (GM) is used to generate broader functionality in oils and to improve their nutritional value using pre-harvest techniques. For example, the presence of linoleic and linolenic acid in soybean oil (SBO) imparts low oxidative stability
towards high heat operations such as frying. Tools of mutagenesis and genetic breeding can be used to develop SBO with lower levels of polyunsaturated fatty acids or higher oleic and stearic levels [33]. These changes alter its functionality by imparting higher shelf life, stability, and increased oxidative stability to the oil needed for frying operations. Researchers have compared the fatty acids in the GM and non-GM oils and have confirmed that the fatty acids from the two sources are chemically and biologically identical. The Omega-9 oil by Bunge is a Genetically modified oil (GMO) which is a high oleic (>70%) low linoleic (<3%) canola oil which has a cleaner taste and lower polymerization over extended use. The Vistive gold from Monsanto is a low saturated high oleic, no trans SBO with higher shelf life and improved nutritional properties. A high stearic (40%) cottonseed oil was developed by Liu et al. [34] using genetic engineering where normal levels of stearic acid in cottonseed oil is only approximately 2-3%. This changed fatty acid profile changes its functionality to a semisolid fat that provides a trans-free oil source for margarine or confectionery applications. A high stearic SBO (45.4%) and a high saturated sunflower oil was also developed by Graef et al. [35] and Osorio et al. [36], respectively using genetic engineering.

The trans free GMOs possess a significant market potential due to better nutritional profile and functionality. Although non-GM oils lack functionality, the GM oils also require metabolic studies for each new oil before introduction in the food market. Thus, each oil type- GM or non-GM has its positives and negatives and their selection for food applications will depend on the functionality, nutritional profile, cost, and availability.
(iii) **Interesterification**

Interesterification is a chemical process used to modify the TAG composition of lipids by repositioning the fatty acids on the TAG while keeping the fatty acid composition constant. The rearrangement of the fatty acids can be done either between two fats or within the same fat. The process can be performed either through chemical route which randomizes the fatty acids or by enzymatic routes using lipases which is more specific on the final arrangement of fatty acids. Enzymatic interesterification is generally preferred over chemical interesterification due to the randomization effect and low specificity of the chemical interesterification process [37]. Most commonly used lipases for interesterification reactions are lipases from Rhizopus sp. and Thermomyces lanuginosa [38]. An interesterification reaction between (a) two TAGs and (b) a TAG and a fatty acid by an enzymatic route is shown in figure 2-2.

The change in the TAG composition affects the melting profiles of the oil to induce different functionality in the oil. The interesterified oil compared to the starting oil has different crystallization properties and hence different microstructure [28, 39], solid fat content [40], rheology [41], hardness and even polymorphism [39]. These properties determine important functional properties such as spreadability, flavor release, and hardness.
Enzymatic interesterification has specific end products in comparison to the randomization effect of chemical interesterification. One of the main reasons that decreases the specificity of the end products of enzymatic interesterification formed is acyl migration. Iwasaki et al. [43] describes the first step in the interesterification of a TAG with a sn-1,3 specific lipase to be the diacylation at sn-1,3 position to form a 2-monoacylglycerol (MAG). Kodali et al. [44] further explains that oxygen atom on the hydroxyl group at the primary positions is a nucleophile that attacks the secondary position, which upon formation of multiple intermediate products, causes the movement of the fatty acid from the secondary to the primary position. Acyl migration can occur in the presence of either an diacylglycerol (DAG) or a monoacylglycerol (MAG) since they both possess the free hydroxyl group which can provide the free lone pair of electrons for the nucleophilic attack [44, 45]. Kodali et al. [44] list the presence of acidic or basic experimental conditions along with the ease of the breakdown of the intermediate compounds responsible for acyl migration. Laszlo et al. [45] studied synthesis of 1,2-DAG and reported the formation of 1,3-DAG as a byproduct of acyl migration. Yang et. al [46] identified high temperature to be an important factor facilitating acyl migration.
Their group studied the acyl migration during the interesterification of tripalmitin (PPP) with caprylic acid and between PPP and conjugated linoleic acid. They [46] found that acyl migration was significantly reduced upon low temperature programming although it could not be completely eliminated. Xu et al. [47] found experimental factors such as water content, the temperature and duration of the reaction, amount of enzyme used along with the substrate ratio to also affect the acyl migration.

Research groups have used interesterification to develop versatile zero trans plastic fats with specific melting profile for end use as base stock fats for margarines, shortenings and spreads using SBO, palm stearin, and fully hydrogenated SBO [39, 48]. The challenge with this process is choosing the optimum ratio of the starting fats to obtain the required functionality. Interesterification does not induce formation of trans fats in the process and has an advantage over PHOs due to their zero-trans content based on non-PHO starting fats.

The interesterification process is also used to produce structured lipids.Structured lipids are composed of TAGs with desired fatty acids at a specific position in the glycerol backbone [49] for improved functionality or nutritional properties. Caprenin is a structured lipid with C8:0 (caprylic acid), C10:0 (capric acid), and C22:0 (behenic acid) [50] along with C20:0 (arachidic acid) and C24:0 (lignoceric acid). This fat has a calorific value of 5 kcal/g due to the poor absorption of behenic acid and faster metabolism of Caprylic and capric acids [51]. Study on the digestion pattern of Caprenin by the body concluded that Caprenin was digested and absorbed similarly to other TAG by hydrolysis in the intestines [52]. However, the poor absorption of the long chain fatty acids (C20-C24), Caprenin only contributes 5 kcal/g compared to the 9 kcal/g for fats
[51, 53]. Marketed by Proctor and Gamble, this low-calorie fat has a melting profile similar to cocoa butter and hence it is used in soft candy and confectionary coatings [54].

Neobee by Stepan Lipid Nutrition is another commercial structured lipid containing C8:0 and C10:0 designed for better fat absorption in the body, ready energy resource, and as an odorless or tasteless solvent for use as a dispersing agent. It finds application in foods, nutrition, and pharmaceutical areas.

Loders Croklaan developed Betapol which is a structured lipids with palmitic acid at the sn-2 position for use in infant food [37]. A clinical study in preterm infants fed with formula containing Betapol was performed [55]. It was found that there was an improvement in the absorption of palmitate while simultaneously reducing the formation of calcium soaps in feces which indirectly improves the calcium absorption in the body [55]. Benefat by Danisco (commercial name for Salatrim) is a structured lipid containing short (C2-C4) and long chain fatty acids (C16-C22), which is a low-calorie substitute fat for shelf life extension applications for certain confectionery products. A clinical study confirmed that in healthy young men, consumption of Salatrim curbed the appetite in comparison with regular fats [56]. The high saturates, low calorie, and zero trans makes this fat a great option for TFA alternatives.

(iv) Combination of processing techniques – Blending

Processing techniques such as interesterification, fractionation, and complete hydrogenation may be combined to develop a fat type with the required melting characteristics.

Reddy and Jeyarani [57] fractionated mango kernel fat and mahua fat and developed shortenings by blending the fractions, or the starting fats with fully
hydrogenated peanut oil to develop shortenings for bakery applications. Jin et al. [58] developed ternary mixtures of 10% palm kernel oil (PKO), 45% tallow, and 45% palm olein and concluded based on their melting behavior, eutectic effects by isosolid diagrams, and polymorphism that the mixture was suitable as a shortening. Aini et al. [59] also developed no trans plastic fat blends with different melting profiles simple blending of different ratios of PO, palm stearin, palm olein and PO, palm stearin and palm kernel olein.

Braipson-Danthine et al. [60] studied the properties of binary blends of hydrogenated soybean oil (HSO), low erucic rapeseed oil (LERO), hydrogenated LERO (HLERO), and hydrogenated palm oil (HPO). Their study showed with the increase in the amount of hydrogenated fats in liquid oils, there was an increase in solid fat content (SFC) of the mixture with a corresponding increase in hardness of the fat. They also observed that at the same SFC level, among blends of HPO, HSO, and HLERO in LERO, the HSO in LERO had a denser crystalline network and hence a harder texture than the others. This study shows that blends with different fat types at the same ratio can induce different texture in the final blend. This happens due to the change in the TAG composition and the way they interact to impart the final texture and affect crystallization behavior. This was also shown by Martini et al. [61] who studied the crystallization behavior of blends of high melting milk fat fraction (HMF) and in sunflower oil (SFO) at increasing concentrations of SFO. They found that due to increase in fraction of lower melting TAGs, it took longer for the samples to crystallize and the microstructure of the blends were different.
Kaufmann et al. [62] also found that a higher level of the liquid oil decreased the hardness of the blend by inducing crystalline clusters. They also showed that a stronger crystalline network could be achieved in the AMF and up to 20% rapeseed oil blend during crystallization by addition of shear [63]. Wright et al. [64] formed blends of milk fat and canola oil (CO) and their research findings showed that along with affecting the texture of the fat, a higher CO content in the blend, changed the polymorphic form of the milk fat in the blend from β’ to β. However, Martini et al. [65] found that different blends of milk fat and SFO crystallized only in the β’ polymorph. However, the ratios of the milk fat and oil used in the two studies were different. Herrera et al. [66] found that additives like sucrose esters may delay crystallization and also lower the SFC of HMF and SFO.

Thus, trans free fats with required plasticity can be developed by combining different fats at different ratios.

**Fats with saturated fatty acid at the sn-2 position**

(i) **Health effects**

Due to increase in the commercial use of interesterified fats in foods, studies are being conducted to study their effect on blood lipids and CHD risk. Sanders et al. [67] concluded based on a study on healthy young subjects that fats with palmitic acid at the sn-2 position caused a decrease in post prandial lipemia thus making them healthier while Zampelas et al. [68] concluded that there was no significant effect of consumption of these fats on post prandial lipemia. Berry et al. [69] suggested that IE fats with higher solid fat content (SFC) at body temperature reduced post prandial lipemia response.
Kritchevsky et al. [70] concluded from animal studies that fat high in palmitic acid at the sn-2 position are more atherogenic than when present at the sn-1 or sn-3 positions.

Meijer and Weststrate [71] studied the effect of interesterified fats with saturated fatty acids at the sn-2 position on healthy adults at realistic consumption levels and found that these fats do not affect the blood lipid levels but increase the levels of fibrin-degradation products, D-dimers. The increase in D-dimers concentration is linked to a higher risk for CHD [71]. Filippou et al. [72] in another study found that replacement of palm olein with interesterified palm olein containing palmitic acid at the sn-2 position did not lower the high density lipoprotein cholesterol nor did it have any negative effects on glucose homeostasis on young subjects. Tomarelli et al. [73] concluded based on animal results that the absorption of palmitic acid improved when it is present at the sn-2 position compared to when it is present at the sn-1 or sn-3 position. Similar results have also been shown in case of human infants [74], however, there is little evidence that these results can be extrapolated to adults.

The relationship between TAG positional isomers and nutritional properties is still controversial but recent research suggest that TAGs with saturated fatty acids at the sn-2 position result in lower postprandial lipemia [67, 75] resulting in a healthier lipid source. IE can be used to generate these lipids with improved nutritional properties through the synthesis of TAGs with saturated fatty acids (SFAs) at the sn-2 position.

(ii) **Interesterification process to produce fats with saturated fatty acid at the sn-2 position**

Ife Düba et al. [76] used Lipozyme TLIM immobilized enzymes to develop two trans free low saturated IE with palmitic and stearic fatty acids at the sn-2 position. Upon
interesterification of the starting materials, high oleic sunflower oil (HOSO) and tripalmitin, IE with 20 and 30% palmitic acid the sn-2 position were made by manipulating the duration of the reaction. Similarly, IE with 20 and 30% stearic acid at the sn-2 position were made with HOSO and tristearin at the sn-2 position. The major fatty acids in these samples were palmitic, oleic, and stearic acid. Although the TLIM enzymes were sn-1,2 specific, non-enzymatic acyl migration caused by the presence of acids, bases or ion exchange resins enriched the saturated fat concentration at the sn-2 position. Upon interesterification, there was a reduction in UUU and SSS (U=unsaturated fatty acids, S=saturated fatty acids) and an increase in USS, SUS, UUS TAGs. The major TAGs in the physical blends (PB) prior to interesterification had extreme melting points and hence the melting thermograms showed two separate melting peaks with large differences in melting temperature. The melting thermograms of the IE fats had a broader melting profile due to formation of new TAGs by rearrangements of the fatty acids in the PB.

Several studies comparing the physical properties of PB and IE have shown that IE are generally softer, with lower melting points and solid fat content than the starting fat [77, 78, 79]. The extent of differences in properties of PB and IE samples depends on the fatty acid and TAG composition of the starting blend [77]. Ifeduba et al. [76] also found that the IE fats had lower oxidative stability than the PB. This may be either due to the loss of inherent antioxidants in the starting fats during interesterification or the presence of free fatty acids in the IE fats [80].
**High Intensity Ultrasound (HIU)**

Ultrasound is defined as sound waves that operate at frequencies above 20 kHz [81]. The end applications of ultrasonic waves depend on their intensity and frequency. The frequency range for high intensity ultrasound, or power ultrasound, is between 20 and 100 kHz while that of low intensity ultrasound is between 100 kHz and 1 MHz, and of diagnostic ultrasound is between 1 and 10 MHz [81]. Low intensity and diagnostic ultrasound are used for nondestructive applications such as sonography, for medical diagnosis of breast or ovarian cancer, to speed up fracture healing [82], jewelry cleaning, and the study of the ocean bed. Low intensity ultrasound also finds applications in food science research for monitoring phase transitions in lipids [83], determination of solid fat content [84], shear modulus [85], and fractal dimension of fats [85], detection of foreign materials in foods [86] and determination of cheese maturity [87] among other applications.

In contrast to the non-invasive low intensity ultrasound, power ultrasound uses high intensity which may induce physical or chemical changes in the medium [88]. Several research groups have demonstrated the use of HIU in food applications. These include inactivation of microbes [89] and enzymes [90], denaturation of proteins [91], homogenization of milk [92], improvement in the efficiency of freezing and thawing of foods [93], and extraction of food proteins [94]. HIU has also been shown to develop nano-emulsification which by adjusting the emulsification duration, increased stability and decreased the droplet size of the emulsion was achieved [95, 96]. In another application of HIU, Ugarte-Romero et al. [97] demonstrated the inactivation of E. coli K12 in apple cider solution by HIU at sub lethal temperatures [97]. The microbial
inactivation was caused by the disruption of biological cell walls by causing perforation, shrinkage and deformation by HIU. Based on this effect of HIU on cell wall disruption, HIU was used to assist in solvent extraction of oil, increasing yield and reducing extraction duration compared to the conventional solvent extraction [98]. Ultrasound has also been used for the extraction of gingerols from ginger [99], soy iso-flavones from freeze-dried soybean [100], and ginsenosides from ginseng [101]. Other applications of HIU include wine ageing [102], food dehydration [103, 104], meat tenderization [105], and juice filtration [106].

HIU is commonly applied using a sonicator connected to a horn/probe dipped in the sample for a pre-determined length of time [107]. The sonicator has a fixed frequency between the 20 kHz to 40 kHz range. The probes used for the application of HIU are available in different length and diameter. The sonicator supplies energy to the probe, which is converted to mechanical energy which causes the probe to vibrate longitudinally. The amplitude of vibration is referred to as the amplitude of the tip and is directly proportional to the acoustic power delivered to the media. At a constant viscosity, a change in diameter of the tip affects the amplitude of vibration which further affects the intensity of sonication. A sonicator tip with a lower diameter has a higher amplitude in the same medium.

High intensity acoustic waves travel longitudinally through the medium and displace particles around their equilibrium position, creating positive and negative pressure zones [108]. An acoustic wave is a sinusoidal wave as shown in Figure 2.3. The number of waves that pass a particular point per second is the frequency (cycles) of the
wave and is a function of the wavelength. When HIU is applied to the medium, the tip immersed in the medium vibrates at a fixed amplitude and the amplitude of vibration corresponds to the amplitude of the acoustic wave in Figure 2-3. The changes in the pressure in the medium produce cavities or bubbles [108] and the size of the bubbles increases across multiple cycles (Figure 2-3). The bubble can reach a resonance size, collapse, and can generate high localized temperature, pressure, and shear forces like an implosion [108, 109]. This is also known as inertial or transient cavitation. This generates spots of extremely high temperatures and pressure at the site of implosion. The rise in temperature in the medium can be used as a factor to calculate the acoustic power delivered to the medium [108]. Acoustic power, $P$ (watts) = $m \times C_p \times \frac{dT}{dt}$, where $m$ is the mass of the medium that was sonicated, $C_p$ is the specific heat capacity of the
sonicated medium and $dT/dt$ is the rise in temperature per unit time upon sonication [108]. Thus, higher sonication duration, increases the temperature in the medium and hence increases the acoustic power delivered to the system. On the other hand, the bubbles can be stable to implosion and can act as nuclei for crystallization. This is referred to as non-inertial or stable cavitation. The inertial bubbles can also collapse into non-inertial smaller bubbles which can act as nuclei for further crystallization [109]. This proposed mechanism of the action of cavitation is shown in Figure 2-4. The formation of cavitation in the system is a function of the viscosity, temperature, and volume of food along with the amplitude, intensity, and frequency of the ultrasonic waves [110]. Cavitation also produces microstreaming of bubbles which leads to effective heat and mass transfer [111].

Research has shown that crystallization of fats in the presence of HIU (also known as sonocrystallization) causes changes in the texture and the crystallization properties of fats. It induces nucleation and crystal growth. Suzuki et al. [112] studied the microstructure of palm kernel oil (PKO) and an all-purpose shortening and compared the crystals formed without HIU application and with the application of HIU at two conditions: [1] when the sample reached the crystallization temperature, and [2] when first crystals were observed in the system. It was found that HIU generated more and smaller crystals and a higher efficiency was obtained when HIU was applied in the presence of crystals. Sonication also increased the hardness of the fat. Similar microstructure was observed for both fats, however the melting enthalpy was higher only for the PKO samples at higher crystallization temperature.
Figure 2-4: The proposed mechanism of the effect of cavitation generated by HIU. (Permission to copy and distribute this work was granted by the copyright owner, see appendix)

Martini et al. [109] studied the outcome of HIU application on the crystallization of anhydrous milk fat (AMF) and found that HIU decreased the induction period of crystallization at intermediate supercooling. However, at the high supercooling level, HIU caused an increase in the induction of crystallization and this was due to the high temperatures generated by sonication which melted the nuclei in the system. Similar to the results observed by Suzuki et al. [112], HIU also induced smaller crystals and promoted crystallization in AMF and increased the viscosity of the AMF. Similar to Suzuki et al. [112], Martini et al. [109] demonstrated that sonication in the presence of crystals was more effective and that by applying HIU at lower intensity and shorter duration it was possible to develop smaller crystals and promote crystallization even under low supercooling conditions.

The induction of crystallization and formation of smaller crystals was also observed by Ye et al. [107] when studying the effects of HIU on the crystallization properties of interesterified SBO. These authors reported a higher reduction in the
induction of crystallization with the increase in HIU power. Higher power levels also induced smaller and more crystals in the system. The authors explained that when sonication occurred in the presence of crystals the shear forces generated by HIU break the existing crystals and thus promotes secondary nucleation. In another study by Ye et al. [113] it was found that a larger tip 1/2” was more effective than a 1/8” tip in inducing crystallization at lower temperatures. The author found no differences in the sonication duration (10, 5, and 2.5 s) on crystal morphology. The change in the microstructure by sonication significantly improved the viscoelastic properties of the fat.

Another study on the effects of HIU in tripalmitin and cocoa butter systems showed that it was possible to induce the formation of a certain polymorphic type of crystals [114]. Induction and promotion of the formation of the β crystals were also shown in tricaprin and trimyristin [115-117].

Chen et al. [118] studied the effect of sonication on palm oil (PO). They found that higher power levels increased the crystallization in PO due to higher shear forces generated which increase the interactions between solid and liquid fat. The solid fat content of the oil increased and the crystal size decreased with the increase in the duration of sonication and HIU power levels.

Patrick et al. studied the effect of using various power intensities of ultrasound (30, 35, 40 and 45 dB) on the crystalline structure of PO [119]. The sample without the application of HIU was characterized by big spherulite crystals. The structure changed from dense clotted cream-like with very few crystals to uniform crystals clumps that settled at the bottom of a clear liquid with the change in HIU intensity from 30 to 35db. At a 40 dB, the structure of PO resembled that of a face cream with very small crystals
and no free liquid. At a 45 dB intensity, there was no clear crystal structure. Thus, Patrick et al. demonstrated that it is possible to change crystal microstructure of a lipid based on HIU intensities [119].

Researchers used sonocrystallization in several food systems. HIU, when applied to agar gel system [120], was effective at inducing nucleation and controlling crystal size distribution. HIU application induced a faster immersion freezing of potato slices [121] and apples [122] along with less cell wall damage [123]. Mortazavi and Tabatabaie [124] studied the effect of the HIU pulse on the freezing of ice cream. They found that HIU was effective in reducing the freezing duration by increased mass transfer and ice crystal size by fracturing the already present crystals. Sensory tests suggested that while the texture and flavor of the control sample was better than the pulsed sample, the mouth feel of the pulsed sample was better.

**Crystallization of fats**

Fat crystals are desirable in food applications such as chocolates, cookies, butter, and shortenings. Fats are composed of TAG and fatty acids which differ based on the chain length and the presence and type [cis or trans] of double bonds in them. The type and the amount of different fatty acids and the TAG composition influence physical characteristics of the fats. The fatty acids with no double bonds are called saturated fatty acids (eg. palmitic acid, stearic acid), those with one double bond are called monounsaturated fatty acids (eg. oleic acid) while those with two or more double bonds are called polyunsaturated fatty acids (eg. linoleic acid and linolenic acid). The melting points of fatty acids increase with chain length and decrease with the increase in unsaturation [125]. For example, butyric acid which has 4 carbon atoms has a melting
point of -7.9 °C while stearic acid which has 18 carbon atoms has a melting point of 69.6 °C [125]. Hence oils with higher amount of long chain saturated fatty acids have high melting points and are usually solid/semi-solid at room temperature [24]. Vegetable and animal fats including PO, coconut oil, milk fat, lard, and tallow have higher amounts of saturated fatty acids such as lauric, myristic, palmitic and stearic acid [24]. Oleic acid is present in olive oil, SFO, and SBO among others at levels of 55-83%, 13-40% and 17.7-25.1% respectively [126]. Oils including those of safflower, sunflower, cottonseed and soybean have higher amounts of linoleic acid and are usually present at levels above 40% [126]. The presence of trans-fatty acids also affects the melting point of fats. Due to higher packing of the trans isomer compared to the cis isomer, trans fatty acids have higher melting points than their cis counterparts and their presence correspondingly affects the melting points of fats [127].

Supercooling is the magnitude of temperature difference between the fat melting point and the temperature at which the crystallization is performed. This temperature difference is the driving force for crystallization. After sufficient supercooling is generated in the fat, formation of nuclei may commence by several ways- [1] Primary homogeneous: where nucleation progresses spontaneously in the absence of any foreign particles and [2] Primary heterogeneous: where the nucleation happens in the presence of foreign materials or impurities, or [3] Secondary nucleation: where the new nuclei is formed on contact with prevailing crystals in the medium. Nucleation is followed by crystal growth. Crystal growth occurs by assimilation of TAGs from the bulk into the crystals’ matrix and is propagated by the supercooling and the viscosity of the solution.
Fat crystals show polymorphic behavior which is the ability to exist in multiple crystalline forms [24]. X-ray diffraction is used to study the polymorphic form of crystals. Common polymorphic forms found in fats include α, β, and β’ with α being the least stable and β form the most stable [24]. The α form has a hexagonal structure while the β’ and β form has an orthorhombic and triclinic structure and their melting points increase in the order of stability. The properties of these polymorphic forms are given in Table 2-1.

Fatty acid and TAG composition along with processing conditions affect the polymorphic form of fats. Due to the lower activation energy associated with the formation of α crystals, these are formed upon rapid cooling, which rapidly converts to the more stable β’ form [24] which is later converted to the β form (Figure 2-5). This transformation occurs because the free energy of β < β’ < α. The polymorphs tend to convert into polymorphs with lower free energy. In food products like the cocoa butter in chocolates, in order to induce the formation of the desired polymorphic form, either tempering or seeding is employed [27].

Several processing conditions affect nucleation and crystal growth which are rate of agitation, cooling rate, crystallization temperature, and type of fat. Herrera et al. [129] studied the effect of different chemical composition, cooling rate, and agitation rate on the blends of high melting milk fat fractions and low melting milk fat fractions. They found that a slow cooling rate produced larger and fewer crystals than a faster cooling rate at the same agitation speed. The increased agitation speed induced smaller crystals mainly due to the increased interactions among the crystallizing TAGs. At the same crystallizing temperature, fats with higher amounts of saturated fatty acids had more
Table 2-1: The properties of the three main polymorphic forms found in fats [128] ("Republished with permission of Marcel Dekker Incorporated, from Crystallography, In Fat crystal networks, Marangoni, AG, 2005; permission conveyed through Copyright Clearance Center, Inc."

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>α Form</th>
<th>β' Form</th>
<th>β Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chain packing</td>
<td>Hexagonal</td>
<td>Orthorhombic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Short spacing(s)</td>
<td>0.415 nm</td>
<td>0.38 and 0.42 nm</td>
<td>0.46 nm</td>
</tr>
<tr>
<td>IR Spectrum (-CH₂-)</td>
<td>Singlet at 720 cm⁻¹</td>
<td>Doublet at 727 and 719 cm⁻¹</td>
<td>Singlet at 717 cm⁻¹</td>
</tr>
<tr>
<td>Density</td>
<td>Least dense</td>
<td>Intermediate</td>
<td>Most dense</td>
</tr>
<tr>
<td>Melting Point</td>
<td>Lowest</td>
<td>Medium</td>
<td>Highest</td>
</tr>
</tbody>
</table>

Figure 2-5: The activation free energy (ΔG#n) of the formation of different polymorphs and the difference in the free energies of the polymorphs [128]. ("Republished with permission of Marcel Dekker Incorporated, from Crystallography, In Fat crystal networks, Marangoni, AG, 2005; permission conveyed through Copyright Clearance Center, Inc.")
crystals than fats with lower amounts of saturated fatty acids. Higher agitation rates [75, 100, and 125 rpm] were also studied by Grall et al. [130] on butterfat crystallization and found that higher agitation induced faster nucleation and subsequent crystallization. Martini et al. [61] showed that with decreasing crystallization temperature ($T_c$), the lowest $T_c$ generated smallest and more crystals. This may be due to the higher supercooling generated with lower $T_c$ which induced nucleation and faster crystallization in the sample.

Thus, processing conditions strongly affect the crystal growth and network in the fat and hence special care must be taken to control these to obtain and maintain the required crystalline network.

**Analyses to quantify physical properties of fats**

The physical properties of fats affect and influence the functionality of the food products. For example, butter is semisolid and has a solid fat content (SFC) between 30-40% at room temperature, which influences its spreadability. An increase in SFC would decrease butter spreadability [131]. For puff pastry applications, harder fats with higher SFC are preferred since they stay solid during kneading but melt on baking which assists in forming multiple layers [132]. Crystal structure also affects the plasticity of fats [133]; while crystal size, especially smaller crystals affect their texture making them harder [107]. The viscoelastic properties of the fat influence their texture and consistency [134].

Liquid oils with higher oxidation stability are usually used in frying applications while powdered fats are used for their ease with handling, storage, and use [135]. The TFA-based shortenings are very versatile and based on the processing conditions and the
amount of TFA in the shortening, they can be used as plasticized semisolids, liquids, or flakes. That is, TFA-based shortenings can be formulated with a wide range of functional properties that make them ideal for any food application. However, with the elimination of PHO, *trans*-fat substitutes may not always have the multi-functionality of *trans*-fats and additional processing techniques such as application of HIU could be necessary to improve or impart certain physical properties. As discussed before, HIU induces the formation of smaller crystals in fats and improves their rheological and textural properties [107]. It is therefore important to characterize fats in terms of their physical properties such as the microstructure, solid fat content, melting enthalpy, and rheological behavior to assess how these properties are affected by processing conditions. Changes in physical properties can be used to evaluate the degree of processing required in a fat for specific food applications. Below is a description of the most common techniques used to characterize physical properties of fats.

(i) **Polarized light microscopy (PLM) to determine crystal microstructure**

The PLM technique is used to visualize crystal shape and size at various stages of the processing or after different processing conditions. It is important to study crystal morphology since the characteristics of the crystal network formed during processing may affect flavor, mouthfeel, and spreadability of food products. In chocolates, fat crystal size plays an important role in providing shine, gloss, and texture [136]. A faster cooling rate induces smaller crystals compared to the large fat aggregates developed during slow cooling [137]. Also, higher agitation rates produce a higher numbers of nuclei which generate greater numbers of small crystals [138]. A change in crystal morphology with the change in processing conditions could indicate a polymorphic transformation;
however, X-ray diffraction should always be accompanied to verify this change [116, 117, 139]. Chemical composition of the fat also influences the crystallization properties of fats. Samples with similar fatty acid composition but different TAG composition can have different crystallization behavior and can form different crystal microstructure under similar crystallization conditions [140]. Ahmadi et al. showed that upon interesterification, the higher melting TAG decreased and new TAGs were formed which decreased the amount and shape of crystals [117]. These authors showed that the shape of crystals changed from spherulitic to needle shaped after the interesterification process [140]. A blend with higher amount of higher melting TAGs had a denser microstructure. Marangoni showed that the microscopic properties of a crystallized fat correlates to the macroscopic properties of the fat and the hardness of the fat is associated with the strength of interaction between the crystal network [141-143]. Thus, fat microstructure is a very important characteristic that affects the eventual texture of the fat and the appeal of the food product it is used in.

(ii) Nuclear magnetic resonance (NMR) to determine solid fat content

Solid fat content (SFC) measures the amount of solid fat fraction of the crystals in the liquid sample. SFC is commonly measured using low resolution nuclear magnetic resonance (NMR). The SFC can be measured isothermally to determine the change in the SFC over time or over a range of temperatures to determine the SFC profile of the sample [144]. The NMR measures the SFC by exposing the sample to a high radio frequency zone causing excitation of H nuclei. The SFC of the sample is measured based on the relaxation time of the H nuclei. The H nuclei in the solid state relax faster than those in the liquid form and thus based on the ratio of the relaxation of the nuclei, the amount of
solid fat in the sample can be calculated [145]. The SFC of the samples is an indicator of
the plasticity, spreadability [137], mouthfeel, and palatability of fat [112]. In many cases,
the SFC can be related to the hardness of the fat [137]. Campos et al. studied the effect of
cooling rates on SFC of lard and AMF and found that for both samples a faster cooling
rate induces higher isothermal SFC [137]. Ye et al. and Suzuki et al. measured the %
solsids in the fat at different temperatures using differential scanning calorimetry (DSC)
[107, 112]. They found that sonicated fat samples had steeper melting profile compared
to the non-sonicated samples due to the faster melting of the smaller crystals generated by
sonication [107, 112].

SFC changes during the crystallization process can be used to quantify the
crystallization kinetics of the fat. In this case, SFC values can be fitted to various types
of equations. The single step Avrami equation is given by equation 1.

\[ s(t) = S_{\text{max}} \left(1 - e^{-kt^n}\right) \]  \[ 1 \]

Where \( s(t) \) is the SFC of the sample at any given time at a particular temperature,
\( S_{\text{max}} \) is the maximum isothermal SFC, \( k \) is the Avrami constant and refers to the rate of
crystallization while \( n \) is the Avrami exponent and explains the type of nucleation
mechanism [146]. This equation explains the kinetics of conversion of liquid fat to solid
and has a dependence on temperature similar to Arrhenius equation [146]. The Avrami
rate constant, \( k \), is temperature dependent and it decreases with an increase in
temperature. The \( n \) assumes values between 1-4 and it explains the growth of crystals as
rods, spherulites or disks with either spontaneous or sporadic nucleation. Chen et al.
[118] and Wu et al. [147] found an increase in the \( n \) values with the increase in
temperature. These authors also showed that \( n \) values were higher for the non-sonicated
compared to the sonicated samples at certain temperatures while contrasting effects were seen at other temperatures. A decrease in the value of $n$ with sonication was also observed by Ye et al. [148] in a continuous sonication system with PO. These observations indicated that sonication affected the crystallization mechanism along with the crystal shapes depending on the crystallization temperatures. Wright et al [149] studied the crystallization of AMF, AMF TAGs and AMF TAGs + AMF diacylglycerols (DAGs) samples which had different levels of minor components, at different temperatures. Avrami exponents of all these samples increased with increase in crystallization temperature. The $n$ values were same among the samples at lower crystallization temperatures. However, the $n$ values varied among the samples at higher crystallization temperature. This study showed that the minor components in the fats also affected the crystallization mechanism in fats.

Multistep crystallization could occur due to crystallization of the higher melting fractions followed by the lower melting fractions [150]. Isothermal polymorphic transformations can also result in multistep crystallization [150]. Marangoni et al. [143] observed a two-step crystallization of cocoa butter at 17.5 °C and in an another study, Herrera et al. [151] observed a two-step crystallization curve when AMF was crystallized isothermally at temperatures below 25 °C. Chen et al. [118] also observed a two-step crystallization of PO at 20 °C, and similar crystallization was observed under both sonication and non-sonication conditions. The multistep crystallization kinetics can be studied using the multistep Avrami equation described by Marangoni (eq 2) [146]
\[ s(t) = S_0 + \sum_{i=1}^{n} s_{\text{max}_i} \times \left( -\left( K_i \times t_i^n \right) \right) \]

[2]

Where \( s(t) \) is the SFC of the sample at any given time at a particular temperature, \( s_0 \) is the initial SFC, \( s_{\text{max}} \) is the maximum isothermal SFC, \( k \) is the Avrami constant and refers to the rate of crystallization while \( n \) is the Avrami exponent and explains the type of nucleation mechanism. The subscript \( i \) refers to the crystallization step and the set of parameters with identical subscript numbers correlate to the same crystallization step. This equation allows quantification of the Avrami parameters for each crystallization step.

Along with the Avrami equation, the reparametrized Gompertz mathematical equation can also be used to quantify a single step isothermal crystallization and is given below [152].

\[ s(t) = s_{\text{max}} \times e^{\left( -\frac{\mu_{\text{max}} \times e}{s_{\text{max}} \times s(t) - \lambda - t + 1} \right)} \]

[3]

Where \( s(t) \) is the SFC at any given time, \( s_{\text{max}} \) is the maximum isothermal SFC, \( \mu_{\text{max}} \) is the maximum rate of crystallization, \( \lambda \) is the induction period of crystallization and \( e \) is 2.718281 [153]. Compared to Avrami, Gompertz equation provides the induction period of crystallization and the maximum rate of crystallization. Gompertz equation has been used before by to study the crystallization kinetics of phospholipids, water and milk fat mixtures by Vanhoutte et al. [154], of the blends of HPO and SFO by Kloek et al. [152]
and by Chaleepa et al. [155] for the crystallization kinetics of the mixtures of coconut oil with additives such as lauric acid and sucrose esters, respectively.

Isothermal SFC data generated from different processing conditions can be fitted with these mathematical equations and the parameters from each condition can be compared to determine the effect of each processing conditions including temperature, sonication, agitation, cooling rate, etc. The Avrami model has been developed and in use the longest to study the crystallization kinetics. Although the Gompertz model was initially used to study the bacterial growth, it was later applied to fat crystallization based on analogues between the two processes [156] and its parameters are easier to translate into physical concepts [156].

(iii) **Differential scanning calorimetry (DSC) to evaluate melting profiles**

Differential scanning calorimetry (DSC) is used to analyze melting and crystallization behavior of fats. DSC measures the difference in heat flux required to maintain a sample and an empty reference pan at the same temperature when the samples are subjected to a set temperature program [157]. The DSC curve obtained with no pans or empty pans represents a baseline curve. Based on the thermal events occurring in the sample such as melting or crystallization, the DSC curve shows deviations from the baseline [158]. The melting and crystallization events are endothermic and exothermic processes, respectively and the direction of the heat flux can explain corresponding thermal phenomena [158]. Some of the important parameters obtained from the DSC thermograms include the peak onset temperature, the peak temperature and the enthalpy of the thermal transition. To quantify these parameters, the software forms an interpolated baseline across the peak [159]. The peak onset temperature is calculated as the
temperature at which the line drawn through the linear ascending part of the peak intersects the interpolated baseline. The peak temperature is the temperature at which the difference between the interpolated baseline and the DSC curve is the maximum. The enthalpy of melting is calculated as the area of the peak integrated and is influenced by the amount of the crystals, and the TAG composition of the fat.

Upon comparison of the DSC thermograms of the non-IE and IE fats [160], it was observed that the peaks were different among the two samples. This indicates the change in the TAG composition of the fats upon interesterification. Changes in the peak melting and onset temperature along with enthalpy values with processing parameters can also give an indication of the effect that processing has on type of crystals generated of the fat compared to control. Campos et al. (2002) compared the thermograms of the fast and slow cooled samples which revealed the co-crystallization of different fat fractions and formation of mixed crystals under the fast crystallization conditions [137]. An exothermic peak after an endothermic peak in a cooling cycle showed a polymorphic transformation and the presence of the least stable α crystals were observed under the faster cooling rate [137].

Ye et al. [113] found that upon sonication, there was a reduction in the peak onset temperature indicating that there was a peak broadening upon sonication. These results indicated that HIU induced the crystallization of lower melting TAGs. Silva et al. [161] studied the effects of different sonication frequencies along with high speed agitation on the crystallization behavior of a commercial interesterified SBO. Sonication at 20 kHz induced the co-crystallization of two fractions observed in the non-sonicated fat.
thermograms while treatment with high speed agitation at 24,000 rpm induced the growth of each of the fractions separately and did not propagate co-crystallization.

(iv) **Rheology to determine viscoelastic properties of fats**

Viscoelastic properties such as the storage (elastic) modulus (G’), loss (viscous) modulus (G’’), phase angle (δ), and viscosity (η) are important in the determination of mechanical properties of foods. For shortenings and butter, viscosity explains how the sample resists deformation when subjected to stress (e.g. during spreading) while hardness explains the compressibility component of these fats. The storage and elastic modulus of the fat explains the stiffness in the fat based on the solid and liquid fractions. The rheology of food is an important factor that affects their organoleptic properties such as texture and mouthfeel [162]. Foods in which texture plays a major role are potato chips, cookies, emulsion based beverages, and pickled cucumbers. Bourne lists descriptors of texture in US, Austria, and Japan and some of the common ones include terms such as crisp, crunchy, hard, soft, and creamy [163]. Addition of hydrocolloids such as gums and stabilizers changes the texture of foods by changing the viscosity [164] although fats also affect the rheological properties of foods depending on the type and the amount of fat [165].

Studies have also shown that the hardness of fats is also dependent on their composition, amount of the crystallized material, and crystallization conditions including temperature and the rate of crystallization [60, 137, 166].

All the levels of network formation including the type and position of fatty acids on TAGs [167], the polymorphic form of the crystals, the amount of liquid and solid fat
in the sample [167], the formation of crystal aggregates affect the texture, hardness and rheology (Figure 2-6).

As described earlier, Herrera et al. [129] showed that a faster cooling rate and increased agitation rate induced more crystals in the system which in a subsequent study [168] translated into an increase in the storage and loss modulus of the samples. Marangoni explained that elasticity of fats are dependent on the amount of solid fat and their interaction which contributes to a network formation which is depicted in their microstructure [170]. Since HIU has been shown to alter the crystalline network by induction of smaller and more crystals in the network, it eventually also affects the macroscopic properties of the fat. This has been confirmed by Martini et al.[109] who showed a similar microstructure induced HIU which increased the viscosity of fat even at

![Figure 2-6: Diagram depicting the dependence of the properties of fat on fat crystalline network [169] ("Republished with permission of Marcel Dekker Incorporated, from Crystallography, In Fat crystal networks, Marangoni, AG, 2005; permission conveyed through Copyright Clearance Center, Inc.")](image-url)
higher crystallization temperatures. Ye et al. [107] showed HIU induced microstructure increased $G'$ and $G''$. Maruyama et al. [171] showed that the effect of HIU in increasing the $G'$ and $G''$ was observed even in the presence of added emulsifiers.

**Fats and sensory**

The taste of the food is perceived by mass transfer of the taste compounds from the food to the receptors on the tongue [172]. The taste of a food product relates to the sensation measured or felt by the tongue [173] and these include basic tastes such as salty, sweet, sour, bitter and umami. The release of volatile compounds along with the inherent taste contribute to the overall flavor of the food [173]. In foods that contain fats, the flavor release strongly depends on the type of fat and the food structure [174] and the fat content of foods affects flavor perception [175-177]. For an emulsion based delivery system, the flavor release also depends on the droplet size, and the dissolution of the matrix surrounding the flavor infused lipid, into in the saliva [178]. The increase in viscosity of foods delays the mass transfer of the flavor components to the saliva and thus the perception of flavor is delayed [179]. In case of solid foods, the partitioning of the flavor from the lipid into the saliva is enhanced during mastication. Saint-Eve et al. [180] also demonstrated that just by changing the texture of candies from hard to soft, the perception of taste and aroma changed though the concentration of the flavor ingredients were constant.

The presence and the amount of fat also affects the perception of other flavors in foods. Studies have shown that with the increase in fat content in the food, the perception of salt increased though the salt concentration was constant [181, 182]. Hoppert et al.
[183] also showed that an increase in sensitivity towards sweetness was observed with the increase in the fat content of the emulsions.

Fat substitutions in food products not only affects sensory attributes, but it also affects the physical and chemical properties of the foods. Waheed et al. [184] compared the physical, chemical, and sensory attributes of cookies made with hydrogenated shortenings with those made with IE of PO and cottonseed oil. The overall acceptability of the cookies substituted with IE was lower than the control, although the overall acceptability of 50:50 IE blend of PO and cottonseed oil was not significantly different to the control. The acceptability ratings of color, flavor, and texture were also lower than the control sample. Upon storage, cookies received lower acceptability scores on all the sensory parameters tested. This study concluded that though the IE had lower overall scores, 50:50 IE blend with PO and cottonseed oil worked better than the other blends and thus interesterified blends can find suitability as fat substitutes for hydrogenated fats.

Shortenings are also important for flavor delivery and mouthfeel of foods. Dogan et al. [185] successfully showed the use of interesterified blends of PO and cottonseed oil as zero-trans substitutes for hydrogenated shortenings in bakery applications such as cakes with acceptable sensory attributes such as moistness, flavor, and mouthfeel. The role of lipids in mouthfeel and texture depends largely on the increased viscosity, spreading, and adhesion of the lipid over the oral surfaces [182, 186, 187]. Frank et al. [188, 189] showed that the release and intensity of volatile compounds from foods decreased as a function of lipid content in oil-in-water emulsions.

Many studies have been performed on the effect of lipids on flavor release, texture, and mouthfeel in model systems that include oil-in-water emulsions, water-in-oil
emulsions, and model foods [190]. However, the effect of lipid structure generated in the presence of HIU and difference of composition-fatty acids and TAG, especially between the IE fats with different TAG compositions, on food sensory properties by a descriptive sensory panel has never been conducted.

Given that flavor release is driven by the nature of the flavor compound (volatility) and the mass transfer of this compound through the food matrix [190], it is important to explore the role of lipid composition and structure on flavor perception in bulk. The flavor compounds 2- butanone, butyric acid, ethyl butyrate and 2-nonanone are commonly found in food products and their lipophilicity coefficients measured as octanol/water partition coefficients are 1, 6.16, 80, and 1380, respectively [189, 191]. Hence, a systematic study to study the flavor release of these compounds from the fats in consideration can provide an insight into the flavor release differences among the samples. It can further elaborate if the differences in the release is based on the flavor compound used or the sample. These compounds were identified and used by Frank et al. (2012) where he studied the differences in the headspace volatiles in fats with different FA and TAG composition using GC-MS SPME. The IE fats processed with HIU can provide an ideal system for a systematic study of the effect of lipid chemical composition and bulk structure on flavor perception by a descriptive sensory panel.
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CHAPTER 3
SONOCRYSTALLIZATION OF INTERESTERIFIED SOYBEAN OIL WITH AND WITHOUT AGITATION

Abstract
Interesterified soybean oil was crystallized at 29, 34, and 35 °C with and without the use of high intensity ultrasound. Samples were crystallized using either: (i) continued agitation for the entire crystallization process (CA), or (ii) agitation for 10 min (A10) followed by static crystallization. Sonication and agitation decreased the induction period of nucleation at higher temperatures and changed crystal morphology, crystallization kinetics, and viscoelasticity of the sample. Sonication reduced crystal sizes and significantly (p < 0.05) increased viscosity (5.2 ± 1.2 to 2369.6 ± 712.1 Pa.s) and elastic modulus (83.2 ± 4.1 to 69236.7 ± 26765 Pa) of the crystalline networks obtained at 29 °C under A10 condition. No differences in the viscosity and elasticity was also observed for sonicated samples crystallized at 34 and 35 °C under A10 and all CA conditions (p > 0.05). Sonication increased crystallization rates for all conditions tested. Kinetic constants obtained from an Avrami fit increased from 1.3 x10^{-5} to 6.8 x10^{-5} min^n for samples crystallized at 29 °C A10 without and with sonication, respectively and from 2.6 x10^{-9} to 2.4 x10^{-7} min^n for samples crystallized at 34 °C A10 without and with sonication, respectively. This increase in crystallization rate was also observed for samples crystallized under the CA condition at 29 °C.


Introduction

Processing conditions including crystallization temperature, agitation rate and duration, cooling rate [1], and application of high intensity ultrasound (HIU) [2-5] along with the triacylglycerol (TAG) composition [6, 7] control the crystallization behavior of edible fats. Since FDA’s removal of GRAS status for partially hydrogenated oil (PHO), food companies are replacing PHO with fractionated oils, interesterified fats, or blends of fats. In addition, consumers are becoming aware of the health implications related to the consumption of fats with high levels of saturation. However, when PHO or fats high in saturated fats are replaced by trans-fat free and low saturated counterparts, many functional properties of the fat such as texture are lost. To improve the functionality of fats with low content of saturation various processing techniques need to be evaluated. The changes induced in fats by processing techniques include size [1, 4, 5], amount [1, 8], shape and polymorphic form of crystals [9]. These microstructural changes in the samples consequently affect the mechanical and functional properties of fats such as hardness, viscoelasticity, and melting behavior [2-5].

High intensity ultrasound (HIU) has been used to induce and promote crystallization in fats [2-5, 10, 11]. It has been concluded that the effect of HIU depends on the type of fat used along with processing conditions employed including crystallization temperature [4, 5], time of ultrasound application [2, 10], and sonication parameters such as the acoustic power [2, 12], tip size [13, 14], sonication duration [13], and ultrasound frequency [13]. Among the ultrasound parameters, Ye et al. [2] showed that a higher acoustic power induced smaller and more crystals and Silva et al. [13] showed that a low ultrasonic frequency of 20 kHz induced smaller crystals compared to
higher frequencies of 40 kHz. Chen et al. [15] compared the effect of 20 and 60 s sonication durations on palm oil crystallization at different temperatures and found that irrespective of the crystallization temperature, the longer sonication pulses significantly reduced the induction times of crystallization and induced smaller crystals. In contrast, Ye et al. [14] did not find any differences in the microstructure of an all-purpose shortening when sonicated with 2.5, 5, and 10 s pulses. They also found that a larger tip size (1/2” diameter tip) induced the formation of more and smaller crystals than a smaller tip (1/8” diameter tip). Authors [2, 3, 10] have also showed that HIU was most effective at forming smaller and more crystals along with increasing the elasticity and hardness of the sample when applied in the presence of crystals. In this case, HIU worked by inducing secondary crystallization where cavities generated by acoustic waves broke the existing crystals and generated smaller crystals which served as nuclei for further crystallization [2-5]. Earlier studies that aimed at understanding the effect of sonication on lipid crystallization were performed under static conditions where the agitation was stopped prior to the application of HIU [2, 3]. Based on the previous research on lipid sonocrystallization it is unknown how sonication will interact with other processing conditions such as agitation.

Lipid crystallization involves four main steps including generation of supercooling (temperature differential between melting point and crystallization temperature ($T_c$)), generation of nuclei, growth of the nuclei into crystals, and recrystallization. Nucleation and crystal growth stages can be manipulated by chemical composition or processing conditions such as $T_c$, cooling rate, agitation rate and duration, sonication, and seeding, etc. Martini et al. [8] compared the crystallization behavior of
two fats: 10-90% and 40-60% blend of sunflower oil in high melting milk fat fraction, at fast or slow cooling rate in the presence or absence of agitation. They found that in fats with different TAG composition, the same processing condition (cooling rate and agitation) affected the TAG interactions differently thus inducing different crystallization behavior, crystal shape or morphology. It was also confirmed that faster cooling rates created smaller, transparent, less dense crystals, while slower cooling generated larger, denser crystals [1, 8]. At the same cooling rate, a higher T_c favored crystal growth and hence larger and fewer crystals were formed compared to a lower T_c which favored nucleation [1, 8]. Increase in agitation speed induced smaller and more crystals [1, 8] while depending on the type of fat, agitation along with a faster cooling rate either changed the crystal shape from spherical in a slow cooling rate to needle like in a fast cooled sample [8] or just size from larger for slow cooling rate to smaller crystals for fast cooled samples [1]. Ye et al. [2] has shown that HIU affects crystal growth and also studied the effect of acoustic power on the microstructure of a commercial interesterified soybean oil. They found that with higher acoustic power, smaller and more crystals were formed in the system and faster crystallization was induced in the sample. They also studied the effect of HIU application at three time points- before crystallization started, when the sample reached crystallization temperature and when the first crystals appeared in the sample. It was found that HIU was most effective in inducing smaller and more crystals when HIU was applied in presence of crystals. Considering how crystallization behavior of a lipid is affected by various processing conditions, it is important to consider the effect observed on lipid crystallization from the interaction of multiple processing conditions.
The objective of the current study was to assess the effect of temperature, agitation, and HIU on the crystallization properties of a low saturated interesterified soybean oil. In this paper, we compared the effect of HIU on a crystallizing lipid in the presence and absence of agitation at different crystallization temperatures. The properties compared were induction period of nucleation, solid fat content, microstructure, melting characteristics, viscoelasticity, and polymorphism.

Materials and Methods

Materials

Interesterified soybean oil (IESBO), an interesterified product of liquid soybean oil and a fully hydrogenated soybean oil (product number 76-240-0) was sourced from Archer-Daniels-Midland (Decatur, IL, USA). TAG and the fatty acid composition of the oil has been discussed elsewhere [2] and has been added to appendix (Table 1 and 2).

Melting point

The melting point of IESBO was measured in a DSC Q20 (TA Instruments, New Castle, DE) differential scanning calorimeter using an empty pan as a reference. The DSC was calibrated with indium using nitrogen as the carrier gas. An aliquot of the IESBO sample was sealed in a Tzero pan with a Tzero hermetic lid and the sealed pan was kept overnight in a freezer at -20 ºC. This step was performed to ensure complete crystallization of the sample. The starting temperature of the DSC was set to -20 ºC. When the instrument reached -20 ºC the DSC pan (at -20 ºC) was quickly transferred to the DSC. The sample was heated from -20 ºC to 80 ºC at the rate of 5 ºC/min. The onset melting temperature of the highest melting peak of the sample was used as the melting
point of the sample. The analysis was performed in duplicates and the melting point was reported as the average of the results from the two runs.

**Crystallization conditions**

The IESBO fat sample was first heated in the microwave to melt and homogenize it for subsequent weighing. One hundred grams of the melted sample was then placed in a clean beaker and kept in the oven at 70 °C for 30 min to remove any crystal memory in the sample. The sample was then placed in a double-walled glass cell (5.0 cm i.d. x 8.6 cm height x 7.3 o.d. = 169 mL volume) with temperature control provided by an external water bath to induce crystallization. The sample was stirred with a magnetic stir rod at 100 rpm. A He-Ne laser system described previously by Kadamne et al. [4, 5] was used in this experiment to track the crystallization in the sample. The sample was sonicated using a 1/8” tip diameter for 10 s at 216 µm amplitude. A Misonix S-3000 sonicator (Misonix Inc., Farmingdale USA) was used to sonicate the samples at a frequency of 20 kHz. Four crystallization conditions were used in the study:

1. No sonication with agitation for 10 min (no HIU-A10): After the sample was introduced into the cell, the sample was stirred (100 rpm) and then the agitation was stopped after 10 min. The sample was crystallized in the cell until 60 min. Agitation was used for 10 min to increase heat transfer and to allow for a constant cooling rate in all conditions tested.

2. No sonication with continued agitation (no HIU-CA): The sample was crystallized with continued agitation (100 rpm) throughout the experiment for 60 min.
3. Sonication at 10 min with agitation stopped at 10 min (HIU@10-A10): After the sample was introduced in the cell, the agitation was stopped after 10 min and at this point HIU was applied. The sample was crystallized in the cell until 60 min without agitation.

4. Sonication at 10 min with continued agitation (HIU@10-CA): The sample was crystallized with continued agitation (100 rpm) throughout the experiment for 60 min and HIU was applied at 10 min.

5. Crystallization experiments to measure the induction period of nucleation were performed in duplicate at temperatures between 29 and 39 °C as will be explained below. Crystallization experiments used to measure physical properties such as melting behavior, SFC, and rheology were performed in triplicate at 29, 34, and 35 °C as described below.

Measurement of induction period of nucleation

The crystallizing sample was placed in the double wall cell which was kept between the He-Ne laser and its receiver (Figure 3-1). Sample temperature was monitored as a function of time during the crystallization experiments using a thermocouple and recorded by a LabVIEW software. When the sample is liquid, the laser output is 10 V and as the sample starts to crystallize, the laser signal started to decrease. The induction period of nucleation (τ) under isothermal conditions was calculated as below:

\[ \tau = \tau_0 - \tau_T \]

Where, \( \tau_0 \) is the time needed for the laser signal to start decreasing and \( \tau_T \) is the time needed to reach crystallization temperature.
**Figure 3-1:** Schematic representation of the experimental set up. 1. Temperature probe 2. Double walled glass cell 3. Sonicator 4. He-Ne Laser

Experiments were performed in duplicates and the induction period values were reported as average of the two replicates along with their standard error.

**Isothermal solid fat content**

The isothermal solid fat content (SFC) of the sample was measured using a p-NMR (NMS 120 minispec NMR Analyzer, Bruker, Rheinstetten, Germany). The crystallizing sample was transferred from the crystallization cell to an NMR tube (to fill approximately 2 cm in the NMR tube) during crystallization at different time intervals using a transfer pipette at early stages of the crystallization process when the sample was liquid. At later stages of the crystallization process when the sample was not fluid enough to be sampled with a transfer pipette, an aliquot was taken using the back of a Pasteur pipette which was then placed inside the NMR tube. The crystallization experiments were performed in triplicates and the SFC values from the three runs were averaged and plotted against time along with their standard errors.
The Avrami equation given below (eq. 1) was fitted to the isothermal SFC data using
GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) to quantify the kinetics of
crystallization.

\[ s(t) = s_{\text{max}} \left( 1 - e^{(-kt^n)} \right) \]  

(1)

Where \( s(t) \) is the SFC at any time \( t \), \( s_{\text{max}} \) is the maximum SFC, \( k \) is the Avrami rate
constant and \( n \) is the Avrami exponent.

**Microstructure**

The microstructure of the sample was studied using a polarized light microscope (PLM)
(Olympus BX 41 Tokyo, Japan) equipped with an Infinity 2 digital camera (Lumenera
Scientific, Infinity 2, Ottawa, ON, Canada). An aliquot of sample was taken from the cell
with a glass pipette and placed between a glass slide and a cover slide to observe its
microstructure at 10X magnification. Samples were taken from the crystallizing cell at 10
min intervals until 60 min of crystallization. The microstructure of IESBO at
60 min after
processing at the different processing conditions were consolidated in a figure using the
Adobe Photoshop CS2 Version 9.0 software.

**Melting characteristics**

The DSC was set to the crystallization temperature and the melting program was loaded
on the instrument prior to weighing the sample. An aliquot of sample (10-15 mg) was
taken from the crystallization cell at 60 min of crystallization using a glass pipette and
weighed into a Tzero pan. The pan was sealed with the Tzero hermetic lid, placed in the
DSC oven immediately, and heated from the crystallization temperature to 80 °C at the
rate of 5 °C/min in a DSC Q20 (TA Instruments, New Castle, DE) differential scanning
calorimeter using an empty pan as a reference. The thermograms were analyzed by the
TA instruments Universal analysis 2000 software (TA Instruments, New Castle, DE) to measure the melting onset temperature ($T_{on}$), peak melting temperature ($T_p$) and the change in enthalpy associated with the melting process ($\Delta H$).

**Rheology**

After 60 min of crystallization the viscosity, storage modulus ($G'$), elastic modulus ($G''$) and phase angle ($\delta$) were measured using a AR-G2 Rheometer (TA Instruments, New Castle, Delaware). A concentric cylinder geometry (gap = 4000 µm) was used for the measurement of the rheological parameters. An adjustable volume Eppendorf pipette was used to pipette 7.35 mL of the fat sample into the concentric cylinder. The viscosity was measured at the crystallization temperature by the steady state flow procedure by increasing the shear rate from 0.01 to 300 s$^{-1}$. The viscosity of the sample at the shear rate of 0.1 s$^{-1}$ was reported. The viscoelastic parameters were measured by oscillatory tests using a strain sweep step. The strain values were increased from 0.008 to 10%. The rheological parameters were measured at the corresponding crystallization temperature. The rheological parameters of IESBO at 29 ºC when crystallized under the HIU@10-A10 condition were measured using a parallel plate geometry (40 mm diameter) using a gap of 1000 µm. The sample was taken with a spoon from the crystallization cell. This change in geometry was necessary since the sample was too hard to be measured using the concentric cylinder geometry.

**X-ray diffraction (XRD) measurement**

After 60 min of crystallization, the sample from the crystallization cell was filtered using a Büchner funnel and Erlenmeyer flask to collect the crystals and separate the liquid fat from the sample to obtain a better resolution in the XRD measurement. The
XRD was performed using XRD Philips X'Pert 3040 MPD (PANalytical, Almelo, The Netherlands) diffractometer system which had a single PW3050/00 (θ/2-θ) goniometer. The analysis was performed similar to that described by Ye et al. [2] and short spacings were observed in the 2θ region of 16-25º. The polymorphic form of the crystals were identified as α with a short spacing at 4.15 Å, as β’ with short spacings at 3.8 and 4.2 Å and as β if the peaks do not satisfy the conditions for α and β’ and has a strong short spacing at 4.6 Å [16].

Results and discussion

Melting point and induction period
The melting point of the sample was 43.9 ± 0.3 ºC, therefore crystallization temperatures were chosen ranging from 29 to 39 ºC at 1 ºC intervals to cover a broad range of supercoolings. Figure 3-2 shows the induction period of nucleation of IESBO as a function of crystallization temperature for the four processing conditions mentioned in the materials and methods section. A He-Ne laser system was used to track sample crystallization. The time point when the laser signal through the sample starts to drop is when crystals are first detected in the system by the laser. This gives a good indication of the time point closer to when the nucleation starts. The induction period of nucleation was calculated as the difference between the time it took for the laser signal to drop and the time it took for the sample to reach crystallization temperature. The data shows an exponential relationship between the induction period of nucleation and the temperature of crystallization. At higher crystallization temperatures, the induction period of nucleation was much higher than that at lower crystallization temperature due to decrease in the driving force with the increase in temperature. Similar results were shown by other
The induction period ranged from about 4 min at 30 °C to about 122.4 ±1.8 min for samples crystallized without HIU at 39 °C with the no HIU-A10 condition. Compared to the other processing conditions, the induction period of nucleation was 75.7 ± 3.9, 66.1 ± 2.2, and 78.3 ± 1.9 min at the 39 °C for the no HIU-CA, HIU@10-A10, and HIU@10-CA conditions respectively. For both the non-sonicated and sonicated conditions, the CA condition had in general a lower induction period compared to the ones observed for the A10 samples. When compared amongst the A10 samples, application of HIU decreased the induction period of nucleation; while the effect of sonication was not as evident for the samples crystallized under the CA conditions. The effects of sonication and agitation on the induction period of nucleation were more pronounced at higher temperatures.

Figure 3-2: Induction period of nucleation of IESBO crystallized under four processing conditions: (1) No HIU-A10 (2) No HIU-CA (3) HIU@10-A10 and (4) HIU@10-CA. Mean values and standard error of the mean of two independent runs are reported. The data was fitted to an exponential curve
To investigate how the differences in the induction period due to application of sonication and agitation translate into changes in physical properties of the crystalline network, three crystallization temperatures were chosen to represent temperatures where changes in induction periods were not observed (\( T_c = 29 \, ^\circ C \)), where small changes in induction periods (\( T_c = 34 \, ^\circ C \)) were observed, and where large changes in induction periods (\( T_c = 35 \, ^\circ C \)) were observed. \( T_c = 35 \, ^\circ C \) was chosen as the highest temperature since the samples were solid-like at this temperature and differences in the physical properties could be observed due to the change in processing conditions. When samples were crystallized at temperatures above 35 °C very few crystals were obtained and samples remained liquid even after 120 min at \( T_c \).

**Isothermal solid fat content (SFC)**

The isothermal solid fat content (SFC) of the IESBO crystallized under different processing conditions and temperatures are presented in Figure 3-3 and the Avrami parameters are tabulated in Table 1. All the Avrami fits had \( R^2 \) values higher than 0.75 except for the non-sonicated samples at 35 °C (Table 1).

The SFC curves start from the time the sample was introduced in the cell up to 60 min of crystallization. The time point when the isothermal SFC curve started to rise gives an indication of crystal growth in the system and in this study, is used to measure the induction time of crystallization. The induction period of nucleation values are lower than the induction time of crystallization as crystallization progressed after nucleation. At 29 °C, the induction time of crystallization was approximately 12 min for the sonicated samples and between 15-17 min for the non-sonicated samples. Figure 3-3A shows a faster rise in the SFC of the sonicated samples compared to the non-sonicated ones when
Figure 3-3: Solid fat content of IESBO crystallized at 29, 34, and 35 °C under four processing conditions: (1) No HIU-A10 (2) No HIU-CA (3) HIU@10-A10 and (4) HIU@10-CA. Plotted values of SFC are the average of triplicates results with their standard error of the mean.
**Table 3-1:** Avrami parameters obtained from the Avrami fit to the isothermal solid fat content data of IESBO crystallized without and with sonication. Tabulated values are average ± standard error of the mean.

<table>
<thead>
<tr>
<th>$T_c$ (°C)</th>
<th>Sample</th>
<th>No HIU-A10</th>
<th>No HIU-CA</th>
<th>HIU@10-A10</th>
<th>HIU@10-CA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$s_{\text{max}}$ (%)</td>
<td>4.0 ± 0.1$^b$</td>
<td>4.7 ± 0.1$^a$</td>
<td>5.1 ± 0.1$^a$</td>
<td>5.2 ± 0.2$^a$</td>
</tr>
<tr>
<td>29</td>
<td>$k$ (min$^{-n}$)</td>
<td>$1.3 \times 10^{-5}$ ± $8.9 \times 10^{-6}$$^b$</td>
<td>$2.8 \times 10^{-7}$ ± $3.0 \times 10^{-7}$$^b$</td>
<td>$6.8 \times 10^{-5}$ ± $1.7 \times 10^{-9}$$^a$</td>
<td>$6.9 \times 10^{-5}$ ± $5.3 \times 10^{-9}$$^a$</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>3.3 ± 0.2$^a$</td>
<td>4.2 ± 0.3$^a$</td>
<td>3.2 ± 0.03$^a$</td>
<td>3.2 ± 0.04$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.97</td>
<td>0.96</td>
<td>0.91</td>
<td>0.88</td>
</tr>
<tr>
<td>34</td>
<td>$s_{\text{max}}$ (%)</td>
<td>1.8 ± 0.2$^c$</td>
<td>2.9 ± 0.1$^{ab}$</td>
<td>3.5 ± 0.2$^a$</td>
<td>2.8 ± 0.2$^b$</td>
</tr>
<tr>
<td></td>
<td>$k$ (min$^{-n}$)</td>
<td>$2.6 \times 10^{-9}$ ± $9.9 \times 10^{-9}$$^a$</td>
<td>$5.1 \times 10^{-7}$ ± $6.3 \times 10^{-7}$$^a$</td>
<td>$2.4 \times 10^{-7}$ ± $6.1 \times 10^{-7}$$^a$</td>
<td>$4.8 \times 10^{-7}$ ± $1.5 \times 10^{-6}$$^a$</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>5.2 ± 1.0$^a$</td>
<td>3.9 ± 0.4$^a$</td>
<td>4.4 ± 0.8$^a$</td>
<td>4.1 ± 0.9$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.80</td>
<td>0.95</td>
<td>0.77</td>
<td>0.82</td>
</tr>
<tr>
<td>35</td>
<td>$s_{\text{max}}$ (%)</td>
<td>0.2 ± 0.1$^b$</td>
<td>NC</td>
<td>1.3 ± 0.0$^a$</td>
<td>1.8 ± 0.3$^a$</td>
</tr>
<tr>
<td></td>
<td>$k$ (min$^{-n}$)</td>
<td>$7.8 \times 10^{-20}$ ± 0.0$^a$</td>
<td>NC</td>
<td>$6.8 \times 10^{-13}$ ± $1.8 \times 10^{-12}$$^a$</td>
<td>$4.4 \times 10^{-11}$ ± $2.6 \times 10^{-10}$$^a$</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>10.8 ± 0.4$^{ab}$</td>
<td>NC</td>
<td>7.3 ± 0.7$^b$</td>
<td>6.2 ± 1.6$^a$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.33</td>
<td>NC</td>
<td>0.95</td>
<td>0.75</td>
</tr>
</tbody>
</table>

$s_{\text{max}}$: maximum solid fat content, $k$: Avrami rate constant, $n$: Avrami exponent

NC: Avrami equation did not converge for the no HIU-CA condition at 35 °C

The Avrami parameters were compared among 29 and 34 °C by a 2-way ANOVA followed by Tukeys’ multiple comparison test at the same temperature among different processing conditions at $\alpha=0.05$. The Avrami parameters were compared among 35 °C by a 1-way ANOVA followed by Tukeys’ multiple comparison test. At the same temperature, each parameter at different processing condition with different superscript alphabets are significantly different.
crystallized at 29 °C. The rise of SFC in the HIU@10-A10 sample can be attributed to secondary crystallization where the existing crystals were broken down during sonication and crystal growth continued on the newly formed nuclei. Since induction time of crystallization in the HIU@10-CA condition started almost at 12 min and sonication was applied at 10 min the bubbles generated during sonication were not affected by the continued agitation and an induction in the crystallization was observed. This correlates well with the Avrami rate constant, $k$ presented in Table 1, which was significantly higher for the sonicated samples compared to the non-sonicated ones ($p < 0.05$). The maximum solid fat content, $s_{\text{max}}$, of the sonicated samples was also higher than the non-sonicated ones. This increase in the final SFC was significant for the A10 samples ($p < 0.05$) but not for the CA samples. Temperature along with agitation promoted the crystallization at this processing condition (CA) and sonication did not contribute to a further rise in SFC of the samples. Agitation promoted crystallization and thus continued agitation induced higher crystallization in the non-sonicated sample compared to the non-sonicated A10 sample ($p < 0.05$, Table 1). The $s_{\text{max}}$ of the non-sonicated samples crystallized under A10 conditions at 29 °C was 4.0 ± 0.1% while that of the non-sonicated one crystallized under CA conditions was significantly higher (4.7 ± 0.1%). The Avrami exponent ($n$) was not significantly different ($p > 0.05$) for all the crystallization conditions tested; however, a slightly higher $n$ value was obtained for the non-sonicated sample crystallized under CA conditions. Marangoni [19] explains that the $n$ value represents the type of crystal growth and the pattern of nucleation which could be either instantaneous or sporadic. The pattern of nucleation either assumes a value of 0 (sporadic) or 1 (instantaneous). The type of crystal growth can assume values including 3 (spherulitic), 2 (disc) or 1 (rod). So, an $n$
value of 3 could be the summation of either 0 and 3 which could indicate instantaneous spherulitic nucleation or 1 and 2 which is sporadic disc shaped crystal nucleation.

The isothermal SFC curve of IESBO crystallized at 34 °C under different processing conditions is presented in Figure 3-3B. Upon comparison of the SFC at 34 °C with that at 29 °C, the maximum SFC attained by the sample at 34 °C was lower due to the higher crystallization temperature. The $s_{\text{max}}$ of the sample was 1.8 ± 0.2, 2.9 ± 0.1, 3.5 ± 0.2 and 2.8 ± 0.1\% for no HIU-A10, no HIU-CA, HIU@10-A10 and HIU@10-CA conditions respectively. The SFC of the IESBO at 34 °C was higher (p < 0.05) when sonicated with A10 agitation compared to the CA condition. During the CA condition agitation was counteractive to the effect of HIU by slightly dissolving the bubbles and thus decreasing the effect of sonication. Hence, less secondary crystallization occurred and hence the HIU@10-CA sample had a lower final SFC than the HIU@10-A10 sample. It should be noted however, that although the overall SFC was lower when sonicated with CA, the rate of crystallization was not significantly different between the two processing conditions indicating that CA did not affect crystal growth in the sample. This also suggests that HIU alone is more efficient at inducing lipid crystallization than just agitation or a combination of agitation and HIU at the intermediate temperature. In general, at 29 °C, the $n$ value was 3 while at 34 °C, the $n$ value is 4. This indicates that the crystallization pattern is sporadic with spherulite nuclei [19]. According to Wright et al. [20] an increase in the Avrami exponent is expected with an increase in the induction period of nucleation. For the no HIU-A10 condition, $n$ value was 5.2 which is higher than the range of values expected for $n$. Higher values of $n$ have been reported by other
authors [4, 20], however it is difficult to predict the growth mechanism based on the higher $n$ values.

The isothermal SFC curve of IESBO at 35 °C is presented in Figure 3-3C and the Avrami parameters are presented in Table 1. There was very small rise in the SFC of the IESBO sample at 35 °C under the no HIU-A10 condition and hence there was a poor fit of the Avrami curve ($R^2 = 0.33$) for this data set. Also, the Avrami fit did not converge for the no HIU-CA condition at 35 °C and hence this data is not presented in Table 1. The $s_{\text{max}}$ and $k$ was lowest amongst the other temperatures studied. The SFC curve for the no-HIU A10 condition showed a minimal increase with an Avrami rate constant of $7.8 \times 10^{-20} \text{ min}^{-n}$ and a $s_{\text{max}}$ of 0.2%. The fit for this data was poor with an $R^2$ of 0.33 and the $n$ value was 10.8 which was vague. There was some crystallization observed in the sample upon sonication and the $s_{\text{max}}$ was 1.3 and 1.8% for the A10 and CA conditions respectively. Crystal growth is driven either by thermodynamic or kinetic factors. At the highest $T_c$, since the thermodynamic factor was weakest, continued agitation and/or HIU helped the crystallizing TAGs to diffuse through the fat to the nucleating crystal and thus promoted crystallization. Hence the no HIU-A10 sample had the least $s_{\text{max}}$ compared to all the other processing conditions (Figure 3-3C and Table 1). There were no significant differences in the $s_{\text{max}}$ or $k$ of the HIU@10-A10 and HIU@10-CA condition at 35 °C. At the highest crystallization temperature, the samples were sonicated prior to nucleation. Ye et al. (2011) studied a similar processing condition and observed that although not as effective when sonicated in the presence of crystals, there was a still slight reduction in crystal size when sonication occurred in the absence of crystals compared to the non-sonicated condition. Further agitation upon sonication increased the interaction among
the crystallizing species promoting crystal growth and hence the formation of a crystalline network. However, more crystals were not induced after sonication and the $s_{\text{max}}$ of the two sonication conditions were not different. The Avrami exponent, $n$, was 7.3 and 6.2 which for the HIU@10-A10 and HIU@10-CA condition respectively, which was higher than the values expected for $n$. As previously discussed, these high values do not have a physical meaning and it is difficult to explain the growth mechanism based on these values.

**Microstructure**

Figure 3-4 shows the microstructure of the IESBO crystallized under different processing conditions at different temperatures. The brighter spots represent the crystals over a darker background of liquid sample. Upon visual observation, these pictures show that the amount of crystals decreased with the increase in the crystallization temperature. When the microstructure was visually compared at the same crystallization temperature, it was found that the different processing conditions generated crystals with different sizes. In the non-sonicated samples, at 29 °C, the morphology was similar among the A10 and CA sample. This correlates well with the $s_{\text{max}}$ data at these conditions. Application of HIU induced the formation of smaller and more crystals in the system as can be observed in Figure 3-4. There was no distinct difference in the microstructure of the A10 and CA sonicated samples. Since spherulite crystals are observed at 29 °C in the microstructure presented in Figure 3-4, and thus an $n$ value of 3 could very possibly stand for instantaneous spherulitic nucleation. At 29 °C, for the no HIU-CA condition, with an $n$ value of 4 and with a microstructure containing spherulite crystals, it would be safe to
Fig. 3-3 Microstructure of IESBO after 60 min of crystallization at 10X magnification. Samples were crystallized at 29, 34, and 35 °C under different processing conditions: (1) No HIU-A10 (2) No HIU-CA (3) HIU@10-A10 and (4) HIU@10-CA
assume that the nucleation was sporadic (4=1+3) [19] or a combination of instantaneous and sporadic nucleation.

When samples were crystallized at 34 °C the size of the crystals was larger for the no HIU-A10 condition compared to the no HIU-CA sample. Larger crystals in the microstructure of the non-sonicated A10 samples compared to the CA samples can be explained based on the theory that temperature was the only driving force for crystallization after the agitation was stopped. The temperature drove the growth of existing crystals instead of inducing additional nucleation in the sample. In the no HIU-CA sample, the agitation along with the temperature induced nucleation in the sample and hence the crystals were smaller. At this temperature, the non-sonicated, both A10 and CA IESBO had a liquid consistency and the crystals were suspended in the solution. Sonication did induce the formation of smaller and more crystals in the sample at 34 °C, especially for the A10 condition. Based on the SFC data, the highest SFC was obtained in the HIU@10-A10 sample followed by the sonicated CA samples. No HIU-A10 condition had the lowest SFC amongst all the processing conditions. SFC values discussed above correlate well with the microstructure shown in Figure 3-4. The microstructure shows spherulite crystals and this correlates well with the Avrami exponent, \( n \) of 4.

Few and large crystals suspended in the liquid sample were observed for samples crystallized at 35 °C. This correlates well with the SFC data. Continued agitation induced more crystallization in the non-sonicated sample compared to the no HIU-A10 condition and smaller crystals were observed in the sample crystallized under CA condition. The consistency of this sample at 60 min of crystallization was liquid with suspended crystals.
Sonicated samples, in general had more crystals than the non-sonicated samples, at this temperature. Since these samples were sonicated prior to nucleation and crystal formation, secondary nucleation did not happen and hence smaller crystals like those seen in the microstructure of HIU@10-A10 at 29 and 34 °C were not observed.

Thus, HIU was effective at inducing the formation of smaller and more crystals in the system at lower temperature (29 °C) irrespective of the agitation condition. At intermediate and higher crystallization temperatures (34 and 35 °C), HIU induced nucleation in the sample and crystal growth was perpetuated by the thermodynamic force in case of HIU@10-A10 and by agitation in case of HIU@10-CA.

**Melting characteristics of IESBO based on differential scanning calorimetry**

**thermograms**

Melting profiles of IESBO samples are presented in Figure 3-5 and the integrated DSC parameters are presented in Table 2. The melting onset temperature could only be determined for the HIU@10-A10 condition crystallized at 29 °C. Based on the shape of the thermograms, it can be observed that there were no significant differences in the crystallizing fractions at 29 or 34 °C with processing conditions. At 35 °C, due to the higher crystallization temperature and hence a lower supercooling, there was a very small amount of crystalline material obtained in the non-sonicated samples. Thermograms of the non-sonicated samples were mostly flat, while thermograms of the sonicated samples were more defined in shape. Different processing conditions did not affect the peak melting temperature of the sample. The enthalpy of melting is an estimate of the amount of crystallinity in the sample. In general, the enthalpy of melting decreased with the
increase in crystallization temperature. Although, smaller and more crystals were observed in the microstructure of the IESBO at 29 ºC under the HIU@10-A10 condition,

![DSC melting thermograms of IESBO](image)

**Figure 3-4** DSC melting thermograms of IESBO after 60 min of crystallization. Samples were crystallized at 29, 34, and 35 ºC under different processing conditions: (1) No HIU-A10 (2) No HIU-CA (3) HIU@10-A10 and (4) HIU@10-CA. Temperatures indicated below the thermograms represent mean values for the peak melting temperatures (Table 2).
Table 3-1: DSC parameters including the melting onset temperature ($T_{on}$), peak melting temperature ($T_p$) and the melting enthalpy ($\Delta H$) obtained from integrating the melting thermograms of IESBO processed at different conditions

<table>
<thead>
<tr>
<th>Melting onset temperature ($T_{on}$) ($^\circ$C) *</th>
<th>HIU@10-A10</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>33.8 ± 1.7**</td>
</tr>
</tbody>
</table>

| Peak Melting temperature ($T_p$) ($^\circ$C) |
|--------------------------------------------|-------------|
| $T_c$ (°C)                                 | no HIU-A10 | no HIU-CA | HIU@10-A10 | HIU@10-CA |
| 29                                         | 45.1 ± 0.2a | 43.2 ± 0.2a | 43.9 ± 0.2a | 44.3 ± 0.4a |
| 34                                         | 47.7 ± 0.4a | 47.0 ± 0.5a | 46.7 ± 0.8a | 46.8 ± 0.9a |
| 35                                         | 45.7 ± 0.2a | 45.2 ± 2.0a | 46.9 ± 1.1a | 46.3 ± 0.7a |

| Enthalpy of melting ($\Delta H$) (J/g) |
|---------------------------------------|-------------|
| $T_c$ (°C)                            | no HIU-A10 | no HIU-CA | HIU@10-A10 | HIU@10-CA |
| 29                                    | 9.1 ± 0.5b  | 13.2 ± 0.6a | 7.6 ± 0.6b  | 7.4 ± 0.6b  |
| 34                                    | 6.6 ± 1.0a  | 6.0 ± 0.8a  | 7.6 ± 1.1a  | 7.3 ± 1.0a  |
| 35                                    | 2.0 ± 0.2b  | 4.6 ± 0.9ab | 5.8 ± 0.4a  | 6.2 ± 0.2a  |

* melting onset temperatures could not be integrated by the software for all the processing conditions
** melting onset temperature could be integrated for only 2 replicates
The $T_{peak}$ and the Enthalpy data was compared across temperatures and processing conditions by 2-way ANOVA followed by Tukeys’ multiple comparison test at the same temperature among different processing conditions at $\alpha=0.05$. At the same temperature, each parameter at different processing condition with different superscript alphabets are significantly different.
the melting enthalpy was not significantly higher than for the no HIU-A10 at 29 ºC. There were no significant differences (p > 0.05) in the enthalpy values obtained for samples crystallized at 34 ºC. At 35 ºC, the melting enthalpy was lower for samples crystallized under the no HIU-A10 condition indicating less crystallinity in the sample than other processing conditions tested at this temperature (p<0.05). This data correlates well with the microstructure of IESBO at 35 ºC for no HIU-A10 where there were very few crystals compared to the other conditions. The enthalpy of melting for HIU@10-A10 was significantly higher than the no HIU-A10 sample at 35 ºC but was not different from the other conditions tested at this temperature. This is an interesting result since it suggests that a similar amount of crystalline material can be obtained when samples are sonocrystallized statically compared to the amount obtained when the sample is crystallized under continuous agitation. These enthalpy results relate well with the SFC data described before.

Rheology

Figure 3-6 shows the viscoelastic data of IESBO samples crystallized at 29, 34, and 35 ºC using the different processing conditions tested. The viscosity of IESBO was highest for the HIU@10-A10 condition at 29 ºC and was 2,369 ± 712 Pa.s. The microstructure of the sample crystallized using this processing condition shows small crystals. Kadamne et al. showed that smaller and more crystals in the microstructure corresponds to higher viscosity and viscoelastic properties [4, 5]. Although smaller crystals were seen in the microstructure of the sonicated samples at 29 ºC, the viscosity of the CA sample was significantly lower than the sonicated A10 sample and was not different from the non-sonicated A10 sample (p< 0.001). Also, the $s_{\text{max}}$ of the no HIU-
Figure 3-5 Rheological parameters of IESBO crystallized at 29, 34, and 35 ºC under different processing conditions: (1) No HIU-A10 (2) No HIU-CA (3) HIU@10-A10 and (4) HIU@10-CA. The rheological parameters were compared by a 2-way ANOVA within each temperature and Tukeys’ multiple comparison test was performed to compare the parameters among the 4 processing conditions at each temperature. Values shown are average ± standard error of the mean. Parametric values at each temperature indicated by different alphabets are significantly different (α= 0.05).
CA, HIU@10-A10 and HIU@10-CA were not statistically different (p > 0.05). This can be explained based on the interactions of the existing crystals and the morphology of the crystalline network. The CA changes the way the existing crystals interact with each other and hence further crystal growth is also affected. This further affects the crystalline network in the system. Fat crystal aggregates can be seen in the microstructure of samples processed by all the processing conditions except HIU@10-A10 which only has smaller crystals. This microstructure gives it a uniform network and hence imparts the higher viscosity to the sample. This indicates that sonication significantly increased the viscosity of the samples when used at the A10 condition. At 34 and 35 ºC, there were no significant differences in the viscosity of the sample with the change in processing condition although there were differences in the microstructure. At 35 ºC, there were fewer crystals in the PLM of the no HIU-A10 sample compared to the no HIU-CA sample and this correlated well with the viscosity readings. In an earlier study, Herrera et al. [1] compared fat crystallization with and without agitation and found that agitation induced the formation of smaller and more crystals in the sample. With more crystals in the system, there may be higher interaction amongst the crystals which might form a network. This higher interaction may have further correlated to an increased viscosity. Recently, Silva et al. [13] also showed that with increased agitation, there was an increase in the crystallinity in the sample that resulted in increased viscosity.

Like the trend in the viscosity data, the storage modulus, G’ of the sample was highest at 29 ºC when processed by the HIU@10-A10 condition (p < 0.001). The G’ of the sample was 69,236.7 ± 26,765.0 Pa and 47.3 ± 20.8 Pa at 29 ºC for the HIU@10-A10 and the HIU@10-CA condition. The magnitude of G’ was highest for HIU@10-A10
conditions also at 34 and 35 °C but the value was not statistically different (p > 0.05) from the other processing conditions at these temperatures.

The elastic modulus, G” was also higher in magnitude for the HIU@10-A10 condition at all temperatures studied. However, it was significantly different only at 29 °C. The G” values were 3564.7 ± 1280.5, 142.8 ± 38.6, 26.6 ± 2.2 Pa at 29, 34 and 35 °C, respectively.

**X-ray diffraction (XRD)**

The IESBO crystals obtained from all the crystallization conditions except the ones obtained at 35 °C no HIU-A10, were analyzed by XRD. Samples crystallized at 35 °C using the no HIU-A10 condition were very liquid and not enough crystals could be obtained to perform the X-ray analysis. The IESBO, irrespective of the crystallization temperature or the processing condition, crystallized only in the β’ polymorphic form. This was confirmed based on the strong XRD signals obtained at 3.8 and 4.2 Å. In addition, the IESBO sample crystallized at 34 °C under the HIU@10-A10 condition was kept at 34 °C for 31 days and checked for the polymorphism of the crystals. It was found that there was no change in the polymorphism of the sample and the crystals maintained the β’ polymorphic form. The results agree with those reported by Ye et al. [2] that IESBO crystallized in the β’ polymorphic form under different crystallization conditions.

**Conclusion**

This study showed that HIU was more effective in inducing crystal growth than nucleation when HIU was applied in the absence of crystals in the system. However, agitation was more effective on the reduction of induction period of nucleation than sonication alone. Sonication significantly improved the rate of crystallization at the
lowest $T_c$ and the $s_{\text{max}}$ at the highest $T_c$ irrespective of the agitation condition. Thus, crystallization kinetics were not affected by the presence of agitation during sonication. Similarly, higher enthalpy values were observed for the sonicated samples at the lowest $T_c$ compared to the non-sonicated samples. The different processing conditions studied here did not change the polymorphic form of the crystals in IESBO. The effect of HIU on improving the rheological properties of fat is more pronounced at a lower crystallization temperature which drives the samples to crystallization. Although agitation alone is known to induce nucleation in a supercooled lipid sample the effect of sonication is more pronounced in the absence of agitation after HIU has been applied. When HIU is applied in the presence of agitation the bubbles formed during sonication that are responsible for inducing changes in the crystallization of the fat might be quickly dissolved by the agitation in the sample. This faster dissolution of the bubbles might result in a lower efficiency of sonication. Also, agitation may have affected the interaction of the crystals resulting in a change of the crystalline network which affected its rheological properties.

**Acknowledgements**

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**References**


CHAPTER 4
SONOCRYSTALLIZATION OF INTERESTERIFIED FATS WITH 20 AND 30% C16:0 AT SN-2 POSITION

Abstract

The objective of this study was to induce crystallization in enzymatically interesterified fats (IE) with 20 and 30% palmitic acid at the sn-2 position using high intensity ultrasound (HIU). The physical blends (PB) used to prepare these two IE consisted of tripalmitin and high oleic sunflower oil and contained 13.2 and 27.1% tripalmitin, respectively. Crystallization behavior of IE was compared with PB at supercoolings of 9, 6 and 3 °C. Results show that the melting point, SFC, and crystallization rate of PB were higher than IE and were driven mainly by tripalmitin content. HIU induced crystallization and generated small crystals in the IE samples. At 9 °C supercooling, sonication did not increase the viscosity of IE C16:0 20%, while that of the IE C16:0 30% increased significantly from 192.4 ± 118.9 to 3297.7 ± 1368.6 Pa·s. The elastic modulus (G’) for IE C16:0 30% increased from 12521 ± 2739.8 to 75076.7 ± 18259 Pa upon sonication at 9 °C supercooling, while the G’ of the IE C16:0 20% did not increase. Similar behavior was observed for the other supercoolings tested. This research suggests that HIU can improve the functional properties of IE with low content of C16:0 creating more viscous and elastic materials. These fats with low C16:0 content and improved functional properties could be used as trans-free fat alternatives.

1Journal of American Oil Chemists’ Society, Sonocrystallization of interesterified fats with 20 and 30% C16:0 at the sn-2 position, 94, 2017, 3-18, Kadamne JV, Ifeduba EA, Akoh CC and Martini S, (original copyright notice as given in the publication in which the material was originally published) “With permission of Springer”
Introduction

In June 2015, the FDA ruled on its 2013 proposal regarding partially hydrogenated oils (PHO) and concluded that PHO are not “generally recognized as safe” due to the presence of trans-fats. FDA has set a three-year compliance period for food companies to reformulate products and eliminate PHOs in any food products unless approved by FDA. Consumption of trans fatty acids is associated with higher chances of coronary heart disease and increase in plasma low density lipoprotein cholesterol [1, 2]. Following FDA’s proposal in 2013, efforts were directed towards replacement of PHO by trans-free alternatives derived by processing techniques including but not limited to blending, genetic modification, fractionation, and interesterification. In order to reformulate food products with trans-free fats, there is no one fat that would fit all applications, rather the replacement fat must be designed for each specific application [3]. The trans-fat substitute for any application must possess similar functionality as trans-fats, including melting, crystallization, oxidative stability, and texture [3].

While reformulating products to replace PHO, the current consumer trend towards healthier food options must be considered and reformulation of products with low saturated, nutritionally beneficial fats seems necessary. Enzymatic interesterification can be employed to develop a structured lipid with nutritional benefits and desired triacylglycerol (TAG) composition which can serve as healthier trans-fat alternatives [4]. The relationship between TAG positional isomers and nutritional properties is still controversial, but recent research has suggested that consumption of fats containing TAG with saturated fat at the sn-2 position by healthy adults decreased postprandial lipema [5] and also did not affect insulin secretion [6,7] or glucose homeostasis [6]. Triacylglycerols
with this unique arrangement of fatty acids are found in human milk fat [8] and lard [9]. Structured lipids with palmitic acid at the sn-2 position developed by enzymatic interesterification can be also used as human milk fat substitutes in infant formulas [9] and to replace lard in food such as sausages. Recently, Ifeduba et al. [10] demonstrated the production of fats with saturated fatty acids at the sn-2 position using Lipozyme TLIM lipase. However, on comparison of the physical properties of the fat before and after interesterification it was found that in general, the interesterified (IE) samples are softer, crystallize slowly, and have lower isothermal solid fat content than the starting fat [11]. Also, lipids with TAG containing saturated fats at the sn-2 position melt at lower temperature which gives them a softer texture and thus restricts their application as trans-fat alternatives.

Previous research has shown that high intensity ultrasound (HIU) can improve the physical properties of fat systems [12, 13]. Crystallization in the presence of ultrasound is termed as sonocrystallization [14] and an excellent compilation of the overview, process parameters, mechanism and applications of sonocrystallization has been provided by Martini [15]. Ye et al. [12] tested different sonication conditions on low saturated interesterified fat and made the following two major conclusions (a) HIU is highly effective when applied at the onset of crystallization in the presence of crystals and (b) HIU generates smaller crystals by secondary nucleation and develops a harder, more viscous, and elastic material. Furthermore, in a separate study it was confirmed that HIU-induced fat crystals were similar in TAG and fatty acid composition to the crystals generated in its absence [16]. HIU was also effective in inducing crystallization in anhydrous milk fat, palm kernel oil and all-purpose shortening [13]. In a study by Patrick
et al. [17] it was found that a range of different crystal structures could be developed in palm oil by varying the applied ultrasonic intensity. In tripalmitin [14, 18], cocoa butter [18], and trilaurin [14] systems it was found that by adjusting the HIU and supercooling conditions, it was possible to obtain a stable polymorphic form.

The research presented here is focused on evaluating the effect that HIU has on functional properties of interesterified fats containing palmitic acid at the sn-2 position. The functional properties evaluated were melting point, isothermal solid fat content, microstructure, rheological properties—viscosity, $G'$ and $G''$, and melting behavior. The fat samples were crystallized at different supercooling levels and HIU was applied in the presence of crystals. The functional properties were compared at the same supercooling between the physical blend and the interesterified samples crystallized with and without HIU.

**Materials and Methods**

**Materials**

Interesterified (IE) and physical blends (PB) of tripalmitin (>85% purity, sourced from TCI American, Portland, OR [10]) and high oleic sunflower oil (HOSO) (Trisun® sourced from Stratas Foods, Memphis, TN [10]) with 20 and 30% palmitic acid (C16:0) were prepared in Dr. Akoh’s laboratory at the University of Georgia according to the methods outlined by Ifeduba et al. [10]. The IE and PB with 20% palmitic acid contained 71.4 and 71.5% oleic acid and 4.6 and 4.7% linoleic acid, respectively while those with 30% palmitic acid contained 61.8 and 61.4% oleic acid 4.0 and 3.9% linoleic acid, respectively [10]. The PB contained 16.8% palmitic acid at the sn-2 position which upon interesterification and acyl migration increased to 21.5% [10]. The major triacylglycerol
(TAG) species in PB with 20% C16:0 samples were OOO (80.8%) and PPP (13.2%) (O = oleic acid, P = palmitic acid) and in IE with 20% C16:0 samples were OOO (45.4%) and OOP + OPP (47.2%) [10]. The major TAG species in PB with 30% C16:0 samples were OOO (68.3%) and PPP (27.1%) (O = oleic acid, P = palmitic acid) and in IE with 30% C16:0 samples were OOO (27.5%) and OOP + OPP (67.1%) [10]. The PB and IE with 30% palmitic acid contained 23.8 and 32.9% palmitic acid at the sn-2 position, respectively [10]. The analysis of the TAG composition was based on the equivalent number of carbons (ECN) following the method described by Ifeduba et al. [19] and hence all the stereochemical isomers were integrated as one peak. The detailed fatty acid and TAG composition of the IE and PB with 20 and 30% palmitic acid has been further discussed in detail by Ifeduba et al. [10].

**Capillary Tube Melting Point**

The melting point of the samples was measured by AOCS Official method Cc 1-25 [20].

**Crystallization and Application of HIU**

All the samples were first melted in the microwave followed by heating in the oven at 80 °C for thirty minutes to allow for complete melting of crystals. The samples were filtered hot to ensure that no impurities were present in them using a Büchner funnel and flask which were also preheated in the oven to prevent crystallization during filtration. The oil samples were filtered by Whatman glass microfiber filters using vacuum suction and stored in glass bottles in the freezer until further use.

For the crystallization experiments, 30 g of the filtered sample was melted in a microwave oven and kept at 80 °C for at least 30 min to allow for complete melting of
the sample. After this period of time, samples were introduced into a double-wall thermostatized cell set at crystallization temperatures (T_c) corresponding to supercooling levels of 3, 6 and 9 °C based on their melting points using a water bath (VWR, Radnor, PA). As soon as the sample was placed into the crystallization cell, samples were stirred with a magnetic stirrer (100 rpm) for 10 min. The crystallization of the sample was monitored using a HeNe laser set up (105-2, Uniphase, San Jose, CA) similar to that described by Herrera [21]. The glass cell containing the crystallizing fat was kept between the laser source and the receiver. The receiver detected the light signal transmitted though the sample. The initial laser signal was approximately 10 V when the fat was completely liquid and started to drop down to 0 V as the sample started to crystallize [22, 23]. Lab view software version 8 (National Instruments Corp., Austin, TX) was used to record the laser signal (V) and sample temperature (°C) as a function of time.

The IE samples were crystallized without and with the application of HIU (20 kHz) while the PB samples were crystallized without HIU application. For the IE samples crystallized with HIU application, HIU was applied when the laser signal dropped to 0.6 V. Previous research [12, 13] has shown that HIU was more effective in the presence of crystals. The decreasing laser signal is an indication of presence of crystals. The HIU was applied just before the laser signal dropped to zero. Slight cloudiness was observed when the Laser signal reached 0.6 V and therefore this value was chosen to apply the HIU at consistent time points and conditions in the different samples and processing conditions. HIU was applied for 5 s using a Misonix S-3000 sonicator (Misonix Inc., Farmingdale, NY) and a 1/8” diameter microtip of 6 ½ inch length and a vibration amplitude of 216
μm. The agitation was stopped just before application of HIU if the laser signal dropped to 0.6 V before 10 min. After HIU was applied, the sample was transferred using transfer pipettes into four centrifuge tubes and six NMR glass tubes, which were maintained at $T_c$ in a water bath. The samples crystalized in these tubes at $T_c$ for the entire duration of the experiment. The samples from the centrifuge tubes were used for rheology and five of the NMR tubes were used for SFC determination. Aliquots of samples were taken from the remaining NMR tube by glass pipettes for microscopic and DSC analysis.

For IE and PB samples crystallized without the application of HIU, agitation was stopped at 10 min and samples were transferred to the tubes immediately after the laser signal dropped to 0.6 V similar to that described above. If the laser signal dropped to 0.6 V before 10 min, agitation was stopped and the sample was immediately transferred to the said tubes.

The samples from the tubes were used to evaluate crystal morphology by polarized light microscopy (PLM), melting behavior using differential scanning calorimetry (DSC), viscoelastic behavior by rheology, and isothermal solid fat content (SFC) using a p-NMR. Each crystallization run followed by the subsequent analyses were performed in triplicates at each $T_c$ for all the samples except for the SFC data which was collected in duplicates.

**Isothermal solid fat content (SFC)**

After the sample was transferred to NMR tubes maintained at $T_c$, the tubes were kept in a water bath and the solid fat content of the sample was measured isothermally every min for 60 min. The samples from the five, numbered NMR tubes, kept at $T_c$ were used repeatedly, in sequence to measure the SFC by NMR every min after the samples
were transferred to the tubes. The NMR tubes for SFC analysis were put back in the water bath after each measurement. An NMS 120 minispec NMR Analyzer (Bruker, Germany) was used for the SFC measurement using the five NMR tubes in succession by the AOCS Direct method Cd 16b-93 [24]. For each sample at each crystallization temperature (Table 4-1), the SFC data were collected in duplicates, from two separate crystallization runs and the data is reported as a mean of data from the two runs. SFC values followed a two-step or a single step crystallization behavior. Curves that showed a two-step crystallization were fit using a multi component Avrami [25] model using Eq. (1) by GraphPad Prism Software (GraphPad Software, Inc., La Jolla, CA):

\[
s(t) = S_0 + \sum_{i=1}^{n} s_{max_i} \times \left(1 - e^{-\left(K_i \times t^n_i\right)}\right)
\]

where \(S_0\) is the SFC at \(t = 0\), \(s_{max}\) is the maximum SFC (%), \(k\) is the Avrami rate constant and \(n\) is the Avrami exponent.

Curves that showed a single step crystallization were fitted to a re-parameterized Gompertz equation [26, 27] (Eq. (2)) also by the GraphPad Prism Software (GraphPad Software, Inc., La Jolla, CA):

\[
s(t) = s_{max} \times e^{e^{-\left(\frac{\mu_{max}}{s_{max}} \times e^{x(\lambda - t) + 1}\right)}}
\]

where \(s(t)\) is the % SFC at time \(t\), \(s_{max}\) is the maximum SFC, \(\mu_{max}\) is the maximum rate of increase in SFC (%/min), \(\lambda\) is the induction time of crystallization (min), \(e = 2.718281\) [27].
Table 4-1: Melting point (Tm) of the samples, crystallization temperatures (Tc) used to achieve supercoolings of 3, 6, and 9 °C and the driving forces (Φ) at each of the crystallization temperatures.

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Tm (°C)**</th>
<th>TC (°C)</th>
<th>Φ</th>
<th>Tc (°C)</th>
<th>Φ</th>
<th>Tc (°C)</th>
<th>Φ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB C16:0 20%</td>
<td>48.8 ± 1.2</td>
<td>40</td>
<td>15.09</td>
<td>43</td>
<td>10.06</td>
<td>46</td>
<td>5.03</td>
</tr>
<tr>
<td>IE C16:0 20%</td>
<td>16.1 ± 0.7</td>
<td>7</td>
<td>39.68</td>
<td>10</td>
<td>26.45</td>
<td>13</td>
<td>13.23</td>
</tr>
<tr>
<td>PB C16:0 30%</td>
<td>55.7 ± 0.7</td>
<td>47</td>
<td>17.00</td>
<td>50</td>
<td>11.34</td>
<td>53</td>
<td>5.67</td>
</tr>
<tr>
<td>IE C16:0 30%</td>
<td>28.0 ± 0.3</td>
<td>19</td>
<td>23.85</td>
<td>22</td>
<td>15.90</td>
<td>25</td>
<td>7.95</td>
</tr>
</tbody>
</table>

*PB C16:0 20%; Physical blend with 20% palmitic acid, IE C16:0 20%; Interesterified sample with 20% palmitic acid, PB C16:0 30%; Physical blend with 30% palmitic acid, IE C16:0 30%; Interesterified blend with 30% palmitic acid. **Melting points are average of triplicate values and are reported along with their standard deviation

Polarized Light Microscopy (PLM)

The effect of HIU on the crystal structure obtained during the crystallization process was observed using a 10× magnification objective in a PLM (Olympus BX 41 Tokyo, Japan) equipped with an Infinity 2 digital camera (Lumenera Scientific, Infinity 2, Ottawa, ON, Canada). An aliquot of the sample was taken from the tubes placed in the water bath using a glass pipette, placed on a glass slide and covered with a micro cover glass (VWR International, Radnor, PA) and microstructure was observed. Samples were taken from the tube at 10-min intervals after the sample was transferred to the tubes up to 60 min of crystallization. The glass pipettes, microscopic slides, and micro cover glass were all maintained at Tc. The microscope camera was operated by the Lumenera software (Lumenera Scientific, Infinity 2, Ottawa, ON, Canada) through a computer interface. The microscope glass slides were placed on an Instec TS62 Microscope thermal stage (Instec, Inc., Boulder, CO) also maintained at the Tc. The images collected
after 60 min of crystallization were assembled for comparison of the microstructure and changed to grayscale using Adobe Photoshop CS2 Version 9.0.

**Differential Scanning Calorimetry (DSC)**

After 60 min of crystallization, approximately 10–15 mg of sample was weighed, placed in a Tzero pan, covered with a Tzero Hermetic lid and hermetically sealed. The melting behavior of the samples was analyzed using a DSC Q20 (TA Instruments, New Castle, DE) by heating the sample from $T_c$ to 80 °C at a heating rate of 5 °C/ min with an empty Tzero sealed pan as reference similar to that described by Ye *et al.* [12]. Each sample was analyzed by the DSC after each of the three-replicate crystallization runs and hence the DSC data is reported as an average of three individual runs along with its standard error of the mean. Hermetically sealed pure Indium standard was used to calibrate the baseline temperature. The instrument was controlled using the TA Instrument Explorer software (TA Instruments, New Castle, DE). The melting curves were analyzed using TA Instruments Universal analysis 2000 software (TA Instruments, New Castle, DE) and the onset melting temperature ($T_{on}$), peak melting temperature ($T_{peak}$) and the melting enthalpy ($\Delta H$) were measured. The temperature of the instrument was controlled using a TA Instruments refrigerated cooling system 90 with N$_2$ as the purge gas.

The values for the IE C16:0 20% samples were analyzed using a two-way ANOVA followed by Tukey’s multiple comparisons as a *post hoc* test. The multiple comparisons compared values at each supercooling level between the PB and IE without and with HIU. The $T_{on}$, $T_{peak}$ and $\Delta H$ values were compared separately. The values for the IE C16:0 30% samples were compared by one-way ANOVA and only at 9 and 6 °C
supercooling levels. The results at supercooling at 3 °C were compared by an unpaired \( t \) test.

**Rheology**

Rheological parameters including viscosity, elastic (\( G' \)) and viscous (\( G'' \)) moduli, and phase angle, delta, were measured using an AR-G2 Rheometer (TA Instruments, New Castle, Delaware) after 60 min of crystallization. The instrument was run using the Rheology Advantage Instrument Control. Air was used as the purge gas at 30 psi. The 8 mm standard steel parallel plate assembly with a gap of 1000 μm was used for measurement of the properties of the interesterified blend with 30% C16:0. The other samples including the PB were analyzed using the standard size recessed end concentric cylinders geometry with gap set to 4000 μm. Viscosity measurements were performed using the standard steady state flow at the crystallization temperature by varying the shear rate from 0.01 to 300 (s⁻¹). The viscosity data was plotted by using a Carreau fit and the viscosity of the sample at a shear rate = 0.1 s⁻¹ was calculated. The viscoelastic parameters (\( G' \), \( G'' \), and delta) were measured at Tc using the strain sweep oscillation procedure by varying the % strain from 8.0 × 10⁻⁴ to 10% at a constant 1 Hz frequency. The Rheology Advantage software (TA Instruments, New Castle, Delaware) was used for data analysis.

The IE C16:0 20% results were analyzed by a two-way ANOVA followed by Tukey’s multiple comparisons. The multiple comparisons compared values at each supercooling level between the PB and IE without and with HIU. A two-way ANOVA was also performed to compare the IE C16:0 30% 9 and 6 °C supercooling levels along
with the Tukey’s multiple comparison test, similar to that of IE C16:0 20%. The results at the supercooling of 3 °C were compared by an unpaired t-test.

**Statistical Analysis**

Rheology and DSC results are reported as mean values and standard errors of the mean of three individual crystallization experiments.

In general, each parameter from the PB and the sonicated and non-sonicated IE C16:0 20% samples were compared at each supercooling level by an ANOVA test and post hoc tests were performed to evaluate significant differences (α = 0.05). Values from each parameter from the PB and the sonicated and non-sonicated IE C16:0 30% samples were compared at supercooling levels of 9 and 6 °C by an ANOVA test. Post hoc tests were performed to compare the parameter values at these supercooling levels separately among the three samples. The parameters for the sonicated and non-sonicated IE C16:0 30% samples at supercooling of 3 °C were compared by an unpaired t test. Detailed explanation of the statistical tests performed is provided in the results and discussion section.

**Results and Discussion**

**Melting Point**

The melting points (MP) of the samples was measured in triplicate and are tabulated in Table 4-1. The MP of the PB and IE C16:0 20% samples was 48.8 ± 1.2 °C and 16.1 ± 0.7 °C while that of the PB and IE C16:0 30% samples were 55.7 ± 0.3 °C and 28.0 ± 0.3 °C, respectively. Narine and Marangoni [28] discussed that melting behavior of fat crystal networks are dependent on the TAG composition and structure. The presence of high melting tripalmitin influenced the melting behavior of the PB compared
to the interesterified samples in which randomization of TAG caused a drop in the melting point due to the depletion in tripalmitin TAG. Also, the MP values of C16:0 30% samples were higher than C16:0 20% samples and this could be due to the higher total saturated fatty acid composition in C16:0 30% samples.

Preliminary crystallization experiments in our laboratory showed that if the PB was crystallized at the same $T_c$ as IE, PB crystallized instantaneously due to the large supercooling attributed to the differences in the MP of the samples. The instantaneous crystallization of the PB made it difficult to move the sample into the tubes for further analysis. Hence, it was decided to compare the crystallization properties of the samples at the three equally spaced supercooling levels (low, intermediate, and high supercooling). The temperatures at which the samples were crystallized are tabulated in Table 4-1. The physical blend with C16:0 30% did not crystallize sufficiently at $\Delta T = 3 \, ^\circ C$ for the laser signal to drop below 0.6 V in the duration of the experiment and hence none of the analytical measurements were performed at this supercooling level for the PB C16:0 30%.

**Isothermal Solid Fat Content (SFC)**

Figure 4-1 shows the SFC of the IE and PB with 20% palmitic acid at different time points throughout the crystallization process. SFC values obtained for the IE were lower than those obtained for PB at all the supercoolings and differences in SFC values were more pronounced at the supercooling level of 3 °C. The high melting tripalmitin (PPP) TAG in PB are driving the crystallization of these samples and act as seeds for further crystallization resulting in a fast crystallization rate. Decrease in the SFC of the
Figure 4-1: Isothermal solid fat content of the interesterified (IE) sample and physical blend (PB) with C16:0 20% crystallized at supercoolings of 9, 6, and 3 °C without and with HIU. The arrow indicates the time point of HIU application.
fat upon interesterification has been observed previously [11] and occurs mostly due to the randomization and formation of low melting TAG. The SFC of the PB increased to its maximum value almost instantaneously while there was a gradual increase in the SFC of the IE to its maximum value. The effect of HIU on the SFC of the IE C16:0 20% sample was most pronounced at $\Delta T = 9 \, ^\circ C$ and at this supercooling, the final SFC of IE at 60 min of crystallization was 5.7 and 7.1% SFC without and with the application of HIU, while SFC values for the PB was 8.0%.

The time point for HIU application for the IE C16:0 20% samples was approximately 5, 6, and 13 min for 9, 6 and 3 °C supercooling, respectively. For the IE C16:0 30% samples, HIU was applied at approximately 7, 16, and 27 min for 9, 6 and 3 °C supercooling, respectively. In order to fit the Avrami/Gompertz models to the SFC data, additional 0% SFC points were added to the data starting at $t = 0$ until before the actual SFC measurements started. From Fig. 4-1 it can be seen that the IE sample with C16:0 20% followed a two-step isothermal crystallization at all the supercooling levels. This could be due to the crystallization of one polymorphic form followed by its change into the more stable polymorph or the initial crystallization of the high melting TAG fractions followed by the low melting TAG at a later time point [29, 30]. The kinetics of the multistep crystallization process was fitted using the multi component Avrami equation as described by Marangoni [25] (Eq. (1)) for $\Delta T = 9$ and 6 °C. $R^2$ obtained for these fits were 0.98 or above. This equation calculated the maximum SFC, Avrami rate constant ($k$) and the Avrami exponent ($n$) for each plateau and is shown in Table 4-2. The Avrami exponent, $n$, for the first step are denoted by the subscript “1” and for the second step by the subscript “2”. The Avrami rate constant, $k$, is associated with the rate of the
Table 4-2: Parameters obtained from the multi component Avrami fit of the isothermal solid fat content data for the IE sample containing 20% palmitic acid without and with HIU. Maximum SFC (s_max), Avrami rate constant k, and Avrami exponent n are shown.

<table>
<thead>
<tr>
<th>ΔT</th>
<th>Sample</th>
<th>s_max 1 (% SFC)</th>
<th>k_1 (x10^-5) (min^-n)</th>
<th>n_1</th>
<th>s_max 2 (% SFC)</th>
<th>k_2 (x10^-7) (min^-n)</th>
<th>n_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>IE C16:0 20% without HIU</td>
<td>1.8 ± 0.0^a</td>
<td>8.6 ± 0.0^a</td>
<td>5.1 ± 0.2^a</td>
<td>3.9 ± 0.1^a</td>
<td>1.1 ± 0.7^a</td>
<td>4.4 ± 0.2^a</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 20% with HIU</td>
<td>2.1 ± 0.1^a</td>
<td>8.6 ± 2.3^a</td>
<td>5.0 ± 0.2^a</td>
<td>5.1 ± 0.1^b</td>
<td>3.6 ± 3.1^a</td>
<td>4.0 ± 0.2^a</td>
</tr>
<tr>
<td>6</td>
<td>IE C16:0 20% without HIU</td>
<td>1.4 ± 0.0^a</td>
<td>8.6 ± 0.0^a</td>
<td>4.5 ± 0.2^a</td>
<td>3.4 ± 0.1^a</td>
<td>7.4 ± 4.3^a</td>
<td>4.0 ± 0.2^a</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 20% with HIU</td>
<td>1.2 ± 0.1^a</td>
<td>8.6 ± 0.0^a</td>
<td>4.3 ± 0.1^a</td>
<td>3.5 ± 0.1^a</td>
<td>9.0 ± 9.5^a</td>
<td>3.9 ± 0.3^a</td>
</tr>
</tbody>
</table>

At each supercooling level, data represented for each parameter with different alphabets are statistically different (α = 0.05).

reaction and is temperature dependent [25]. The multistep Avrami equation did not provide a good fit for the data obtained for the IE sample crystallized at ΔT = 3 °C and therefore, these data were fitted with the Gompertz model and reported in Table 4-3a. Each parameter from the two step Avrami fit of the 9 and 6 °C supercooling data were analyzed separately. These were entered as mean, standard deviation and number of replicates in the Prism Graphpad software and analyzed using two-way ANOVA. As a post hoc test, the Sidak’s multiple comparison was performed and each parameter was compared only among different samples at each supercooling level. Based on the values listed in Table 4-2, the rate constant (k_1) for the first step was two orders of magnitude higher than that for step two (k_2) at supercooling levels of both 9 and 6 °C. The value of k_1 at ΔT = 9 °C for IE without HIU for the first step was 8.6 × 10^-5 min^-1 and that of k_2 was
**Table 4-3:** Gompertz parameters – Maximum SFC ($s_{\text{max}}$), maximum rate of crystallization ($\mu_{\text{max}}$) and Induction period ($\lambda$) obtained from the Gompertz fit to the isothermal solid fat content data of the sonicated and non-sonicated IE and PB samples with 20% palmitic acid (a) and for the IE and PB samples with 30% palmitic samples (b).

<table>
<thead>
<tr>
<th>$\Delta T$</th>
<th>Sample</th>
<th>$s_{\text{max}}$ (%SFC)</th>
<th>$\mu_{\text{max}}$</th>
<th>$\lambda$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td><strong>Table a.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PB C16:0 20%</td>
<td>8.5 ± 0.1</td>
<td>6.7 ± 1.8</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>PB C16:0 20%</td>
<td>7.5 ± 0.0</td>
<td>2.9 ± 0.2</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>PB C16:0 20%</td>
<td>6.6 ± 0.0$^a$</td>
<td>1.1 ± 0.1$^a$</td>
<td>10.5 ± 0.2$^a$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 20% without HIU</td>
<td>3.8 ± 0.3$^b$</td>
<td>0.1 ± 0.0$^b$</td>
<td>16.5 ± 0.8$^b$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 20% with HIU</td>
<td>3.6 ± 0.1$^b$</td>
<td>0.1 ± 0.0$^b$</td>
<td>15.4 ± 0.7$^b$</td>
</tr>
<tr>
<td></td>
<td><strong>Table b.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PB C16:0 30%</td>
<td>12.1 ± 0.1$^a$</td>
<td>3.8 ± 0.3$^a$</td>
<td>5.7 ± 0.1$^a$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 30% without HIU</td>
<td>7.6 ± 0.0$^b$</td>
<td>0.4 ± 0.0$^b$</td>
<td>9.2 ± 0.2$^b$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 30% with HIU</td>
<td>7.8 ± 0.0$^b$</td>
<td>0.6 ± 0.0$^b$</td>
<td>9.7 ± 0.3$^b$</td>
</tr>
<tr>
<td>6</td>
<td>PB C16:0 30%</td>
<td>10.6 ± 0.1$^a$</td>
<td>1.7 ± 0.1$^a$</td>
<td>10.1 ± 0.2$^a$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 30% without HIU</td>
<td>6.0 ± 0.0$^b$</td>
<td>0.4 ± 0.0$^b$</td>
<td>22.6 ± 0.2$^b$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 30% with HIU</td>
<td>5.9 ± 0.0$^b$</td>
<td>0.7 ± 0.0$^b$</td>
<td>15.9 ± 0.2$^c$</td>
</tr>
<tr>
<td>3</td>
<td>IE C16:0 30% without HIU</td>
<td>3.4 ± 0.4$^a$</td>
<td>0.1 ± 0.0$^a$</td>
<td>34.4 ± 0.7$^a$</td>
</tr>
<tr>
<td></td>
<td>IE C16:0 30% with HIU</td>
<td>4.5 ± 0.0$^a$</td>
<td>0.4 ± 0.0$^b$</td>
<td>26.8 ± 0.3$^b$</td>
</tr>
</tbody>
</table>

At each supercooling level, data represented for each parameter with different alphabets are statistically different ($\alpha = 0.05$).
1.1 × 10⁻⁷ min⁻ⁿ. The application of ultrasound did not affect the rate of crystallization in the first step at ΔT = 9 °C while k₂ increased from 1.1 × 10⁻⁷ min⁻ⁿ to 3.6 × 10⁻⁷ min⁻ⁿ though the difference in k₂ after HIU application was not significantly different (p > 0.05). The application of HIU did increase the maximum SFC of the IE at the supercooling level of 9 °C in the first step from 1.8 to 2.1% and later in the second step from 3.9 to 5.1% (p < 0.05). Although there was no change in the values of the parameters determined by the two step Avrami at 6 °C supercooling with the application of HIU, there was a slight rise in the k₂ value (not statistically significant).

The Avrami exponent n indicates the type of nucleation and the dimension of the crystal growth. While an integer value is expected for n values, fractional values are usually obtained based on the fit which could be attributed to different crystal types forming together or change in the shape of the crystals [25]. The n values for the crystallization curves reported in Fig. 4-1 ranged from 3.9 to 5.1 and similar values have been obtained before in AMF, AMF-TAG and AMF-TAG with DAG systems at high crystallization temperatures [31]. The Avrami exponent changed with each step of crystallization indicating that either the nucleation or the crystal growth geometry changed from the first step to the other [31]. This was true for both supercoolings (9 and 6 °C). However, there was no change in the Avrami exponent with the application of HIU at both the steps and supercoolings.

Even though IE at ΔT = 3 °C did crystallize in two steps, Eq. (1) did not converge for the data. Hence a single step re-parameterized Gompertz equation was used to fit this data. The Gompertz equation calculates the maximum SFC, rate of crystallization and the induction period of crystallization of the sample. On the other hand, the PB C16:0 20%
crystallized in a single step process at all the supercoolings and hence was also fitted with the Gompertz equation and the parameters obtained from this equation and are tabulated in Table 4-3.a and the plots are in Fig. 4-1. The Gompertz equation was a better fit to this data compared to the single step Avrami equation with a goodness of fit of 0.94 and higher. Since, the Gompertz parameters for all the three samples were available only at the supercooling of 3 °C, one-way ANOVA was performed to compare each parameter from the $\Delta T = 3$ °C curves separately followed by Tukey’s multiple comparison test to evaluate the differences in the samples.

From Table 4-3a it can be seen that the maximum solid fat content, $S_{\text{max}}$ of PB correlated with the degree of supercooling and was highest at $\Delta T = 9$ °C and decreased with the decrease in the driving force. Also, with the decrease in the supercooling from 9 to 6 °C, the maximum rate of crystallization, $\mu_{\text{max}}$ of PB decreased from 6.7 to 2.9%/ min and further down to 1.1%/min at supercooling of 3 °C and a corresponding increase in the induction period ($\lambda$). On comparison of the Gompertz parameters for the IE at $\Delta T = 3$ °C we see that the $\mu_{\text{max}}$ was not affected by sonication. Also, there was no significant change in the $S_{\text{max}}$ and the induction period of crystallization. The PB crystallized faster than the IE at $\Delta T = 3$ °C and had a lower induction time (10.5 min), higher $S_{\text{max}}$ (6.6%), and higher $\mu_{\text{max}}$ (1.1%/min).

The isothermal solid fat content of the PB and IE C16:0 30% is shown in Fig. 4-2. The SFC of the PB was higher than that of IE. Upon sonication, there was a faster rise in the SFC of the IE samples. At the supercooling level of 3 °C, the application of HIU did increase the final SFC of the IE. We speculate that if the SFC of the sample was tracked beyond 60 min of crystallization, the SFC of the IE with and without HIU might plateau.
**Figure 4-2:** Isothermal solid fat content of the Interesterified (IE) sample and physical blend (PB) with C16:0 30% crystallized at supercoolings of 9, 6 and 3 °C without and with HIU. The arrows indicate the time point of HIU application.
and reach a similar SFC. Contrary to the results reported for the samples with 20% C16:0, most of the IE samples showed a single step crystallization process with the exception of IE samples crystallized at the supercooling level of 9 °C. Even though the IE at ΔT = 9 °C followed a multistep crystallization, the two step Avrami equation did not converge to the experimental data. Hence, the single step Gompertz equation was used to fit the IE data. Also from Fig. 4-2 it can be seen that the PB C16:0 30% sample followed a single step crystallization as reported for the 20% PB samples. In order to evaluate the crystallization kinetics, all the SFC curves in Fig. 4-2 were fitted to the Gompertz equation (Eq. (2)). The equation fit well to the data with a goodness of fit (R2) of 0.93 and above. The Gompertz parameters including the Smax, μmax, and λ, were calculated by the fit and are tabulated in Table 4-3.b As the driving force (degree of supercooling) decreased, the maximum rate of crystallization μmax and the maximum solid fat attained by the sample (Smax) also decreased. The Smax of PB ranged from 12.0 to 10.6% while the μmax ranged from 3.8 to 1.7%/min for 9 and 6 °C supercooling, respectively while the induction period of crystallization increased (Table 4-3.b). At both the supercooling levels of 9 and 6 °C, the μmax of PB was the highest compared to that of IE without and with HIU. For e.g., at ΔT = 6 °C, the PB had the highest μmax of 1.7%/min while the IE without and with HIU had a μmax of 0.4 and 0.6%/min. The application of HIU did increase the μmax at all supercooling levels but the increase was only statistically significant at ΔT = 3 °C (p < 0.05). Based on the induction period values, PB crystallized before IE at all the supercoolings. Also, HIU was effective at inducing crystallization in IE at the intermediate and the lower supercooling by significantly lowering the induction period of crystallization (Table 4-3.b).
As previously mentioned, it is very likely that the crystallization behavior of the PB is driven by the presence of PPP (~13 and 27% for the 20 and 30% samples) and that OOP + OPP (~47 and 67% for the 20 and 30% samples) are responsible for the crystallization behavior of IE samples. The higher content of palmitic acid and the given TAG content was responsible for the higher SFC of the IE with 30% palmitic acid than the IE with 20% palmitic acid content at all the supercoolings [10]. At 60 min of crystallization, the SFC % at 9 and 6 °C supercoolings without HIU were 5.7 and 4.9% for the IE C16:0 20%, respectively and were 7.9 and 6.0% for IE C16:0 30%, respectively. However, at 3 °C supercooling, the SFC % was 2.9 and 2.4% for IE C16:0 20% and IE C16:0 30% sample, respectively. In addition, HIU induced the crystallization of 30% IE at low (ΔT = 3 °C) and intermediate (ΔT = 6 °C) supercoolings and of 20% IE at high supercoolings (ΔT = 9 °C). It is not clear why HIU affects the crystallization behavior of these samples in a different manner. Both samples (IE 20 and 30%) have very similar TAG composition with the difference that IE 30% has a greater amount of OOP and OPP (67.1 and 47.2% for the 30 and 20% sample, respectively) which would explain the higher SFC obtained for the 30% samples. However, the effect of higher content of palmitic fatty acids in the 30% samples is confounded by the driving force for crystallization. Even though both samples are crystallized under the same degree of supercooling, the driving force of crystallization was higher for the IE 20% than for the 30% samples due to the lower crystallization temperatures (Table 4-1). The driving force of crystallization (Φ) in lipids is a function of the supercooling, enthalpy of melting, and the melting point of the samples and is given by the equation below [32, 33]:

\[ \phi = \Delta H_m \times \frac{\Delta T}{T_m} \]
where $\Delta H$ is the enthalpy of melting of the sample, $\Delta T$ is the degree of supercooling: $T_m - T_c$, and $T_m$ is the melting point of the sample.

The enthalpy of melting calculated by DSC were $81.82 \pm 0.75$ and $70.97 \pm 0.06$ J/g for the PB and IE C16:0 samples and was $105.23 \pm 0.47$ and $74.21 \pm 0.83$ J/g for the PB and IE C16:0 30% samples. Equation (3) shows that for the same sample, the driving force increases with supercooling. When compared among the different samples, the driving force was highest for the IE C16:0 20% followed by IE C16:0 30%, PB C16:0 20% and PB C16:0 30% samples in that order. The higher driving force for crystallization resulted in shorter induction times of crystallization for the IE 20% samples compared to the 30% ones. Even though the onset of crystallization occurred sooner in the IE 20% samples, the final SFC was lower as mentioned above. This lower amount of crystalline material observed in the 20% samples can be attributed to the lower content of OOP and OPP TAG (47.2% compared to 67.1% in the IE C16:0 30% samples) and to the molecular kinetics events that occur during the crystallization of these samples [32].

The different effect that sonication has on the samples can be explained by understanding the sonication conditions. In the case of the IE 20% sample at a supercooling of 9 °C, sonication was applied at approximately 5 min when the laser signal was close to 0.6 V. During this time, the sample temperature had not reached the crystallization temperature and was approximately 24.4 °C. The viscosity in the sample was 0.063 Pa·s at the time of application of ultrasound and was low enough to allow for cavitation bubbles to form and therefore to induce crystallization in the samples. When the sample was crystallized at 6 and 3 °C of supercooling, HIU was applied at
approximately 6 min and 13 min and the temperature of the samples was 19.6 and 16.9 °C, respectively. The viscosities in the samples at the time of application of HIU at Δ\(T = 6\) and 3 °C were 0.077 and 0.086 Pa·s. In these cases, the slightly higher viscosities associated with these lower temperatures might have hampered the formation of cavitation bubbles and hence less crystallization was induced in the samples at these temperatures. In the case of the IE 30% samples the temperature and viscosity of the sample at the time of HIU application were 24.3, 24.6 and 27 °C and 0.065, 0.064 and 0.058 Pa·s at 9, 6 and 3 °C supercooling levels, respectively. The lower viscosities associated with these samples allowed for the formation of cavitation bubbles. In these samples, however, when the supercooling was too high, sonication did not affect the crystallization behavior of the sample. These results suggest that HIU effectiveness depends on: (a) supercooling and driving force for crystallization, (b) temperature of the material, (c) viscosity of the material, and (d) molecular thermodynamic and kinetic events.

**Microstructure**

In Fig. 4-3, the PLM images in the first and second column correspond to crystals obtained from IE samples crystallized without and with HIU, respectively, while the third column shows crystals obtained for the PB samples. Images in the same row correspond to the same supercooling level. Fat crystals are seen as bright spots on the dark background. Upon comparison of the microstructure among the IE and PB at each supercooling, it was seen that the microstructure of the PB samples was denser than the IE samples. Also, the size of the crystals increased with the decrease in supercooling. This correlated with the finding by several authors that indicate that crystallization
Figure 4-3: Polarized light microscopy pictures of crystals obtained for the interesterified (IE) sample and physical blend (PB) with C16:0 20% after 60 min at supercooling of 9, 6 and 3 °C. (The white bar represents 100 μm)

temperature affects crystal size and that larger crystals are formed at higher crystallization temperature [12, 34–36]. There were fewer crystals at the lowest supercooling compared to those at 9 and 6 °C. Although a few bright spots are visible in the PLM microstructure of the IE sample without HIU, upon careful observation, it can be seen that the microstructure is filled with smaller crystals in the background of the bright spots (larger crystals). Upon sonication, more and brighter crystals were observed in the IE samples at all supercooling levels tested. This is an interesting result since even though the SFC of the samples was not significantly affected by sonication (Fig. 4-1) an increase in crystal numbers is observed. Figure 4-4 shows the microstructure of the IE
and PB C16:0 30% samples crystallized at different supercoolings. PB samples at both the supercooling levels had a very dense microstructure. The difference in the microstructure may be attributed to the presence of the tripalmitin in the PB which may have formed the larger crystals. The amount of crystals formed decreased significantly in IE sample crystallized at the lowest supercooling (3 °C). Based on the PLM data, it can be concluded that HIU induced crystallization in these samples and generated smaller crystals, which was also observed by other authors [12, 13, 37, 38] and this effect was consistent at all supercooling levels tested. On comparison of the microstructure of the crystals at the same higher $T_c$ values, Ye et al. [12] also reported formation of smaller and
more crystals compared to the non-sonicated samples in the IESBO sample when HIU was applied in the presence of crystals. Some larger crystals are also observed in the PLM pictures of the IE with HIU at $\Delta T = 3 \, ^\circ C$. These larger crystals may have been the ones formed prior to the application of HIU. Although as part of secondary nucleation, HIU breaks the larger crystals into smaller crystal size, not all crystals may have been broken during this mechanism.

**Melting Characteristics by Differential Scanning Calorimetry (DSC)**

The melting thermograms of IE and PB C16:0 20% are shown in Fig. 4-5 and those of PB and IE C16:0 30% are shown in Fig. 4-6. The different melting parameters calculated from these thermograms including the onset melting temperature ($T_{on}$), peak melting temperature ($T_{peak}$) and the melting enthalpy ($\Delta H$) are tabulated in Table 4-4.a for the 20% IE samples. The DSC thermograms of the C16:0 20% in general, consisted of a single major peak. The $T_{on}$ and $T_{peak}$ values of PB samples increased in general with the decrease in supercooling (Table 4-4.a). Melting profiles were sharper for IE samples crystallized without HIU than those for IE crystallized with HIU. The sonicated samples at all supercoolings had shoulder peaks indicating some level of fractionation. This effect was also seen in the non-sonicated sample at $\Delta T = 9$ and 6 $^\circ C$ while at $\Delta T = 3 \, ^\circ C$, a single sharp melting peak was observed. The $T_{on}$, $T_{peak}$ and $\Delta H$ for PB was higher and significantly different ($p < 0.05$) than IE at all supercooling levels due to the difference in TAG composition. Application of HIU to IE lowered the $T_{on}$ temperature at $\Delta T = 9 \, ^\circ C$. There was also a slight decrease in the $T_{peak}$ and an increase in the $\Delta H$ upon sonication; although, these changes were not statistically significant ($p > 0.05$) (Table 4-4a). Also,
Figure 4-5: DSC melting profiles of interesterified (IE) samples and physical blends (PB) with C16:0 20% crystallized at supercoolings of 9, 6 and 3 °C.
Table 4-4: DSC melting parameters- $T_{\text{onset}}$, $T_{\text{peak}}$ and enthalpy ($\Delta H$) for the interesterified (IE) and physical blend (PB) with C16:0 20% (Table 4.a.) and for the IE and PB with C16:0 30% (Table 4.b.) at supercooling of 9, 6 and 3 °C

| $\Delta T$ ($^\circ$C) | $T_{\text{onset}}$ ($^\circ$C) | | $T_{\text{peak}}$ ($^\circ$C) | | $\Delta H$ (J/g) |
|----------------------|-------------------------------|---------------------|-------------------------------|---------------------|
|                      | PB               | IE                 | PB               | IE                 | PB               | IE                 |
|                      | no HIU | HIU    | no HIU | HIU    | no HIU | HIU    |
| 9                    | 48.6±1.5$^a$ | 21.8±2.4$^b$ | 20.1±0.6$^b$ | 55.9±0.2$^a$ | 24.1±2.3$^b$ | 23.5±0.6$^b$ | 23.5±0.1$^a$ | 4.8±1.1$^b$ | 4.3±0.3$^b$ |
| 6                    | 48.6±0.8$^a$ | 20.5±0.0$^b$ | 23.0±0.7$^b$ | 57.5±0.4$^a$ | 23.6±0.3$^b$ | 25.9±0.7$^b$ | 24.6±1.0$^a$ | 4.2±0.3$^b$ | 2.7±0.2$^b$ |
| 3                    | 50.1±0.3$^a$ | 23.2±0.9$^b$ | 23.2±1.2$^b$ | 59.3±0.5$^a$ | 27.3±1.7$^b$ | 26.8±0.5$^b$ | 22.4±1.4$^a$ | 2.5±0.5$^b$ | 2.5±0.6$^b$ |
| 9                    | 54.7±1.1$^a$ | 24.3±0.5$^b$ | 26.3±0.6$^b$ | 59.6±0.4$^a$ | 27.7±0.4$^b$ | 28.7±0.4$^b$ | 44.3±1.7$^a$ | 8.5±1.1$^b$ | 9.1±0.1$^b$ |
| 6                    | 54.9±0.5$^a$ | 27.2±0.4$^b$ | 26.2±0.0$^b$ | 60.3±0.3$^a$ | 32.6±0.8$^b$ | 31.9±0.2$^b$ | 45.2±3.4$^a$ | 9.0±1.4$^b$ | 7.5±0.1$^b$ |
| 3                    | NA               | 28.9±0.4$^a$ | 29.4±0.9$^a$ | NA               | 35.5±0.7$^a$ | 33.2±0.1$^b$ | NA               | 4.2±0.5$^a$ | 6.3±0.1$^b$ |

At each supercooling level, for each parameter, data represented with different alphabets are statistically different ($\alpha = 0.05$).
there were no significant differences \((p < 0.05)\) in the \(T_{\text{on}}, T_{\text{peak}}\) and \(\Delta H\) of the IE without and with the application of HIU at other supercoolings.

The melting enthalpy relates to the amount of energy absorbed during melting and is associated with the amount of crystalline material present in the sample. At \(\Delta T = 9 \, ^\circ\text{C}\), the melting enthalpy of the PB was \(23.5 \pm 0.1 \, \text{J/g}\) while that of IE without and with HIU were not significantly different \((p > 0.05)\) with values of \(4.8 \pm 1.1\) and \(4.3 \pm 0.3 \, \text{J/g}\), respectively. The higher melting enthalpy of PB could be due to combination of the presence of the high melting tripalmitin fraction and the large amount of crystalline material as seen in the dense crystal microstructure of PB (Fig. 4-3). Even though smaller and more crystals were seen in the microstructure of sonicated IE, the melting enthalpies of the sonicated samples were not higher than those obtained for the non-sonicated samples at \(\Delta T = 6\) or \(3 \, ^\circ\text{C}\). The lack of difference in enthalpy values correlate well with the SFC values reported in Fig. 4-1 where only slight or no differences in SFC are observed between the sonicated and non-sonicated samples.

The IE C16:0 30% melted over a broad temperature range (Fig. 4-6). The thermograms corresponding to the sonicated IE samples had sharper peaks and defined shoulders compared to IE samples crystallized without HIU, at all the supercooling levels. These results suggest that HIU may have induced some degree of fractionation of the lipid blend. This suggest that HIU induced co crystallization of TAG of similar structural characteristics that melt over a narrower temperature range resulting in defined peaks and shoulders that correspond to different groups of TAG packed together. However, fractionation was observed only at \(\Delta T = 9\) and \(6 \, ^\circ\text{C}\) while the thermograms of sonicated IE samples crystallized at \(\Delta T = 3 \, ^\circ\text{C}\) had a single sharper peak compared to IE
samples crystallized without HIU. Also, at supercooling of 3 °C, due to the higher crystallization temperature, the low melting TAG did not crystallize and hence peaks corresponding to these fractions were not observed. Since these TAG did not crystallize, the melting enthalpy was also lower at this supercooling level compared to the others (Table 4-4.b). The single peak observed in the thermograms of the IE C16:0 30% at ΔT = 3 °C represent the melting of a crystalline network of highly organized TAG molecules obtained by sonication. The different melting parameters including the onset melting temperature ($T_{on}$), peak melting temperature ($T_{peak}$) and the melting enthalpy ($\Delta H$) are tabulated in Table 4-4.b for the 30% IE samples. The $T_{on}$, $T_{peak}$ and $\Delta H$ of the PB were higher and significantly different than IE at all the supercooling levels mainly due to the differences in the TAG composition. At ΔT = 9 °C, the $T_{on}$ for the PB was 54.7 ± 1.1 °C and that of the IE without HIU was 24.3 ± 0.5 °C. This corresponds well with the higher MP of the physical blend (Table 3-1). The application of HIU at ΔT = 9 °C increased the enthalpy from 8.5 ± 1.1 to 9.1 ± 0.1 J/g though the increase in enthalpy was not statistically significant (p < 0.05). However, at ΔT = 6 °C, the enthalpy decreased from 9.0 ± 1.4 to 7.5 ± 0.1 J/g but differences were not statistically significant either.

However, at ΔT = 3 °C, there was a significant (p < 0.05) increase in enthalpy from 4.2 ± 0.5 to 6.3 ± 0.1 J/g. This increase in enthalpy corresponded well with the increase in the number of crystals as seen in the microstructure of the sonicated IE C16:0 30% samples and with the increase in final SFC. Previous research in our laboratory has
Figure 4-6: DSC melting profiles of interesterified samples (IE) and physical blends (PB) with C16:0 30% at supercooling of 9, 6 and 3 °C
shown that the effect of sonication on enthalpy values is very variable and it can be affected by the level of supercooling. Ye et al. [12] reported that sonication did not affect enthalpy values in a commercial interesterified soybean oil. Similar results were reported by Suzuki et al. [13] in an all-purpose shortening. However, significant increases in enthalpy values were obtained by Suzuki et al. [13] in palm kernel oil and in anhydrous milk fat, especially at low supercoolings. Other authors have reported significant increases in enthalpy values in sonicated samples [39–41].

**Rheological Properties**

The rheology data is presented on a log scale vs degree of supercooling in Fig. 4-7 for the samples with 20% palmitic acid and that for samples with 30% palmitic acid is shown in Fig. 4-8. The error bars represent the standard error of the mean. At ΔT = 9 °C, the viscosity of the PB 20% C16:0 was 12.8 ± 1.9 Pa.s and that of IE was significantly higher at 266.9 ± 32.4 Pa.s. However, there was no statistical influence of HIU on the viscosity of the IE C16:0 20% sample (p > 0.05). Similar trends in the viscosity of the PB and IE-without and with HIU were observed at other supercooling levels. Figure 4-7 shows that the viscosity of 20% PB was lower than the one observed for the IE samples even though more crystalline material was obtained (see SFC values in Fig. 4-1). The differences in the viscosity of the samples could be attributed to differences in the microstructure of the samples as presented in Fig. 4-3. Smaller crystals were observed in the IE samples which imparted a slightly higher viscosity to the IE compared to the PB. Slightly higher viscosities were obtained in the sonicated IE 20% C16:0 samples compared to the non-sonicated ones. Since no difference in the final SFC was observed between the sonicated and non-sonicated samples it is likely that the slight difference
Figure 4-7: Rheology parameters, viscosity, G’, G’’ and tanδ for the interesterified (IE) samples and physical blends (PB) with C16:0 20% crystallized at supercooling of 9, 6 and 3 °C. At each supercooling level, data represented with different alphabets are statistically different (α = 0.05).
observed in viscosity values is driven by the difference in crystal microstructures (Fig. 4-3).

$G'$ represents the storage or the elastic modulus of the sample. Based on the data presented in Fig. 4-7 it can be seen that $G'$ for 20% C16:0 PB was lower than the IE at supercoolings of 9 and 6 °C but was higher at $\Delta T = 3$ °C. The differences in the $G'$ values between the PB and the IE samples were statistically significant ($p < 0.05$) only at $\Delta T = 9$ °C. The $G'$ of PB at $\Delta T = 9$ °C was $210.7 \pm 89.2$ Pa and of IE without and with HIU was $16038 \pm 7062.6$ and $19404.3 \pm 10489.3$ Pa. With the application of HIU, the SFC of the IE slightly increased, which increased the $G'$ of the sample. Thus, similar to the trend observed for viscosity, HIU did increase the $G'$ of the sample, but was not statistically significant. Similar trend in the loss and viscous modulus $G''$ was observed. At a higher and intermediate supercooling level, IE had a higher $G''$ than PB and it increased with the application of HIU but the increase was not statistically significant. Delta refers to the phase angle between the maximum stress and the maximum strain during the oscillatory test. All the samples at all the supercooling levels have $0^\circ < \delta < 90^\circ$ which indicates that all the samples had viscoelastic behavior. The magnitude of $\delta$ for the PB was $46.5 \pm 1.6^\circ$ and that of IE without and with HIU was $15.5 \pm 1.7$ and $16.5 \pm 2.0^\circ$ at $\Delta T = 9$ °C. Thus, based on this data it can be stated that the IE had a more elastic behavior while PB showed a stronger viscous behavior.

The viscosity of the PB and IE with C16:0 30% at $\Delta T = 9$ °C was $123.5 \pm 32.2$ and $192.4 \pm 119$ Pa·s, respectively, and with the application of HIU, the viscosity of IE samples increased to $3297.7 \pm 1368.6$ Pa·s. Sonication of IE significantly increased ($p < 0.05$) the viscosity at all the supercoolings. The $G'$ value of the IE and the PB sample
were comparable and $G'$ values increased in sonicated samples. This increase in $G'$ was statistically significant ($p < 0.05$) at all the supercooling levels. At $\Delta T = 3 \, ^\circ C$, the $G'$ value of IE was $1944.6 \pm 1153.8 \, Pa$ and was raised to $48180 \pm 12962.7 \, Pa$ with HIU application. The loss modulus ($G''$) was higher for the PB than the IE. There was an increasing trend in the $G''$ of the IE with the application of HIU, but the increase was statistically significant only at $\Delta T = 3 \, ^\circ C$. HIU induced the formation of smaller crystals in the IE based on the PLM pictures in Fig. 4-4. The increase in viscosity, $G'$ and $G''$, correlates well with small crystal size and increased SFC of the IE samples. The rheology measurements were performed after 60 min of crystallization and at that time, the SFC of

![Figure 4-8](image)

**Figure 4-8**: Rheology parameters, viscosity, $G'$, $G''$ and $\tan \delta$ for the interesterified (IE) samples and physical blends (PB) with C16:0 30% crystallized at supercooling of 9, 6 and $3 \, ^\circ C$. At each supercooling level, data represented with different alphabets are statistically different ($\alpha = 0.05$).
IE at $\Delta T = 3 ^\circ C$ was higher for the sonicated sample. This difference in the SFC of the sonicated sample correlates well with the increase in the viscosity. The phase angle $\delta$ of PB and IE at all supercoolings was $0^\circ < \delta < 90^\circ$ and thus all the samples showed viscoelastic behavior at all the supercoolings. However, based on the magnitude of $\delta$ (Fig. 4-8), it can be seen that IE samples showed a more elastic behavior than PB samples. Similar to the IE C16:0 20% samples, the phase angle $\delta$ decreased significantly upon interesterification while HIU did not influence it.

**Conclusion**

This study demonstrates that ultrasound along with supercooling can induce a change in the physical properties of interesterified samples including microstructure, isothermal solid fat content, and rheological properties. Although the application of HIU did change the physical properties of both IE with 20 and 30% palmitic acid, the effect was more pronounced on the IE with 30% palmitic acid. This could be due to differences in TAG composition between PB and IE samples. Palmitic acid was mostly present in the OOP + OPP TAG in IE samples while PB samples were composed mainly of PPP. These TAG species are responsible for driving the crystallization of the samples. The higher concentration of these TAG in the IE 30% palmitic acid sample may have corresponded to more nuclei and thus higher degree of crystallization, which may have affected the physical properties of these samples upon sonication. Results from this research suggest that HIU effectiveness is driven by two main factors: (a) the generation of supercooling, and (b) the presence of sufficient saturated fats. That is, HIU is not effective at inducing crystallization in samples with low content of palmitic acid. It is possible that the lack of effect under these conditions is due to physical properties of the material such as its
viscosity, which will affect the formation of cavities during sonication. However, with slightly higher content of palmitic acid (30%), sonication and processing conditions can be tailored to obtain various physical properties. By changing the HIU conditions, the extent of change may be modified and this processing technique can be extended to the healthier IE samples for use as trans-free fat alternatives.

Acknowledgements

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REFERENCES


CHAPTER 5
SONOCRYSTALLIZATION OF INTERESTERIFIED FATS WITH 20 AND 30% OF STEARIC ACID AT THE SN-2 POSITION AND THEIR PHYSICAL BLENDS

Abstract

Physical blends (PB) of high oleic sunflower oil and tristearin with 20 and 30% stearic acid and their interesterified (IE) products where 20 and 30% of the fatty acids are stearic acid at the sn-2 position crystallized without and with application of high intensity ultrasound (HIU). IE samples were crystallized at supercooling temperatures ($\Delta T$) of 12, 9, 6, and 3 °C while PB were crystallized at $\Delta T = 12$ °C. HIU induced crystallization in PB samples, but not in the IE ones. Induction in crystallization with HIU was also observed at $\Delta T = 6$ and 3 °C for IE C18:0 20 and 30% and at $\Delta T = 9$ °C only for the 30% samples. Smaller crystals were obtained in all sonicated samples. Melting profiles showed that HIU induced crystallization of low melting triacylglycerols (TAGs) and promoted co-crystallization of low and high melting TAGs. In general, HIU significantly changed the viscosity, $G'$, and $G''$ of the IE 20% samples except at $\Delta T = 12$ °C. While $G'$ and $G''$ of IE 30% did not increase significantly, the viscosity increased significantly at $\Delta T = 9$, 6, and 3 °C from $1526 \pm 880$ to $6818 \pm 901$ Pa.s at $\Delta T = 3$ °C.

The improved physical properties of the sonicated IE can make them good contenders for trans-fatty acids replacers.

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1Journal of American Oil Chemists’ Society, Sonocrystallization of interesterified fats with 20 and 30% of stearic acid at the sn-2 position and their physical blends, 94, 2017, 1045-1062, Kadanne JV, Ifeduba EA, Akoh CC and Martini S, (original copyright notice as given in the publication in which the material was originally published) “With permission of Springer
Introduction

Modification of the physical properties of fats is often desired to obtain specific functionalities for use in various food applications. Enzymatic interesterification is a widely used processing technique to achieve this [1]. Interesterification changes the triacylglycerol (TAG) composition of the fat without changing its fatty acid composition [2]. In 2016 Ifeduba et al. [3] enzymatically interesterified physical blends of (a) high oleic sunflower oil (HOSO) and tripalmitin and (b) HOSO and tristearin to develop fats containing TAGs with palmitic or stearic acid at the sn-2 position. Several studies have evaluated the effect of IE fats consumption with saturated fatty acids at the sn-2 position. Results from these studies are variable and no consensus about the nutritional properties of these IE fats has been achieved. However, some studies show that TAGs with saturated fats at the sn-2 position can either reduce [4, 5] or have no effect on postprandial lipemia [6, 7]. Increasing uses of interesterification by the lipid industry and consumer demands for healthier fats prompts the need of exploring the functionalities and physical properties of these new fats. Changes in TAG composition of fats upon interesterification affects their crystallization behavior [8] and depending on the new TAGs formed, the resulting IE fats could have slower crystallization behavior than their corresponding physical blends (PB) [9]. Therefore, IE fats are in general softer than their PB counterparts and the interesterification process limits their uses in many foods where harder fats are needed.

Extensive research has been performed related to the use of high intensity ultrasound (HIU) to induce crystallization of ice [10], sucrose [11], and fats such as cocoa butter [12], anhydrous milk fat [13, 14], palm kernel oil [13], and interesterified soybean
HIU has been shown to change the crystallization behavior of lipids by inducing and accelerating the formation of smaller [15] and more fat crystals [14], creating harder fats [13], increasing viscosity [14, 16], viscoelastic properties [15] and solid fat content [17]. Choosing appropriate sonication conditions such as size of the sonicator tip, amplitude of sonication, duration, crystallization temperature and amount of crystallizing material is essential for improved results [15, 17]. However, the role that fat chemical composition plays on lipid sonocrystallization still remains unknown.

The authors of this paper previously studied the crystallization behavior of interesterified (IE) fats with palmitic acid at the sn-2 position and the corresponding physical blends [18]. This study allowed us to compare the crystallization behavior of fats with similar fatty acids but different TAG composition along with the comparison of fats with different content of saturated fatty acids (SFA). The palmitic containing IE fats were found to be softer than their physical blends and the hardness of the IE samples was increased by using HIU. In the present study, the tripalmitin previously used by Kadamne et al. [18] in the PB was replaced by tristearin with the assumption that the higher melting stearin in the corresponding IE will provide a harder texture compared to the palmitic containing IE. Using interesterification conditions reported in Ifeduba et al. [3] IE fats with low total saturated fatty acids (20–30%) and stearic acid at the sn-2 position were produced.

The objective of this research is to evaluate the crystallization behavior of the IE fats containing 20 and 30% stearic acid at the sn-2 position and of the physical blends used to synthesize these IE samples. The effect of HIU on their crystallization behavior was studied at different supercooling levels. Crystal microstructure, solid fat content,
viscosity, elastic and storage modulus, and melting behavior were evaluated. The fats used in this study differ from those in the previous study based on the major saturated fatty acid at the sn-2 position, which is stearic acid in the present and palmitic acid in the former [18]. Along with the characterization of the physical properties of the IE fats, these studies will help us to understand the effectiveness of ultrasound induced crystallization with changes in type and amount of SFA.

Materials and Methods

Starting Materials

Dr. Akoh’s laboratory from the University of Georgia provided the interesterified (IE) and physical blends (PB) of tristearin (>99% purity, Spectrum Chemicals, Gardena, CA, USA) and high oleic sunflower oil (Stratas Foods, Memphis, TN, USA). The two PB samples contained a total of 20 and 30% stearic acid while in the IE prepared by the interesterification of PB using Lipozyme TLIM [3], among the fatty acids at the sn-2 position, about 20 and 30% were occupied by stearic acid. The PB used to prepare the IE samples containing 20% stearic acid at the sn-2 position (IE C18:0 20%) will be referred to as PB C18:0 20% while the physical blends used to prepare the IE samples containing 30% stearic acid at the sn-2 position (IE C18:0 30%) will be referred to as PB C18:0 30%.

Melting Point Determination

The IE and PB samples were melted completely upon reception, filtered while hot to remove any foreign impurity and stored at −20 °C until further use. The AOCS
Official Method Cc 1-25 was used to measure the melting point of the IE and PB samples.

**Fatty Acid Analysis and Triacylglycerol Composition**

The samples were analyzed for their fatty acid composition and triacylglycerol content according to the methods outlined by Ifeduba *et al.* [3].

**Crystallization Experiment**

Crystallization experiments were performed in a double wall glass cell with an external water bath to control the sample temperature. A magnetic stirrer was used to provide agitation at 100 RPM. Thirty grams of filtered sample was melted in the microwave and later kept in the oven at 100 °C for 45 min to remove crystal memory. The melted sample was then placed in the crystallization cell. Crystallization was performed at supercooling levels (ΔT) of 12, 9, 6, and 3 °C. Crystallization temperatures (T_c) used for each sample at each supercooling are shown in Table 5-1. The crystallization behavior of the samples was monitored using a He–Ne laser source (105-2 Uniphase, San Jose, CA, USA) as previously described by Wagh *et al.* [19]. The temperature of the sample was monitored by the thermocouple along with the laser signals and recorded by Lab- VIEW 8.0 software (National Instruments Corp., Austin, TX, USA). Sonication was performed using a Misonix 3000 sonicator (20 kHz, Misonix Inc., Farmingdale, NY, USA) and 3.2 mm diameter tip operating at 216 μm vibration amplitude for 5 s.

Prior to crystallization, the experimental set up was set at the desired temperature along with the sonication equipment with the stirrer. The position of the laser was arranged such that a maximum laser signal output of 10 V was obtained through the
empty cell. After the sample was introduced in the crystallization cell, the laser signal was monitored. The laser signal remained at its highest value until the sample started to crystallize. At this point, the laser signal decreased steadily. When the laser signal reached a value of 0.6 V, which corresponds to a slight amount of turbidity in the media, the agitation was stopped and HIU was applied to the sample. The 0.6 V laser output was chosen as the time point for HIU application since it corresponds to a slight turbidity indicative of the onset of crystallization. This allows for consistent sonication conditions for all the samples. Immediately after sonication, the sample was transferred into five nuclear magnetic resonance (NMR) tubes and three centrifuge tubes which were pre-warmed at the crystallization temperature and kept in the water bath until 60 min from the start of the experiment. The NMR tube samples were used to measure solid fat content (SFC) while the samples in the centrifuge tubes were used for microscopy.

Table 5-1: Melting point (Tm), crystallization temperatures (Tc), melting enthalpy (ΔH) and the driving force of crystallization (ϕ)* at different supercooling levels

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tm (°C)</th>
<th>ΔH (J/g)</th>
<th>ΔT = 12 °C</th>
<th>Tc (°C)</th>
<th>ϕ (J/g)</th>
<th>ΔT = 9 °C</th>
<th>Tc (°C)</th>
<th>ϕ (J/g)</th>
<th>ΔT = 6 °C</th>
<th>Tc (°C)</th>
<th>ϕ (J/g)</th>
<th>ΔT = 3 °C</th>
<th>Tc (°C)</th>
<th>ϕ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB C18:0 20%</td>
<td>53.6 ± 0.4</td>
<td>105.2 ± 1.8</td>
<td>42.0</td>
<td>23.6</td>
<td>45.0</td>
<td>17.7</td>
<td>48.0</td>
<td>11.8</td>
<td>51.0</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE C18:0 20%</td>
<td>38.0 ± 0.3</td>
<td>106.5 ± 2.2</td>
<td>26.0</td>
<td>33.6</td>
<td>29.0</td>
<td>25.2</td>
<td>32.0</td>
<td>16.8</td>
<td>35.0</td>
<td>8.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB C18:0 30%</td>
<td>60.0 ± 0.4</td>
<td>126.4 ± 1.6</td>
<td>48.0</td>
<td>25.3</td>
<td>51.0</td>
<td>19.0</td>
<td>54.0</td>
<td>12.6</td>
<td>57.0</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE C18:0 30%</td>
<td>43.2 ± 0.6</td>
<td>105.2 ± 1.3</td>
<td>31.0</td>
<td>29.2</td>
<td>34.0</td>
<td>21.9</td>
<td>37.0</td>
<td>14.6</td>
<td>40.0</td>
<td>7.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The calculation of the driving force of crystallization is explained in the solid fat content discussion section.
melting characteristics, and rheology. If the laser signal reached 0.6 V after 10 min of crystallization, the agitation was stopped at 10 min and the sample was crystallized further without agitation.

Samples evaluated in this study were crystallized without and with sonication. The non-sonicated samples were transferred to the tubes immediately after the laser signal reached 0.6 V. The crystallization experiment at each processing condition was performed in triplicates and the analyses were performed once after each of the triplicate runs.

**Solid Fat Content**

The five NMR tubes were kept in the water bath and the SFC of the sample in tube was measured every 2 min until 60 min of crystallization using Minispec mq20 (Bruker Biospin GmbH, Rheinstetten, Germany). The measurement of SFC started after the laser signal reached 0.6 V. For the sake of curve fitting, SFC points of 0% SFC were added to time point prior to the start of measurement. The tubes were put back into the water bath after SFC was measured and the tubes were used in rotation for further time points. The mean SFC values along with their standard error were plotted against time and the reparametrized Gompertz equation [20] was fitted to the data. Equation (1) shows the reparametrized Gompertz equation:

\[
s(t) = s_{\text{max}} \times e^{\left(1 - \frac{\mu_{\text{max}} \times e}{s_{\text{max}} \times (\lambda - t) + 1}\right)}
\]
where \( s(t) \) is the \( \% \) SFC at time \( t \), \( s_{\text{max}} \) is the maximum SFC, \( \mu_{\text{max}} \) is the maximum growth rate \( (% \text{ SFC/min}) \), \( \lambda \) is the induction time of crystallization \( \text{(min)} \), and \( e = 2.718281 \) [20].

**Polarized Light Microscopy**

Sample aliquots were taken from the centrifuge tube in the water bath every 10 min until 60 min of crystallization using pre-warmed glass pipettes and placed onto glass slides and then covered with cover slides. The microstructure of the sample was observed by the Olympus BX41 polarized light microscope (PLM) (Olympus, Tokyo, Japan) at 10X magnification. The microscope was fitted with an Instec TS62 thermal stage (Instec, Inc., Boulder, CO, USA) that was set to the crystallization temperature to prevent any change in the crystallization conditions in the slides due to temperature fluctuations.

**Differential Scanning Calorimetry**

The melting behavior of the samples was analyzed after 60 min of crystallization by a DSC Q20 (TA Instruments, New Castle, DE, USA). The sample was sealed hermetically in a Tzero pan with a Tzero hermetic lid and heated in the DSC from the crystallization temperature to 80 °C at 5 °C/min. The melting peaks were integrated to quantify the peak melting temperature \( (T_p) \), onset temperature of melting \( (T_{on}) \) and the melting enthalpy \( (\Delta H) \). For the calculation of the driving force of crystallization, the melting enthalpies were calculated by equilibrating the sample in the hermetically sealed pans overnight at \(-20 \, ^\circ\text{C}\) and followed by heating in the DSC from \(-20\) to \(80\, ^\circ\text{C}\) at 5 °C/min. The driving force for the crystallization of fats can be calculated using Eq. (2):

\[
\phi = \frac{\Delta H \times \Delta T}{T_m}
\]  

(2)
where $\Delta H$ is the change in enthalpy associated with the melting (J/g); $\Delta T$ is the supercooling (°C); and $T_m$ is the melting point of the sample (°C).

**Rheology**

Rheological parameters including viscosity, storage ($G'$), and loss ($G''$) moduli and the phase angle ($\delta$) were measured using a AR-G2 Rheometer (TA Instruments, New Castle, DE, USA). The viscosity was measured by a steady state flow procedure by increasing the shear rate from 0.01 to 300 (s$^{-1}$) at the crystallization temperature. Sample viscosity at 0.1 s$^{-1}$ shear rate was reported. The measurement of the viscoelastic parameters ($G'$, $G''$, and $\delta$) was performed at $T_c$ by a strain sweep oscillation procedure where the strain values changed from 0.008 to 10% at constant frequency of 1 Hz. The rheological parameters of the IE samples were measured using a parallel plate geometry (40 mm diameter) using samples from the centrifuge tubes in the water bath after being 60 min at $T_c$. The PB had a crumbly texture and, therefore, these samples were transferred to 20 mm diameter molds after the laser reached 0.6 V to obtain a more uniform network. The molds were maintained at $T_c$ for the duration of the experiment (60 min). The samples from the molds were used to measure the rheological parameters of the PB samples using parallel plate geometry (20 mm diameter). The rheology data was collected after each of the three separate runs at each processing condition. Thus the rheological data was collected and presented as the mean of the triplicate values along with its standard error of the mean.

**Statistical Analysis**

At $\Delta T = 12$ °C IE and the PB samples were compared within each fatty acid content using a two-way ANOVA followed by Tukey’s post hoc test at $\alpha = 0.05$. Results
for IE C18:0 20% samples at ΔT = 9, 6, and 3 °C were compared using a two-way ANOVA followed by the Sidak’s multiple comparison test to compare the effect of sonication at each supercooling level. Similar statistics were performed for the IE C18:0 30% samples at ΔT = 9, 6, and 3 °C.

Results and Discussion

Melting Point

The melting point of the PB C18:0 20% sample was 53.6 ± 0.4 °C while that of the IE C18:0 20% sample was 38.0 ± 0.3 °C. The PB C18:0 30% and IE C18:0 30% sample had melting points of 60.0 ± 0.4 and 43.2 ± 0.6 °C, respectively (Table 5-1). The melting point decreased upon interesterification due to the decrease in the amount of Tristearin in the samples while the samples containing 30% stearic acid had a higher melting point than the 20% samples due to their higher percentage of stearic acid.

Fatty Acid Composition

The fatty acid composition of the PB samples was reported earlier by Ifeduba et al. [3]. The major fatty acids, oleic and stearic acid were present at 68.1 and 21.0% level in the PB C18:0 20% and at 58.8 and 30.1% level in the PB C18:0 30%. In the PB C18:0 20% and the 30% samples, 11.7 and 19.8%, respectively, of the fatty acids at the sn-2 position were occupied by stearic acid. The total and sn-2 fatty acid composition of the IE samples is presented in Table 5-2. Oleic acid from the high oleic sunflower oil starting material was the highest in the IE sample and was present at 70.2 and 60.7% in the IE C18:0 20 and 30% sample, respectively. The next fatty acid in the highest concentration
Table 5-2: Total and sn-2 fatty acid composition of IE C18:0 20% and IE C18:0 30% samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total fatty acid composition (mol%)</th>
<th>Positional (sn-2) fatty acid composition (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:0</td>
<td>C18:0</td>
</tr>
<tr>
<td>IE C18:0 20*</td>
<td>4.9 ± 0.0</td>
<td>19.2 ± 0.1</td>
</tr>
<tr>
<td>IE C18:0 30*</td>
<td>5.9 ± 0.1</td>
<td>28.3 ± 1.5</td>
</tr>
</tbody>
</table>

*Trace amounts of C14:0 and C15:0; Mean ± SD, n = 2; ND not detected

Table 5-3: Triacylglycerol (TAG) composition of the IE C18:0 20% and the IE C18:0 30%

<table>
<thead>
<tr>
<th>Sample</th>
<th>TAG Molecular Species (peak area %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOO + LPO</td>
</tr>
<tr>
<td>IE C18:0 20%</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>IE C18:0 30%</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>
was stearic acid, derived from the tristearin starting material and the total level of stearic acid in the IE C18:0 20 and 30% samples was 19.2 and 28.3%, respectively. In the IE C18:0 20 and 30% samples, 17.0 and 33.2% respectively of the fatty acids at the sn-2 position were occupied by stearic acid. The major fatty acid at the sn-2 position was oleic acid and was present at 75.2 and 60.0% in the 20 and 30% samples, respectively.

**Triacylglycerol Composition**

The TAG composition of the PB samples has been discussed elsewhere [3]. The major TAGs in the PB C18:0 20% were OOO (79.4%) and SSS (11.4%) and the corresponding levels of these TAGs in the PB C18:0 30% sample were 68.5 and 22.3%, respectively [3]. The TAG composition of the IE samples is presented in Table 5-3. Upon interesterification, SSS in the PB C18:0 20% samples changed from 11.4 to 1.2% and the OOO decreased from 79.4 to 69.0%. New TAG species were formed in the IE samples including OOS and OSS at 23.7 and 4.0% levels, respectively. The amount of SSS, OOO, OOS, and OSS in the IE C18:0 30% was 2.3, 39.9, 42.7, and 14.3%, respectively. Lower content of SSS (1.2%), OSS (4.0%), OOS (23.7%) while higher contents of OOO (69.0%) and LOO/LPO (2.1%) were obtained for the IE C18:0 20%.

**Solid Fat Content**

In order to compare the results with our previous study [18], the samples from these study were crystallized at supercooling levels of 9, 6, and 3 °C. However, at these supercooling levels, the physical blends did not crystallize into a uniform crystalline network that allowed the characterization of its physical properties. The PB was rich in SSS and OOO which have melting points of 73.5 and 4.5–5.7 °C, respectively [21]. Because of large differences in the melting points of these TAGs, the PB crystallized in
two separate fractions: the stearin and the olein fraction and did not form a continuous network of crystals. Because of this discontinuous network, the laser signal did not drop as expected and hence similar crystallization conditions could not be generated in the PB at different supercooling levels. Hence, the samples were also crystallized at $\Delta T = 12 \, ^\circ\text{C}$ where the PB did not fractionate and generated a turbid crystalline sample which reproducibly decreased the laser signal over time. Thus, the IE were crystallized at four supercooling levels while the PB was crystallized at only $\Delta T = 12 \, ^\circ\text{C}$.

The solid fat content (SFC) of the IE and PB samples at $\Delta T = 12 \, ^\circ\text{C}$ are shown Fig. 5-1, while the SFC of IE samples at supercoolings of 9, 6, and 3 $^\circ\text{C}$ are shown in Fig. 5-2. The

**Figure 5-1** Solid Fat content of the IE and PB C18:0 20% and 30% samples at $\Delta T= 12 \, ^\circ\text{C}$. The point of application of HIU for the PB sample is indicated with a dotted arrow on the time axis while that of the IE samples is indicated with a solid arrow. Mean values and standard errors of three experimental replicates are reported.
time point of application of HIU is shown by an arrow pointing at the time axis. The SFC data was fitted to the Gompertz equation as described in the “Materials and Methods” section above (Eq. 1) and the parameters obtained are tabulated in Table 5-4. The maximum SFC, $s_{\text{max}}$, of PB crystallized at $\Delta T = 12 \, ^\circ\text{C}$ was higher than that of the IE samples for both the C18:0 20 and 30% samples (Fig. 5-1; Table 5-4) ($p < 0.05$). When samples were crystallized without sonication the $s_{\text{max}}$ of the PB C18:0 20% sample was 13.5% while that of the IE C18:0 20% sample was 8.6%. Similarly, the $s_{\text{max}}$ of the PB C18:0 30% sample was 18.2% while that of the IE C18:0 30% sample was 10.8%.

Application of HIU to the PB samples did not induce crystallization in the 20% (Fig. 5-1.a) while an induction was observed for the 30% ones (Fig. 5-1.b). A significant ($p < 0.0001$) decrease in the induction period of crystallization ($\lambda$) was observed for the sonicated PB C18:0 30% sample from 11.9 to 8.7 min and an increased growth rate from 3.2 to 4.8% SFC/min (Table 5-4). The maximum growth rate ($\mu_{\text{max}}$) of the PB C18:0 20% samples increased significantly ($p < 0.05$) from 3.33 to 10.12% SFC/min even though there was no significant change in the induction period of crystallization (Table 5-4) ($p > 0.05$). At $\Delta T = 12 \, ^\circ\text{C}$, HIU did not affect the crystallization kinetics of IE samples (Fig. 5-1a, b; Table 5-4). Based on the similarity in the isothermal SFC curves of the IE samples at $\Delta T = 12 \, ^\circ\text{C}$ (Fig. 5-1.a, b) and no the lack of difference in the crystallization kinetics upon sonication (Table 5-4) it can be concluded that at $\Delta T = 12 \, ^\circ\text{C}$ supercooling and not sonication was the dominant force that drove the crystallization of IE samples. In general, the $s_{\text{max}}$ of the IE C18:0 30% samples were higher than those of IE C18:0 20% samples (Fig. 5-1.a, b; Table 5-4). This can be due to the higher stearic acid content and the slightly higher content of SSS in the 30% samples.
Table 5-4: Gompertz parameters – Maximum SFC ($s_{\text{max}}$), rate of crystallization ($\mu$) and Induction period ($\lambda$) obtained from the Gompertz fit to the solid fat content data of the sonicated and non-sonicated IE and PB samples.

<table>
<thead>
<tr>
<th>Gompertz Parameters</th>
<th>IE- no HIU</th>
<th>IE- with HIU</th>
<th>PB- no HIU</th>
<th>PB- with HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C18:0 20% samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta T = 12 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>8.56 ± 0.07$^c$</td>
<td>8.62 ± 0.06$^c$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.98 ± 0.07$^b$</td>
<td>0.90 ± 0.05$^b$</td>
<td>3.33 ± 0.30$^b$</td>
</tr>
<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>2.07 ± 0.29$^b$</td>
<td>2.15 ± 0.25$^b$</td>
<td>7.33 ± 0.20$^a$</td>
</tr>
<tr>
<td>$\Delta T = 9 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>7.51 ± 0.05$^a$</td>
<td>7.62 ± 0.04$^a$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.89 ± 0.05$^b$</td>
<td>1.18 ± 0.06$^a$</td>
<td>3.33 ± 0.30$^b$</td>
</tr>
<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>3.99 ± 0.21$^a$</td>
<td>4.33 ± 0.15$^a$</td>
<td>7.33 ± 0.20$^a$</td>
</tr>
<tr>
<td>$\Delta T = 6 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>6.44 ± 0.04$^a$</td>
<td>6.51 ± 0.02$^a$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.76 ± 0.04$^b$</td>
<td>1.61 ± 0.08$^a$</td>
<td>3.33 ± 0.30$^b$</td>
</tr>
<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>8.36 ± 0.22$^a$</td>
<td>8.39 ± 0.10$^a$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td>$\Delta T = 3 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>5.52 ± 0.05$^a$</td>
<td>5.55 ± 0.02$^a$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.46 ± 0.03$^b$</td>
<td>1.54 ± 0.08$^a$</td>
<td>3.33 ± 0.30$^b$</td>
</tr>
<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>13.03 ± 0.40$^b$</td>
<td>11.77 ± 0.11$^a$</td>
<td>13.54 ± 0.11$^a$</td>
</tr>
<tr>
<td><strong>C18:0 30% samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta T = 12 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>10.81 ± 0.06$^b$</td>
<td>10.74 ± 0.06$^b$</td>
<td>18.21 ± 0.15$^a$</td>
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<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
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<td>0.84 ± 0.03$^c$</td>
<td>3.24 ± 0.23$^b$</td>
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<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>2.17 ± 0.27$^c$</td>
<td>2.76 ± 0.28$^c$</td>
<td>11.91 ± 0.21$^a$</td>
</tr>
<tr>
<td>$\Delta T = 9 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>9.00 ± 0.08$^a$</td>
<td>9.05 ± 0.04$^a$</td>
<td>18.21 ± 0.15$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.66 ± 0.03$^b$</td>
<td>1.38 ± 0.06$^a$</td>
<td>3.24 ± 0.23$^b$</td>
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<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>9.41 ± 0.37$^a$</td>
<td>9.56 ± 0.14$^a$</td>
<td>11.91 ± 0.21$^a$</td>
</tr>
<tr>
<td>$\Delta T = 6 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>7.17 ± 0.05$^a$</td>
<td>7.37 ± 0.05$^a$</td>
<td>18.21 ± 0.15$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.48 ± 0.02$^b$</td>
<td>0.93 ± 0.05$^a$</td>
<td>3.24 ± 0.23$^b$</td>
</tr>
<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>15.55 ± 0.27$^a$</td>
<td>14.13 ± 0.21$^a$</td>
<td>11.91 ± 0.21$^a$</td>
</tr>
<tr>
<td>$\Delta T = 3 , ^\circ\text{C}$</td>
<td>$s_{\text{max}}$ (%SFC)</td>
<td>5.19 ± 0.27$^a$</td>
<td>5.58 ± 0.09$^a$</td>
<td>18.21 ± 0.15$^a$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ (%SFC/min)</td>
<td>0.17 ± 0.01$^b$</td>
<td>0.43 ± 0.04$^a$</td>
<td>3.24 ± 0.23$^b$</td>
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<tr>
<td></td>
<td>$\lambda$ (min)</td>
<td>29.30 ± 0.53$^a$</td>
<td>21.39 ± 0.60$^b$</td>
<td>11.91 ± 0.21$^a$</td>
</tr>
</tbody>
</table>

At $\Delta T = 12 \, ^\circ\text{C}$, each parameter viz. $s_{\text{max}}$, $\mu$, and $\lambda$ was compared between the IE and PB (sonicated and non-sonicated samples) for both the IE C18:0 20 and 30% samples by a 2-way ANOVA followed by Tukey’s multiple comparison test. At $\Delta T = 9, 6$ and 3 $^\circ\text{C}$, each was compared among all the supercooling by 2-way ANOVA followed by Sidak’s multiple comparison between the sonicated and the non-sonicated samples at each supercooling separately.
HIU induced crystallization in the IE C18:0 20% samples at supercooling of 6 and 3 °C and significantly decreased the $\lambda$ from 9.36 to 8.39 min at $\Delta T = 6$ °C ($p < 0.05$) and from 13.03 to 11.77 min at $\Delta T = 3$ °C ($p < 0.05$). HIU also significantly increased the rate of crystallization from 0.76 to 1.61 at $\Delta T = 6$ °C and from 0.46 to 1.54% at $\Delta T = 3$ °C (Table 5-4) ($p < 0.05$). Sonication also induced crystallization in IE C18:0 30% samples at supercooling levels of 9, 6, and 3 °C. The maximum growth rate ($\mu_{\text{max}}$) increased significantly ($p < 0.0001$) from 0.66 to 1.38% SFC/min at $\Delta T = 9$ °C upon sonication and the effect was also observed at the lower supercooling levels (Fig. 5-2d; Table 5-4).

Although the $s_{\text{max}}$ of IE C18:0 30% samples slightly increased with sonication at $\Delta T = 9,$ 6, and 3 °C, the increase was not statistically significant ($p > 0.05$). In addition, the induction period of crystallization decreased significantly ($p < 0.0001$) only for $\Delta T = 3$ °C from 29.3 to 21.4 min (Fig. 5-2.f; Table 5-4).

Similar results were obtained in the previous study with IE C16:0 30% samples at $\Delta T = 3$ °C where even though no significant increase in the $s_{\text{max}}$ was observed with sonication, the induction period of crystallization decreased from 34.4 to 26.8 min [18]. The crystallization behavior observed at the different supercooling levels can be explained based on the driving force of crystallization reported in Table 5-1. The enthalpy of melting used to calculate the driving force of crystallization was measured using the DSC and were 105.2 and 106.5 J/g for the PB and IE C18:0 20% samples and 126.4 and 105.2 J/g for the PB and IE C18:0 30% samples (Table 5-1). Thus, based on Eq. (2), for a specific sample, high supercooling levels can be obtained by lowering the crystallization temperature, thereby creating a higher driving force for crystallization. As the driving force increased, there was an induction in the crystallization of the samples.
Figure 5-2: Solid Fat Content of IE C18:0 20% and 30% samples at ΔT= 9, 6, and 3 °C. The point of application of HIU is indicated with an arrow on the time axis. Mean values and standard errors of three experimental replicates are reported.
For example, at $\Delta T = 12 \, ^\circ \text{C}$ the driving force for the non-sonicated IE C18:0 20% was 33.6 J/g (Table 5-1) and the induction period of crystallization was approximately 2 min (Table 5-4) while at subsequent supercooling levels of 9, 6, and 3 °C, the induction period increased to 4, 9.4 and 13 min, respectively (Table 5-4) due to the decreasing driving force of 25.2, 16.8 and 8.4 J/g (Table 5-1). The driving forces for the 30% stearic samples were lower, but in the same order of magnitude, than the corresponding 20% stearic samples for the same supercooling. This was due to the similar melting enthalpy and the higher melting point of the C18:0 30% samples. The driving force for the IE C18:0 30% samples at supercooling levels of 12, 9, 6, and 3 °C were 29.2, 21.9, 14.6, and 7.3 J/g, respectively (Table 5-1) and the corresponding induction period of non-sonicated crystallization were 2.2, 9.4, 15.5, and 29.3 min, respectively (Table 5-4). The driving force of the IE C18:0 30% samples was lower compared to the IE C18:0 20%. It took longer for the IE C18:0 30% samples to crystallize compared to the IE C18:0 20% samples at all the supercooling levels. The PB samples had lower driving force than the corresponding IE samples and hence the induction period of the PB was higher than those of the IE at $\Delta T = 12 \, ^\circ \text{C}$.

At $\Delta T = 9 \, ^\circ \text{C}$, the driving force of the IE C18:0 20% sample was 25.2 J/g and based on the SFC curves in Fig. 5-2.a it can be seen that there was no difference in the crystallization kinetics of the sonicated and non-sonicated sample. This suggests that similar to the IE samples at $\Delta T = 12 \, ^\circ \text{C}$, the supercooling dominated crystallization of IE C18:0 20% sample at 9 °C and sonication had no effect on the induction of crystallization. However, for the PB C18:0 30% sample at $\Delta T = 12 \, ^\circ \text{C}$, the driving force was 25.3 J/g and HIU induced crystallization despite the high driving force. This was due
to the greater percentage of the higher melting SSS fraction. The cavitation generated by the HIU induced secondary crystallization of the SSS in the supercooling PB sample. Induction in the crystallization of the SSS was not observed at $\Delta T = 9 \, ^\circ\text{C}$ in the IE C18:0 20% sample due to the low amount of SSS compared to the PB C18:0 30% sample.

The $s_{\text{max}}$ of the samples was higher with higher driving force in the case of IE samples. However, the $s_{\text{max}}$ of the PB samples was higher than the IE samples, even though the driving force of the IE was higher. This suggests that the driving force of crystallization was an important factor for the induction of crystallization. However, the composition of the fat played a bigger role in the overall SFC of the fat samples. In the case of the PB samples, the higher SSS content induced a higher $s_{\text{max}}$ in the PB samples and higher content of SSS in IE C18:0 30% compared to IE C18:0 20% resulting in higher $s_{\text{max}}$.

Compared to the previous crystallization studies by the current authors [18], the IE samples with 20% palmitic acid at the sn-2 position crystallized in two steps while the stearic samples crystallized in a single step. The driving force for the IE C16:0 20% sample was 39.68 J/g while that for the IE C18:0 20% sample was 25.2 J/g at 9 °C supercooling level. The lower driving force obtained in the stearic sample for the same degree of supercooling may have allowed sufficient time for the low and high melting TAGs to crystallize together and evidenced as a single-step growing curve. The IE C16:0 30% sample also crystallized in two steps at $\Delta T = 9 \, ^\circ\text{C}$ for the palmitic based samples. However, similar to the IE C18:0 30% samples, due to the decrease in the driving force, with lower supercooling levels, the IE C16:0 30% crystallized in a single step. The $\mu_{\text{max}}$ and the $s_{\text{max}}$ of the IE C18:0 samples were higher than those of the IE C16:0 samples [18].
and these differences can be attributed to the presence of the higher melting TAGs in the samples in the current study. Interestingly, sonication did not induce crystallization of samples with 20% of C16:0 for any of the supercooling levels tested but did have an effect on the crystallization of samples with 20% C18:0. Similar to the previous discussion, the presence of C18:0 with a higher melting point than C16:0 might be responsible for this different effect.

**Microstructure**

Crystal microstructures obtained for the PB and IE samples crystallized at \( \Delta T = 12 ^\circ C \) after 60 min of crystallization are presented in Fig. 5-3. The bright structures in the picture represent the crystals while the dark background represent the liquid part. Upon visual comparison, the PB had larger crystals than the IE samples. Small and large number of crystals were present in the microstructure of the IE C18:0 20% sample without and with sonication. Similar to the SFC, sonication did not affect the microstructure of IE C18:0 20% at the highest supercooling. The crystal size of the IE C18:0 30% seemed larger than those obtained for the IE C18:0 20% samples. The induction period of crystallization of the IE C18:0 30% samples were slightly higher than those of the IE C18:0 20% samples. This provided more time for the TAGs to rearrange and hence the crystals of the IE C18:0 30% were slightly larger than the IE C18:0 20% samples. Based on the induction of secondary nucleation caused by HIU slightly smaller crystals were observed in the sonicated IE C18:0 30%. Although there was a change in the microstructure of the sample, there was no change in the SFC of the sample. The crystals of PB C18:0 30% were larger than all the samples at \( \Delta T = 12 ^\circ C \) and smaller crystals were observed in sonicated PB C18:0 20 and 30% samples.
Figure 5-3: PLM of sonicated and non-sonicated IE and PB C18:0 20% and 30% at $\Delta T = 12 \, ^\circ C$ after 60 minutes of crystallization. (The white bar represents 100 $\mu$m)
Figure 5-4: PLM of sonicated and non-sonicated IE and PB C18:0 20% at $\Delta T = 9$, 6 and 3 °C after 60 minutes of crystallization. (The white bar represents 100 µm)
From Fig. 5-4, it can be seen that there was formation of smaller crystals in the IE C18:0 20% samples upon sonication at supercooling levels of 9, 6, and 3 °C. Although the amount of crystals did not decrease with the decrease in supercooling, slightly larger crystals can be seen in non-sonicated samples at the lowest supercooling. When compared to the previous study involving samples containing 20 and 30% palmitic acid at the sn-2 position [18], the IE C16:0 20% samples had fewer crystals compared to the IE C18:0 20% samples. With decreasing supercooling, there was a decrease in the amount of crystals in the IE C16:0 20% samples while in the case of IE C18:0 20% samples, the decrease in the supercooling increased the size of the crystals while there was no visible change in the amount of crystals in the microstructure. Although HIU application induced the formation of smaller crystals in the IE C18:0 20% sample at all the supercooling levels, the HIU was not as effective in the case of the IE C16:0 20% samples. These results correlate well with the higher SFC of the IE C18:0 20% samples (5.5 and 5.6% for non-sonicated vs. sonicated samples, respectively at ΔT = 3 °C) compared to the IE C16:0 20% samples from the previous study [18] (3.8 and 3.6% for non-sonicated vs. sonicated samples, respectively at ΔT = 3 °C).

The microstructure of the IE C18:0 30% samples at ΔT = 9, 6, and 3 °C are presented in Fig. 5-5. Compared to the ΔT = 12 °C, slightly larger crystals were formed in the non-sonicated samples at all supercooling levels. Similar results were observed by Herrera et al. [22] and Martini et al. [23] in milk fat and, milk fat fractions and sunflower oil blends, respectively. According to Martini et al. [23] at low supercooling levels, or at a higher crystallization temperature, fewer nuclei were formed. This condition favors the
Figure 5-5: PLM of sonicated and non-sonicated IE and PB C18:0 30% at ΔT = 9, 6 and 3 °C after 60 minutes of crystallization. (The white bar represents 100 µm)
growth of the already formed nuclei resulting in fewer and bigger crystals. HIU induced smaller and more crystals in IE C18:0 30% at all the supercooling levels. Compared to the previous study with palmitic samples [18], at ΔT = 3 °C, higher amount of crystals can be seen in the stearic samples and this correlates well with the higher SFC of these samples at the end of crystallization. IE C18:0 30% samples had SFC values of 5.2 and 5.6% for the non-sonicated and sonicated samples, respectively; the IE C16:0 30% had SFC values of 3.4 and 4.5% for the non-sonicated and sonicated samples, respectively.

Increase in the number of smaller crystals upon sonication has been reported previously by several authors [13–15, 17, 18, 24]. In the current study, HIU was applied in the presence of crystals similar to experimental conditions used by Suzuki et al. [13] and Ye et al. [15] According to Suzuki, HIU increased the amount of nuclei in the system by inducing secondary nucleation by breaking the existing nuclei in the system along with primary nucleation.

**Differential Scanning Calorimetry**

The melting thermograms of the IE and PB samples at ΔT = 12 °C are shown in Fig. 5-6 and the corresponding $T_{on}$, $T_P$ and the enthalpy ($\Delta H$) of melting of the samples are presented in Table 5-5. The PB C18:0 20% samples had a single peak for both the sonicated and the non-sonicated sample at ΔT = 12 °C with a peak melting temperature of 61.2 ± 0.4 °C and 61.4 ± 0.1 °C, respectively (Fig. 5-6a; Table 5-5). The melting thermograms of the sonicated PB sample shows a shoulder next to the peak melting temperature which was absent in the non-sonicated sample (Fig. 5-6a). This indicates that there was a slight induction in the crystallization of the lower melting components in the fat such as PSS (2.7%) or PPS + OPS (2.5%) [3]. The IE C18:0 20% samples had a $T_P$ of
Figure 5-6: DSC thermograms of sonicated and non-sonicated IE and PB C18:0 20% and 30% at ΔT = 12 °C

52.8 ± 0.1 and 52.7 ± 0.1 °C with an enthalpy of 9.2 ± 0.5 and 9.4 ± 0.1 J/g without and with sonication, respectively. The majority of TAGs in the IE C18:0 20% sample were SSS (1.2%), OSS (4.0%), OOS (23.7), and OOO (69.0%). This sample showed a single broad melting peak indicating that these TAGs co-crystallized (Fig. 5-6a). Thus, HIU did not affect the crystallization behavior of the IE samples and this confirms the previous speculation that at a ΔT = 12 °C, supercooling dominated the crystallization of the IE C18:0 20% samples. The PB C18:0 30% samples had two well-defined melting peaks with the first peak melting temperatures of 58.1 ± 0.7 °C and the second peak at 64.9 ± 0.5 °C for the non-sonicated sample (Fig. 5-6b; Table 5-5). The higher melting peak corresponds to the crystallization of the SSS TAG while the lower melting peak corresponds to crystallization of PSS (3.8%) and PPS + OPS (2.1%) [3]. For the non-sonicated and sonicated PB C18:0 30%, the melting enthalpy of the first peak was 21.9 ± 3.8 and 36.1 ± 7.1 J/g, respectively and that of the second peak was 10.8 ± 4.8 and 13.0 ± 5.3 J/g, respectively. The IE C18:0 30% sample at ΔT = 12 °C also had two peaks in the
Table 5-5: DSC melting parameters Ton, Tp and enthalpy (ΔH) for the interesterified (IE) samples and physical blends (PB) at ΔT = 12 °C. Each parameter is compared between the sonicated and non-sonicated PB and IE within the same group (C18:0 20% or C18:0 30%). The parameters represented with different alphabets are statistically different (α = 0.05).

<table>
<thead>
<tr>
<th>Peak 1</th>
<th>No HIU</th>
<th>With HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB C18:0 20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ton (°C)</td>
<td>53.3 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.0 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tp (°C)</td>
<td>61.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>ΔH (J/g)</td>
<td>33.8 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.3 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IE C18:0 20%</td>
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<tr>
<td>Ton (°C)</td>
<td>39.8 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Tp (°C)</td>
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<td>ΔH (J/g)</td>
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<tr>
<td>Ton (°C)</td>
<td>54.0 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.1 ± 0.1&lt;sup&gt;a&lt;/sup&gt;*</td>
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<tr>
<td>Tp (°C)</td>
<td>58.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ΔH (J/g)</td>
<td>21.9 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.1 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>IE C18:0 30%</td>
<td></td>
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<tr>
<td>Ton (°C)</td>
<td>38.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.9 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tp (°C)</td>
<td>43.1 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔH (J/g)</td>
<td>2.1 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The Ton temperature of only two replicates were calculated by the software.
melting thermograms (Fig. 5-6b). The IE C18:0 30% sample had 2.3% SSS which drives
the crystallization of the sample. The higher melting peak corresponds to the
crystallization of SSS while the lower melting peaks may comprise of OSS, SOS, OOS,
and OSO. The other TAGs including OOO (melting point = 4.5–5.7 °C), LOO (melting
point 5.1 °C), and LOP (melting point = 13.3 °C) had melting points below the
crystallization temperature and do not contribute to the crystallization behavior of these
samples. However, the changes in the enthalpy of melting in sonicated samples were not
as drastic as compared to the ones observed in the PB sample indicating that sonication
did not alter the crystallization of the samples.

The melting thermograms of the IE C18:0 20% and the 30% samples at
supercooling levels of 9, 6, and 3 °C are shown in Fig. 5-7a–f and the corresponding data
is presented in Table 5-6. At ΔT = 9 °C, IE C18:0 20% showed a single melting peak
similar to the behavior observed at ΔT = 12 °C (Fig. 5-6a). The peak melted at 53.1 ± 0.4
°C and upon sonication, this peak had a lower melting enthalpy that decreased
significantly from 6.8 to 4.1 J/g (p < 0.001). Also, sonicated sample showed a shoulder
peak at 41.2 ± 0.7 °C with a low melting enthalpy of 0.8 J/g (Fig. 5-7a; Table 5-6).
Although it was observed that HIU did not affect the SFC or the microstructure at this
supercooling, the DSC data suggests that sonication induced the crystallization of lower
melting TAGs (OSS and SOS) at this supercooling which was not observed at ΔT = 12
°C. This effect was even more prominent at ΔT = 6 °C and a new peak was formed upon
sonication at 44.7 ± 0.3 °C which was not seen in the thermograms of the non-sonicated
sample (Fig. 5-7b; Table 5-6). The melting enthalpy of the low temperature peak
**Figure 5-7:** DSC thermograms of sonicated and non-sonicated IE C18:0 20% and 30% at $\Delta T = 9, 6$ and $3 \, ^\circ C$
Table 5-6: DSC melting parameters Tonset, Tpeak and enthalpy (ΔH) for the interesterified (IE) samples C18:0 20% and 30% at ΔT = 9, 6 and 3 °C. Within a sample each parameter is compared between the sonicated and non-sonicated sample at each supercooling. The parameters represented with different superscripts are statistically different (α = 0.05)

<table>
<thead>
<tr>
<th>ΔT (°C)</th>
<th>IE C18:0 20%</th>
<th>Peak 1</th>
<th></th>
<th>Peak 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tonset (°C)</td>
<td>Tp (°C)</td>
<td>ΔH (J/g)</td>
<td>Tonset (°C)</td>
</tr>
<tr>
<td>9</td>
<td>No HIU</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>37.7 ± 0.4</td>
<td>41.2 ± 0.7</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>6</td>
<td>No HIU</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>41.5 ± 1.5</td>
<td>44.7 ± 0.3</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>No HIU</td>
<td>42.7 ± 0.4a</td>
<td>47.8 ± 0.1a</td>
<td>1.0 ± 0.4a</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>42.5 ± 0.6a</td>
<td>46.5 ± 0.3a</td>
<td>6.4 ± 0.034b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ΔT (°C)</th>
<th>IE C18:0 30%</th>
<th>Peak 1</th>
<th></th>
<th>Peak 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tonset (°C)</td>
<td>Tp (°C)</td>
<td>ΔH (J/g)</td>
<td>Tonset (°C)</td>
</tr>
<tr>
<td>9</td>
<td>No HIU</td>
<td>39.9 ± 0.5</td>
<td>45.2 ± 0.3a</td>
<td>3.8 ± 0.6a</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>43.3**</td>
<td>46.3 ± 0.2b</td>
<td>13.0 ± 0.3b</td>
</tr>
<tr>
<td>6</td>
<td>No HIU</td>
<td>N/A***</td>
<td>48.6 ± 0.3a</td>
<td>11.3 ± 0.8a</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>N/A***</td>
<td>47.3 ± 0.1b</td>
<td>12.9 ± 0.5a</td>
</tr>
<tr>
<td>3</td>
<td>No HIU</td>
<td>43.3**</td>
<td>51.0 ± 0.3a</td>
<td>9.4 ± 1.0a</td>
</tr>
<tr>
<td></td>
<td>With HIU</td>
<td>41.2 ± 0.002***</td>
<td>48.0 ± 0.1b</td>
<td>12.9 ± 0.3b</td>
</tr>
</tbody>
</table>

* certain peaks were not detected at all the processing conditions; ** The Tonset temperature of only one replicate was calculated by the software; *** For peaks where the Tonset temperature could not be determined by the software, it is denoted by N/A; **** The Tonset temperature of only two replicates was calculated by the software
was 4.9 J/g which was higher than the peak at $\Delta T = 6 ^\circ C$. At the lowest supercooling ($\Delta T = 3 ^\circ C$), sonication favored the crystallization of the lower melting fractions and decreased the size of the higher melting peak from an average enthalpy of 1.2 to 0.1 J/g (Fig. 5-7c; Table 5-6). Although sonication did not affect the $T_p$, the $T_p$ increased with the decrease in supercooling. This indicates that sonication did not fractionate the sample into new TAG fractions but favored the crystallization of the already crystallizing lower TAGs.

The non-sonicated IE C18:0 30% samples, on the other hand, crystallized in two fractions with peak melting temperatures of 45.2 ± 0.3 and 54.5 ± 0.3 °C at $\Delta T = 9 ^\circ C$ (Fig. 5-7d; Table 5-6). This behavior was similar to that observed for the sample crystallized at $\Delta T = 12 ^\circ C$ (Fig. 5-6). In general, upon sonication of the IE C18:0 30% samples, there was an increase in the enthalpy of the first peak while the enthalpy of the second peak decreased. Also, there was a significant increase in the $T_p$ of the first peak indicating that HIU induced the co-crystallization of these two fractions ($p < 0.01$). At $\Delta T = 9 ^\circ C$, the IE C18:0 30% sample melted in two peaks with peak melting temperatures of 45.2 and 54.5 °C (Fig. 5-7d) and upon sonication, the enthalpy of the higher melting peak decreased from 2.6 to 0.1 J/g and the enthalpy of the first peak at 46.3 °C increased from 3.8 and 13 °C (Table 5-6). Although a small second peak was seen at $\Delta T = 6 ^\circ C$ in the thermograms of the IE C18:0 30% sample, this peak disappeared in the sonicated sample along with a slight increase in the enthalpy of the first peak from 11.3 to 12.9 J/g, although not statistically significant (Fig. 5-7e; Table 5-6). At $\Delta T = 3 ^\circ C$ there was only one peak in the sonicated and non-sonicated IE C18:0 30% melting thermograms (Fig. 5-7f). However, there was a significant increase in the melting enthalpy of this peak from
9.4 to 12.9 J/g in the sonicated sample indicating that HIU induced crystallization (Table 5-6). This correlates well with the PLM data where a more crystalline material can be observed in the sonicated sample compared to the non-sonicated one.

The differences in the melting behavior of the IE C18:0 20% and the IE C18:0 30% samples can be explained based on the differences in the SSS content of the samples: 1.2 and 2.3%, respectively. The thermograms of the IE C18:0 20% samples indicate that the higher driving force of the samples favored the crystallization of the higher melting TAG, SSS (1.2%) along with the OSS (4.0%). However, due to the lower amount of OSS, the lower melting peak was not as prominent. Upon sonication, secondary nucleation was induced and the crystallization along with the growth of OSS around these secondary nuclei was favored. As the temperature of crystallization increased, the system had sufficient time to allow for the crystallization of the lower melting TAGs. On the other hand, due to the higher concentration of SSS (2.3%) and OSS (14.3%) in the IE C18:0 30% sample at this supercooling, there were two peaks in the melting thermograms (Fig. 5-7d). Similar to the IE C18:0 20% and the ability of the HIU to induce secondary crystallization, the crystallization of the OSS was favored and the Tp matched with the melting point of OSS (45 °C). Also, in both the IE C18:0 20 and 30% samples HIU induced the crystallization of the lower melting TAGs (OSS) and promoted the incorporation of higher melting point TAGs (SSS) into the crystalline network. This co-crystallization resulted in an increase in size of the first melting peak and a decrease in the size of the second melting peak.

**Rheology**

The rheological parameters of the IE and the PB samples at ΔT = 12 °C are presented in Fig. 5-8. Viscosity and $G'$ values of IE C18:0 20% samples were significantly higher
Figure 5-8: Rheology parameters, viscosity, $G'$, $G''$ and of sonicated and non-sonicated IE and PB C18:0 20% and 30% at $\Delta T = 12$ °C. Mean values and standard errors of three experimental replicates are reported. For samples within each group (C18:0 20% or C18:0 30%), parameters with different alphabets are statistically different ($\alpha = 0.05$)
than those observed in PB samples ($p < 0.05$) (Fig. 5-8a, b). Although the SFC of the PB samples was higher than the IE samples, the rheological parameters for the IE were an order of magnitude higher than the PB ones. The PB samples contained about 11.4% SSS which contributes to the majority of the SFC of the PB samples. However, it also contains 79.4% of OOO, which had a melting point of 4.5–5.7 °C and may be entrapped along with the SSS crystalline matrix. However, due to the big difference in the melting points of the TAG fractions in the PB samples, there may not be a uniform strong crystalline matrix. Hence, the overall rheological parameters were weaker than the corresponding IE samples which had TAG fractions such as OSS, OOS with melting points in the vicinity of each other and may have led to the co-crystallization of several TAG species together. The differences in the rheological properties can also be attributed to the differences in the microstructure of the samples. Based on the PLM pictures presented in Fig. 5-3, it can be seen that the microstructure of the IE samples was comprised of smaller and more crystals compared to those of the PB samples. It has been shown before [15, 18] that smaller crystal microstructure increases the rheological properties of fats.

The viscosity of the non-sonicated PB C18:0 20% sample was 85 ± 37 Pa.s while that of the IE C18:0 20% sample was 736 ± 143 Pa.s at $\Delta T = 12$ °C. The rheological parameters did not change upon sonication. This correlates well with the SFC and the PLM data. There was no change in the final SFC of either the IE or the PB samples with sonication due to the high supercooling. The PLM of the IE samples were also similar without and with HIU. Sonication induced the formation of smaller crystals in PB
microstructure which did increase the magnitude of the rheological parameters, but this increase was not statistically significant ($p > 0.05$).

On the other hand, the magnitude of the rheological parameters was higher for the PB C18:0 30% samples compared to the IE C18:0 30% samples. This may correspond to the higher SSS content (22.3%) in the PB C18:0 30% which was almost twice the amount in the PB C18:0 20% samples. Crystallization of this high melting TAG may have contributed to the rheological properties of the fat blend. The viscosity of the non-sonicated PB C18:0 30% sample was $19,430 \pm 4950$ Pa.s while that of the IE C18:0 30% sample was $1160 \pm 201$ Pa.s. Upon sonication, although there was induction of smaller crystals in the PB C18:0 30% samples (Fig. 5-3), the viscosity significantly decreased to $2481 \pm 997$ Pa.s ($p < 0.05$). However in the IE C18:0 30% samples, there were smaller crystals in the microstructure upon sonication at $\Delta T = 12 \, ^\circ C$ (Fig. 5-3) and the viscosity of the sonicated sample was $2963 \pm 758$ Pa.s. The $G'$ and the $G''$ of the PB C18:0 30% sample were $1.9 \times 10^6 \pm 4.9 \times 10^5$ and $3.4 \times 10^5 \pm 9.1 \times 10^4$ Pa, respectively, and were much higher than those of the IE C18:0 30% which were $7.7 \times 10^4 \pm 5.1 \times 10^3$ and $4.2 \times 10^3 \pm 324$ Pa, respectively. Upon sonication, there was no significant increase in these rheological properties in either of the samples. The phase angle ($\delta$) of the PB and IE C18:0 30% sample were $10.2 \pm 0.6$ and $3.2 \pm 0.1$, respectively and these did not change significantly ($p < 0.05$) upon sonication (Fig. 5-8d). Since these values were $0^\circ < \delta < 90^\circ$, both samples were considered viscoelastic.

The rheology data for the IE C18:0 20% and the 30% samples at supercooling levels of 9, 6, and 3 $^\circ C$ are presented in Fig. 5-9. It has been shown by several authors
Figure 5-9: Rheology parameters, viscosity, $G'$, $G''$ and of sonicated and non-sonicated IE C18:0 20% and IE C18:0 30% at $\Delta T = 9, 6$ and $3 \, ^\circ\text{C}$. Mean values and standard errors of three experimental replicates are reported. Parameters at each supercooling represented with different alphabets are statistically different ($\alpha =0.05$) improves the hardness of the fat.
[13, 15, 18] that HIU induces the formation of smaller and more crystals in the system which improves the hardness of the fat. Based on the statistics indicated in Fig. 5-9, it can be seen that sonication significantly increased the viscosity, $G'$ and the $G''$ (Fig. 5-9a–c), and decreased $\delta$ values for the IE C18:0 20% samples at all the supercooling levels (Fig. 5-9d). For example, the viscosity of the IE C18:0 20% sample increased significantly from 296 ± 32 to 1606 ± 96 Pa.s and the $G'$ increased significantly from 5226 ± 429 to 43,893 ± 2533 Pa upon sonication at $\Delta T = 6$ °C. The $G''$ of the IE C18:0 20% samples increased significantly from 460 ± 23 to 3337 ± 380 Pa. This correlates well with the change in the microstructure of the samples upon sonication to smaller crystals which improved the rheological properties of the samples.

The viscosities, $G'$, and the $G''$ of the IE C16:0 20% samples from the previous study [18] were in general lower than those of the IE C18:0 20% samples at all the supercooling levels. This effect may be due to the higher SFC of the C18:0 20% samples compared to the C16:0 20% samples at all the supercooling levels [18]. Also, in contrast, sonication did not significantly affect any of the rheological properties of the IE C16:0 20% samples at any of the supercooling levels tested. This effect can be associated with the crystallization temperatures of the samples. The IE C16:0 20% samples were crystallized at 7, 10, and 13 °C [18] while the samples in this study were crystallized at 29, 32 and 35 °C at supercooling levels of 9, 6, and 3 °C. The lower crystallization temperatures create higher viscosities in the sample during sonication, which impedes the formation of cavities in the system. Because of this effect sonication was not very effective in the IE C16:0 20% samples.
The viscosity of the IE C18:0 30% sample increased significantly ($p < 0.05$) at all supercooling levels upon sonication similar to the previous study [18] with the IE C16:0 30% samples (Fig. 5-7e). For example, the viscosity of IE C18:0 30% at $\Delta T = 6$ °C was $1901 \pm 186$ which increased to $6756 \pm 595$ Pa.s upon sonication. Along with the final SFC, the viscosity of the IE C18:0 30% samples were also higher than the IE C18:0 20% and IE C16:0 30% [18] at all the supercooling levels. Both $G'$ and the $G''$ were higher for the IE C18:0 30% samples compared to the IE C18:0 20% and the IE C16:0 30% samples. This effect could be due to the differences in the TAG composition and the presence of higher melting TAGs that give the sample a harder texture or due to the differences in the microstructure. The SFC of the IE C18:0 30% samples were higher than that of the IE C18:0 20% and the IE C16:0 30% samples [18]. While the elastic modulus, $G'$ and the viscous modulus, $G''$ of the IE C18:0 30% samples did increase upon sonication Fig. 5-9 f, g), the increase in these parameters was not statistically significant ($p > 0.05$). On the other hand, in the previous study [18], the $G'$ and $G''$ of the IE C16:0 30% samples increased significantly at $\Delta T = 3$ °C upon sonication. Sonication was effective in inducing nucleation and formation of smaller crystals along with changing the melting characteristics of the sample. These changes did increase the viscosity of the sample, however it remains uncertain why the changes in the $G'$ and the $G''$ were not significant. The phase angle ($\delta$) was $0^\circ < \delta < 90^\circ$ indicating that the sample maintained its viscoelastic behavior (Fig. 5-9h).

**Conclusion**

This study shows that HIU affects the crystallization behavior and rheological properties of fats with low content of saturation by not only generating small crystals but
also by promoting the induction of crystallization of certain TAG fractions. Tristearin was the highest melting TAG in all the samples and the amount of SSS in the IE samples drove the crystallization behavior and influenced the rheological properties of the samples. Sonication promoted crystallization of low melting TAGs and the incorporation of SSS into the crystalline network.

The IE samples with stearic acid at the $sn$-2 position have superior crystallization properties including SFC and rheology than the IE with palmitic acid at the $sn$-2 position which were evaluated in an earlier study by the same authors. Although HIU was not as effective at inducing crystallization in the IE C16:0 20% samples due to the lower amount of saturated fats in the system, HIU induced crystallization in both the IE C18:0 20 and 30% samples. This could have been due to the higher melting point of the stearic containing samples compared to the palmitic ones. The induction of superior crystallization properties in these samples upon sonication can make them great candidates as ingredients for trans-fat free applications.

**Acknowledgements**

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REFERENCES


Abstract

The objective of this study was to fractionate a purified interesterified fat to eliminate tristearin (SSS) and to evaluate the crystallization behavior of the tristearin-free fat. The fractionated sample was crystallized with and without the application of high intensity ultrasound (HIU) by supercooling the sample at 2 °C. In the absence of SSS, the crystallization process was driven by low melting point triacylglycerols (TAGs) such as OSS and OOS (O: oleic and S: stearic acid). Although no differences were observed in the crystallinity in the sample based on the solid fat content (SFC) (p > 0.05), but there were no microstructural differences. In addition, an increase in the enthalpy of melting was observed upon sonication, also indicating higher crystallinity (p<0.05). Stronger intramolecular forces were formed in the sonicated samples as evidenced by increased viscoelastic parameters such as the elastic (G’) and storage modulus (G”) (p<0.05). G’ values increased from 138.25 ± 41.30 to 939.73 ± 277.45 Pa while the G” increased from 39.15 ± 8.98 to 149.77 ± 16.00 Pa (p < 0.05). Change in viscosity was not observed as a consequence of sonication (p>0.05). This study showed that HIU was effective in changing the crystallization behavior of SSS-free fats with low-melting TAGs.

Introduction

Crystallization in the presence of HIU waves or sonocrystallization has been widely studied as a processing technique to improve the texture of fats and induce hardness [1-3]. Acoustic cavitation is the main phenomenon responsible for inducing
nucleation [1] and secondary crystallization [2] in sonicated samples [4]. In combination with supercooling and agitation, HIU can reduce the induction period of crystallization [4], increase solid fat content [5], increase the hardness [1], viscosity and viscoelasticity [2, 3, 5] and can generate small crystals [1-3, 5] and stable polymorphs [6] in the system. In lieu of these HIU induced changes, sonication appears to be an efficient way to tailor low saturated, lower melting point fats for use as trans-fat alternatives.

In 2015, Ifeduba et al. [7] developed interesterified fats (IE) of high oleic sunflower oil and tristearin as trans-fat alternatives with low content of saturated fatty acids. Among the fatty acids present at the sn-2 position in this IE fat, approximately 30% was stearic acid. However, these fats were too soft for food use. Kadamne et al. [5] studied the crystallization behavior and properties of these fats under non-sonicated and sonicated conditions at different supercooling levels. The authors found that HIU was most effective in increasing the rate of crystallization at the lowest supercooling. HIU also induced smaller crystals in the system and significantly improved the viscosity of the sample, even though the driving force was the lowest at this supercooling level. In addition, Kadamne et al. [6] showed that HIU affected triacylglycerol (TAG) interactions as evidenced by changes observed in the melting behavior of the crystalline network formed. The IE samples studied by these authors had approximately 2.3% of tristearin (SSS) [5] which drove the crystallization of the sample by forming nuclei at initial stages of crystallization. It is unknown if the same effect of sonication will be observed in a sample without SSS. Therefore, the objective of the current study was to remove SSS from the IE sample using dry fractionation and to study the effect of sonication on the
crystallization behavior of the SSS-free IE sample. Functional properties including solid fat content, microstructure, melting behavior, and rheology were studied.

Materials and Methods

Starting material

Interesterified fat of high oleic soybean oil and tristearin containing 30% stearic acid at the sn-2 position was developed by Dr. Akoh at the University of Georgia [7] and described in detail by Ifeduba et al. [7] and Kadamne et al. [5].

Dry Fractionation

Approximately 100 g of the IE sample was kept in a glass bottle in a water bath maintained at 44 ºC for 2 weeks to induce the crystallization of SSS. This temperature was chosen since it was above the melting point of SOS (43 ºC) and below that of SSS (63 ºC). After 2 weeks, the sample was filtered and the liquid permeate (olein fraction) was collected and refrigerated. A warmed Bucher funnel and Erlenmeyer flask was used in the filtration experiment to prevent recrystallization of the fractioned sample. The liquid permeate was used for the experiments described in this study and from this point forward we will refer to it as fractionated IE sample (f-IE sample).

Melting point

The melting point (Tm) of the f-IE sample was measured by the AOCS official method Cc 1-25 [8].

Fatty acid composition and triacylglycerol composition
The total fatty acid, positional fatty acid (sn-2) and the triacylglycerol composition of the f-IE sample was measured according to Ifeduba et al [7, 9, 10].

For the measurement of fatty acid composition, fatty acid methyl esters (FAMES) were produced from the fat sample. The FAMES were analyzed by an Agilent Technology 6890 gas chromatograph with a Supelco SP-2560 100 mm capillary column with 0.25 mm internal diameter and 0.20 µm film thickness. Sample injection volume was 1 µL with 5:1 split ratio with Helium as the carrier gas. The heating program used was isothermal holding at 140 ºC for 5 min followed by ramp at 4 ºC/min to 240 ºC and isothermal holding at 240 ºC for 15 min. The volatiles were analyzed by the flame ionization detector [9].

The positions of the fatty acids were determined based on the bands formed on Silica Gel G plates. The method has been described in detail by Ifeduba et al. [9].

The TAG analysis was performed using a reverse phase-HPLC (Agilent 1260 Infinity HPLC system) with an evaporative light scattering detector (Sedex Model 85). An ultrasphere C18 column was used for the separation with Acetonitrile and acetone as the mobile phase for the separation. The solvent flow system and the temperature program for the separation is described by Ifeduba et al.[10]. The separated peaks were identified based on their comparisons with the retention times of standard TAG.

**Crystallization**

Approximately 30 g of the f-IE was melted and kept in the oven at 100 ºC for 45 min to remove the crystal memory of the sample. The sample was then transferred to a double wall glass cell kept at 27 ºC by an external water bath. The sample was stirred at 100 RPM and the crystallization in the sample was tracked by a He-Ne laser system
described by Kadamne et al. [3]. The laser output was maximum (10 V) when the sample was completely liquid but started to drop to 0 V as the crystallization progressed. When the laser output reached 0.6 V, the agitation was suspended and HIU was applied at 216 µm amplitude for 5 s. The sample was transferred from the glass cell to centrifuge tubes and five NMR tubes. The non-sonicated sample was transferred directly to the tubes as the laser signal reached 0.6 V and the agitation was stopped. The 0.6 V laser condition ensured a consistent amount of crystals in the system. The centrifuge tubes and the NMR tubes were kept in the water bath at crystallization temperature and the fat from these tubes were used for further analyses. The tubes were maintained in the water bath for 90 min. Each crystallization experiment (sonicated and non-sonicated) were performed in triplicates and analysis of physical properties were performed on the samples after each replicate resulting in triplicate measurements for each analytical test performed.

**Solid fat content (SFC)**

The NMR tubes were numbered from 1-5 and used in succession to measure the solid fat content every 2 min until 90 min of crystallization using NMS 120 minispec NMR Analyzer (Bruker, Germany). The NMR tubes were placed back in the water bath after each measurement, and each tube was used every 10 min. Data was collected under the same experimental condition from three separate runs and was presented as the mean SFC of the triplicate results with its standard error. The isothermal SFC data was plotted against time (min) using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) and the Avrami equation (below) was fitted to this data.

\[ s(t) = s_{max}(1 - e^{-kt^n}) \]  

(1)
Where \( s(t) \) is the SFC at any time \( t \), \( s_{\text{max}} \) is the maximum SFC, \( k \) is the Avrami rate constant, and \( n \) is the Avrami exponent.

**Microstructure**

The microstructure of the crystals formed during the crystallization process was observed on Instec TS62 Microscope thermal stage (Instec, Inc., Boulder, CO) which maintained the sample in the slide and cover slide at the crystallization temperature. The microstructure was observed at 10X magnification by a polarized light microscope (PLM) (Olympus BX 41 Tokyo, Japan). The pictures collected at 90 min of crystallization of the sonicated and non-sonicated samples were grouped together for comparison using Adobe Photoshop CS2 Version 9.0.

**Differential scanning calorimetry (DSC)**

A small amount of sample (10-15 mg) was taken from the centrifuge tubes after 90 min of crystallization and placed in Tzero pans, covered with Tzero hermetic lids, and closed hermetically. These pans were placed in the DSC oven and heated from the crystallization temperature (27 °C) to 80 °C by DSC Q20 (TA Instruments, New Castle, DE) at 5 °C/min. The thermograms were integrated by TA Instruments Universal analysis 2000 software (TA Instruments, New Castle, DE) to compute the onset melting temperature (\( T_{\text{on}} \)), peak melting temperature (\( T_p \)), and the change in enthalpy associated with the phase transition (\( \Delta H \)). Each of these measurements was presented as average of the three crystallization runs with their standard error.
Rheology

At 90 min of crystallization, samples from the centrifuge tubes were analyzed for their viscosity and viscoelastic properties using AR-G2 Rheometer (TA Instruments, New Castle, DE) using a 40 mm parallel plate geometry. The viscosity was measured by steady state flow at 27 ºC with shear rate changing from 0.01 to 300 s\(^{-1}\). The viscoelastic parameters elastic (G’) and viscous (G’’) moduli were measured by strain sweep oscillation at 1 Hz frequency with the percent strain changing from 8.0 \(\times\) 10\(^{-4}\) to 10\%. Each analysis was run after the crystallization experiment and the data was presented as a mean of triplicate runs with their standard error.

Statistics

The triplicate results between the sonicated and the non-sonicated samples were compared by a students’ t-test at \(\alpha = 0.05\). Statistical analysis was performed by GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Values presented in the tables and figures are average of the triplicates \(\pm\) standard error of the mean.

Results and Discussion

Melting point and crystallization

The melting point of the f-IE sample was 28.7 \(\pm\) 0.4 ºC, while that of the non-f-IE sample was 43.2 \(\pm\) 0.6 ºC [5]. The f-IE sample was obtained from an interesterified fat (IE C18:0 30%) previously studied by our research group [6]. In the previous study the IE C18 30% was crystallized at various supercooling levels \((\Delta T = 9, 6, \text{and } 3 \, ^\circ\text{C})\). Preliminary experiments showed that the f-IE sample crystallized very fast at \(\Delta T = 3 \, ^\circ\text{C}\);
hence a lower supercooling level (2 °C) was selected to allow for a slower crystallization rate needed to study the effect of sonication on physical properties of the sample.

**Fatty acid composition**

The total and the positional fatty acid (sn-2) composition of the f-IE sample is presented in Table 6-1. Kadamne et al. [5] reported the fatty acid composition of the non-f-IE sample. On comparison of the total fatty acid composition of the fractionated (Table 6-1) and non-fractionated sample [5], showed a decrease in the higher melting stearic fatty acid from 28.31 to 24.31 % and an increase in the lower melting oleic fatty acid from 60.70 to 65.83 %.

Comparison of the positional fatty acid composition, among the fatty acids present at the sn-2 position showed that stearic acid levels decreased from 33.17 to 26.39% while the oleic acid levels increased from 59.98 to 67.84%.

Comparison of the positional fatty acid composition, among the fatty acids present at the sn-2 position showed that stearic acid levels decreased from 33.17 to 26.39% while the oleic acid levels increased from 59.98 to 67.84%.

**Triacylglycerol composition**

The objective of fractionation was to remove all the SSS from the IE C18:0 30% sample. The IE C18:0 30% sample had 2.30% SSS [5] which was completely removed upon fractionation (Table 6-1). The OOO fraction increased from 39.94 to 42.19%, OOS fraction increased from 42.65 to 44.87% and the OSS fraction decreased from 14.26 to 11.87% upon fractionation (Table 6-1).
Table 6-1: Fatty acid and Triacylglycerol composition of the fractionated IE C18:0 30% sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1n9</th>
<th>C18:2n6</th>
<th>C20:1</th>
<th>C21:0</th>
<th>C22:1n9</th>
<th>C24:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>f-IE</td>
<td>5.57 ± 0.12</td>
<td>24.31 ± 0.60</td>
<td>65.83 ± 1.54</td>
<td>3.23 ± 0.24</td>
<td>0.11 ± 0.16</td>
<td>0.11 ± 0.15</td>
<td>0.46 ± 0.65</td>
<td>0.27 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1n9</th>
<th>C18:2n6</th>
</tr>
</thead>
<tbody>
<tr>
<td>f-IE</td>
<td>2.49 ± 0.10</td>
<td>26.39 ± 0.67</td>
<td>67.84 ± 0.98</td>
<td>3.29 ± 0.22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>LOO + LPO</th>
<th>OOO</th>
<th>OOS</th>
<th>OPS</th>
<th>OSS</th>
<th>SSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>f-IE</td>
<td>0.79 ± 0.12</td>
<td>42.19 ± 0.29</td>
<td>44.87 ± 0.27</td>
<td>ND</td>
<td>11.87 ± 0.33</td>
<td>ND</td>
</tr>
</tbody>
</table>

Solid fat content (SFC)

The isothermal SFC curves of the non-sonicated and sonicated samples are presented in Figure 6-1. The Avrami equation was fitted to the data. HIU was applied at 26 min after the start of crystallization indicated by an arrow on the graph. The Avrami parameters derived from these curves are shown in Table 6-2. The Avrami rate constant $k$ was $3.4 \times 10^{-6} \text{min}^{-n}$ and $2.4 \times 10^{-6} \text{min}^{-n}$ for the non-sonicated and sonicated sample, respectively ($p > 0.05$). In addition, no significant
Figure 6-1: Solid fat content (SFC) of fractionated IE C18:0 30% at 27 °C, without and with sonication. The arrow represents the time point of application of HIU.

Table 6-2: Avrami parameters obtained from the Avrami fit of the isothermal solid fat content data of the fractionated IE C18:0 30% at 27 °C. Means with different superscript alphabets are statistically different (α = 0.05).

<table>
<thead>
<tr>
<th>Avrami Parameters</th>
<th>no HIU</th>
<th>with HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>k ( (x 10^{-6}) (\text{min}^{-n}) )</td>
<td>3.4 ± 2.6(^a)</td>
<td>2.4 ± 1.5(^a)</td>
</tr>
<tr>
<td>n</td>
<td>3.0 ± 0.2(^a)</td>
<td>3.0 ± 0.2(^a)</td>
</tr>
<tr>
<td>S(_{max}) (%)</td>
<td>3.7 ± 0.3(^a)</td>
<td>4.1 ± 0.3(^a)</td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.95</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^a\) S\(_{max}\): maximum solid fat content, K: Avrami rate constant, n: Avrami exponent
differences were observed among the $S_{\text{max}}$ values of the non-sonicated and sonicated sample- 3.7% and 4.1%, respectively ($p > 0.05$).

As reported by Kadamne et al. [6] the $S_{\text{max}}$ was 5.19 and 5.58% for the non-sonicated and sonicated non-fractionated IE C18:0 30% sample at $\Delta T = 3$ ºC. This higher SFC could be due to the higher crystallinity in the IE C18:0 30% based on its SSS content or due to the slightly higher supercooling in the sample. There was a wider gap in the isothermal SFC curves of the non-sonicated and sonicated IE C18:0 30% compared to the f-IE sample. This may be due to HIU being more effective at promoting crystallization in IE C18:0 30% than f-IE. As described by Kadamne et al. [6], this promotion in the crystallization could be due to the presence of SSS. That is, HIU induced the nucleation of SSS which ultimately result in a higher SFC. The induction time of crystallization based on the SFC curve as determined by the Gompertz equation for the IE C18:0 30% sample was 29.30 and 21.39 min. Based on the SFC curves shown in Figure 6-1, an estimate of this induction time of crystallization can be made and was close to 18 min. This indicates that although there was no SSS in the f-IE sample and that it was crystallized at a lower supercooling, f-IE crystallized sooner than the IE C18:0 30% sample. The major crystallizing TAGs in the fractionated sample are OOS and OSS.

It is our hypothesis that the simple TAG composition and the similarity of these TAG species, might lead to a faster and better molecular orientation in the absence of SSS and results in a faster nucleation and growth. Even though the nucleation and the growth was slightly faster in the fractionated sample, the final SFC was not higher since additional supercooling and high melting point TAG, such as SSS, was needed to achieve a higher final SFC. The Avrami exponent was 3 for both processing conditions indicating that
they either had the same crystal type and nucleation pattern (instantaneous spherulites or sporadic disc like) or they had either one of these patterns [11].

**Microstructure**

The microstructure of the sonicated and non-sonicated f-IE sample after 90 min of crystallization is shown in Figure 6-2. Upon visual comparison, the microstructure of the samples crystallized under both processing conditions had spherical crystals. The crystals in the sonicated samples seemed slightly smaller than the ones in the non-sonicated samples but the differences were not as pronounced as those seen for the interesterified sample reported by Kadamne et al. [5]. This may be due to the lower supercooling used in this study along with the absence of the high melting SSS in the sample. These two conditions in the non-fractionated sample may have formed a larger number of nuclei in the sample which grew over the 90 min duration. Hence, more crystals were seen in IE C18:0 30% at ΔT = 3 ºC [5] than in the f-IE at ΔT = 2 ºC. Upon comparison of the microstructure of the sonicated samples of the IE C18:0 30% at ΔT= 3 ºC [5] to the f-IE at ΔT = 2 ºC, it can be inferred that crystallization in the non-fractionated sample was driven by the nucleation of SSS (many nuclei resulting in many small crystals) while the crystallization of the fractionated samples have fewer nuclei and crystal growth was promoted (larger spherulitic-like crystals). The use of HIU in the non-fractionated sample generate smaller crystals by breaking down existing crystals in the system [2]. In the case of the f-IE sample, crystals would be mainly composed of OSS and OOS. These 2 TAGs have a lower melting point compared to SSS present in the non-fractionated IE C 18:0 30%. These low melting point TAGs could have partially melted upon sonication due to the slight increase in temperature observed during sonication. Based on the Avrami
exponent of 3, this sample would have instantaneous spherulitic nucleation which correlates with the spherulites seen in the microstructure of the sample. Similarly, spherulites were observed in the microstructure of IE C18:0 30% samples [5].

**Differential scanning calorimetry (DSC)**

The melting thermograms of the non-sonicated and sonicated f-IE sample are shown in Figure 6-3 and the parameters integrated from these thermograms are shown in Table 6-3. For both the samples, the peak onset temperature ($T_{on}$) fell outside the temperature interval of the DSC run and was not integrated by the software; therefore, these values were not reported in Table 6-3. The thermograms of both samples were almost identical in shape (Figure 6-3). The peak melting temperature of the non-sonicated sample was 38.3 ºC while that of the sonicated sample was 36.5 ºC and these values were not statistically different ($p > 0.05$). The peak melting temperature of IE C18:0 30% sample at $\Delta T = 3$ ºC was between 48-51 ºC for the sonicated and non-sonicated samples.
**Figure 6-3:** DSC melting profiles of fractionated IE C18:0 30% without and with sonication

**Table 6-3:** DSC melting parameters including peak melting temperature, $T_p$ (°C) and enthalpy (ΔH) for the fractionated IE C18:0 30%. Parameters with different superscript alphabets are statistically different ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>$T_p$ (°C)</th>
<th>Enthalpy (ΔH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no HIU</td>
<td>with HIU</td>
</tr>
<tr>
<td>38.3 ± 0.2$^a$</td>
<td>36.5 ± 0.4$^a$</td>
</tr>
<tr>
<td>4.9 ± 0.2$^b$</td>
<td>7.5 ± 0.1$^a$</td>
</tr>
</tbody>
</table>
at all the supercoolings due to the SSS in the sample [5]. Upon fractionation, the removal of SSS decreased the peak melting temperature. The major TAGs in the f-IE sample were OOO (melting point 4.5-5.7 °C), OOS (melting point = 25 °C) and OSS (melting point = 45 °C). At the crystallization temperature of 27 °C, OSS must have crystallized along with some OOS which decreased the $T_p$ of the crystallized sample to lower than the melting point of OSS alone. For the sonicated sample, HIU must have induced more OOS along with OSS to crystallize which slightly decreased the $T_p$ and this increased crystallinity contributed to the increased enthalpy of the sample. The enthalpy of the sonicated sample was higher than the non-sonicated sample ($p = 0.004$) indicating higher crystallinity in the sonicated f-IE sample. At $\Delta T = 3 \, ^\circ C$, enthalpy values of the IE C18:0 30% samples were 9.4 and 12.9 J/g for the non-sonicated and sonicated samples, respectively [5]. The f-IE melting enthalpy values were 4.9 and 7.5 J/g for the non-sonicated and sonicated sample, respectively. The presence of SSS along with generation of more nuclei due to a slightly higher supercooling of 3 °C in IE C18:0 30% vs. 2 °C in f-IE may have contributed to a higher enthalpy of IE C18:0 30%. A denser packing of the crystalline material in the crystals of the IE C18:0 30% sample may also have contributed to a higher enthalpy.

Although the type and amount of fatty acids are important factors that drive the melting behavior of fats, their position on the glycerol backbone is crucial at governing their melting properties. Even though changes in FA and TAGs were not extreme when comparing the fractionated with the non-fractionated samples, the removal of SSS and the corresponding change in the TAG composition had a significant impact in the melting point of the sample. Changes in FA and TAGs were sufficient to create a significant
difference in the samples’ melting point. The melting point of the f-IE sample was more than 14 °C lower than the melting point of the non-fractionated IE C18:0 30% sample.

**Rheology**

Kadamne et al. [5] showed that the viscosity of the IE C18:0 30% sample increased significantly upon sonication at 9, 6, and 3 °C supercoolings since there were large differences in the microstructure. The viscosities increased from 1526 ± 880 to 6818 ± 901, 1901 ± 186 to 6756 ± 594 and 2065 ± 498 to 4947 ± 613 Pa.s at supercoolings of 9, 6, and 3 °C, respectively. Several studies [1, 2, 12, 13] have also

![Graphs showing viscosity, G', G'', and delta for the fractionated IE C18:0 30% at 27 °C without and with sonication. Data represented with different alphabets are statistically different (α = 0.05)](image)

**Figure 6-4**: Rheological parameters, viscosity, G’, G” and delta for the fractionated IE C18:0 30% at 27 °C without and with sonication. Data represented with different alphabets are statistically different (α = 0.05)
shown that small crystals formed by sonication significantly improved the viscosity of the sample. However, in our study there were no differences in the viscosity of the non-sonicated and sonicated f-IE sample (Figure 6-4). The viscosity of the non-sonicated sample was $9.81 \pm 0.87$ Pa.s while that of the sonicated sample was $14.73 \pm 2.06$ Pa.s. However, the viscoelastic properties, $G'$ and $G''$ significantly improved upon sonication. The $G'$ of the non-sonicated and sonicated samples were $138.25 \pm 41.30$ Pa and $939.73 \pm 277.45$ Pa, respectively while the $G''$ values were $39.15 \pm 8.98$ and $149.77 \pm 16.00$ Pa, respectively. The higher enthalpy of the sonicated sample indicates higher crystallinity. This increase in enthalpy suggests increased intermolecular interactions in the sonicated samples and hence higher $G'$ and the $G''$ values. The phase angle $\delta$, was $90^{\circ} < \delta < 0^{\circ}$ which means that the sample maintained its viscoelasticity upon fractionation.

**Conclusion**

This study shows that HIU was effective in changing the crystallization behavior of a tristearin-free sample with lower saturation levels (<30%) and containing low melting TAGs. However, these effects were not as significant as the ones observed in the presence of tristearin. Sonication did not affect the crystallization kinetics of the f-IE. However, a significant increase in the melting enthalpy and elastic modulus was observed suggesting that HIU promoted the formation of stronger intermolecular forces. Overall, this study shows that HIU has the potential to increase the rheological properties of fats with low levels of saturation and with no trans-fats.
Acknowledgements

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REFERENCES


CHAPTER 7
EFFECT OF FATTY ACID AND TRIACYLGLYCEROL COMPOSITION AND PHYSICAL STRUCTURE ON FLAVOR INTENSITY AND RELEASE OF COMPOUNDS WITH DIFFERENT LIPOPHILICITY

Abstract

Headspace volatile analysis by gas chromatography-mass spectroscopy solid phase micro extraction (GC-MS SPME) and descriptive sensory analysis were performed to study the release and flavor intensity of 2-butanone and 2-nonanone in bulk fat. Fats used in the study were physical (PB) and interesterified blends (IE) of high oleic sunflower oil (HOSO) and tripalmitin and HOSO and tristearin. Each of the PB and IE had either 20 or 30% saturated fats. The flavor intensity was studied in all the IE and PB liquid samples to understand the effect of fatty acid and triacylglycerol composition and physical state on flavor perception. The IE samples were also crystallized with and without sonication to understand the effect of physical state and crystalline network on flavor release. The amount of volatiles released, in general, increased with the decrease in lipophilicity of the flavor compounds. Based on the sensory data, the flavor intensity was higher for the crystallized samples than the liquid samples due to the higher solid fat content and poor penetration of the flavor into the bulk crystals.

Introduction

Lipid shortenings are widely used in the food products such as cakes, icing, puff pastries, bread, whipped cream, and frying to name a few (1). Shortenings are composed of solid fat and liquid oil at different proportions depending on the final application (1).
Most commonly, soybean oil, palm oil fractions, cottonseed oil, and canola oil along with their hydrogenated derivatives and emulsifiers are used in shortenings (1, 2). With the food industry considering zero trans- and low saturated shortening alternatives, several different fat types can be developed using blending, fractionation, or interesterification. The presence of fat also affects the perception of other taste and according to Suzuki et al. (3), presence of fats increased the saltiness perception in emulsions compared to aqueous solutions.

Recently Ifeduba et al. (4) developed two types of interesterified fats with different levels of either palmitic or stearic acid at the sn-2 position as low saturated healthier alternatives for trans fats. These fats differ from each other in terms of their fatty acid (FA) and triacylglycerol (TAG) composition. These IE fats are softer that renders them unusable as trans fat alternatives. In a previous study, Kadamne et al. (5, 6) (Chapters 4 and 5) formed smaller and more crystals and increased the SFC and viscosity of these IE fats using high intensity ultrasound (HIU) thus imparting structural features that can allow them to serve as trans fat alternatives.

Relkin et al. (7) studied the flavor release at different temperatures from fats such as hydrogenated palm kernel oil and anhydrous milk fat in emulsion systems which differ in their FA and TAG composition. They found that the amount of volatiles from these two fat systems were not identical. The authors explained higher volatile release based on the four parameters: 1. The higher the amount of liquid fat at the given temperature 2. bigger droplet size 3. Lower levels of soluble proteins which tend to bind esters and 4. the type of volatile compound. Roberts et al. (8) showed that with the increase in the lipophilicity of the flavor, there is a decrease in the amount of volatiles released from the
sample. They also showed that increased fat levels in emulsions decreased the volatiles released and the lipophilicity of the added flavor influenced how much fat was needed to limit the released volatiles (8). Frank et al. (9) also observed that with the increase in fat content in an emulsion, the amount of volatiles released decreased. They used compounds with different levels of hydrophobicity (k_{o/w}) and similar trend was observed for all the compounds. Roberts et al. (8) discussed that solids in the lipid matrix entraps the volatiles and hence the release of volatiles was lower compared to liquid samples. However, all these studies were performed by instrumental techniques, mostly gas chromatography and using emulsions. The current study is aimed at understanding the perception of flavor of compounds with extreme lipophilicity which were identified by Frank et al. (9) – 2-butanone and 2-nonanone in bulk fats with different FA and TAG composition by descriptive sensory analysis. The volatiles from the headspace of these samples were also quantified by GC-MS SPME analysis.

Narine and Marangoni (10) evaluated how various crystalline networks affect the macroscopic properties of the material such as rheology. The mechanical strength of the crystalline network are also known to affect the sensory attribute such as mouthfeel and texture (11). Research conducted by Martini’s lab (5, 6, 12, 13) has shown that sonication (application of HIU) generates a crystalline network different from the one obtained in non-sonicated samples affecting the mechanical properties of the fat. Hence, another objective of this study was to understand the release of flavor from non-sonicated and sonicated crystalline lipids by descriptive sensory analysis.

From this study, we will gain an insight into how flavor perception from bulk fats is affected by FA and TAG composition along with crystalline network. This study may
put forward approaches for direct replacement of fats and optimization for flavor release when replacing with fats with different TAG or FA composition or with a different crystalline network.

**Materials and Methods**

**Materials**

The physical (PB) and interesterified (IE) blends of (i) high oleic sunflower oil and tripalmitin and (ii) high oleic sunflower oil and tristearin were provided by Dr. Akoh’s laboratory from University of Georgia. Two types of samples were used in this study. First IE samples containing either 20 or 30% palmitic acid at the sn-2 position and their corresponding PB samples were studied. These were labelled IE C16:0 20%, IE C16:0 30%, PB C16:0 20% and PB C16:0 30% samples. The second type were the IE samples containing either 20 or 30% stearic acid at the sn-2 position and their corresponding PB samples. These were labelled IE C18:0 20%, IE C18:0 30%, PB C18:0 20% and PB C18:0 30% samples. All these eight fats were evaluated as liquids and all the IE fats were also analyzed as crystallized fats with and without the application of HIU. Thus, depending on the physical state and the crystallization conditions, a total of 16 fats (in duplicates) were analyzed in this study. The physical and chemical properties of the IE and PB samples were described in detail by Kadamne et al. (5, 6) (Chapters 4 and 5). The sensory training was performed with soybean oil (Pure Wesson Vegetable Oil, Conagra Brands, Chicago, IL, USA) which was purchased from local market. Food grade 2-butanone, 2-nonanone, ethyl butyrate, and butyric acid were purchased from Sigma-Aldrich, St. Louis, Mo, USA.
Solid Phase micro-extraction

(i) Sample preparation

Liquid samples: All the samples were melted in a microwave oven and 1500 µL of oil was pipetted in the GC-MS glass vial. The flavor standards used in this study were 2-butanone, 2-nonanone, butyric acid and ethyl butyrate. The standard (1 µL) was pipetted into the oil while making sure that the oil was liquid while the standard was being added. The standards were analyzed individually in all the oils used in this study and were not mixed. The sample vials were capped immediately after the standard was added and the samples were gently swirled.

Sonicated and non-sonicated crystallized samples: The IE C16:0 30% and the IE C18:0 20 and 30% samples were crystallized with and without the application of HIU. The IE samples (30 g) were melted and kept at 100 ºC for 30 min and then transferred to a double glass wall cell maintained at crystallization temperature. The sample was stirred at 100 rpm and the crystallization was monitored by a He-Ne laser as described by Kadamne et al. (5) (Chapter 4). As the laser signal reached 0.6 V, non-sonicated samples were transferred to centrifuge tubes which were kept at crystallization temperature. Crystallization temperature for IE C16:0 20% and 30% samples were 13 and 25 ºC while that of IE C18:0 20% and 30% samples were 35 and 40 ºC. For the sonicated samples, when the laser signal reached 0.6 V, the agitation was stopped and high intensity ultrasound was applied at an amplitude of 216 µm for 5s and then transferred to the centrifuge tubes in the water bath. To avoid pipetting errors at very low volumes, 1500 µL of liquid IE was weighed and was 1.248 g and the weight of the samples was used to prepare the sample for the SPME analysis. After the samples were weighed carefully into
the glass vials, 1 µL of standard was added to the sample and gently stirred with a spatula and capped.

(ii) Instrumentation

Liquid samples: Thirty sample vials were loaded on the auto sampler tray which were maintained at room temperature for overnight analysis. Each sample was loaded into the incubator by the Gerstel automated sampler (MPS, Linthicum, MD) at 70 °C and agitated at 250 rpm. The samples crystallized while in the auto sampler tray and were melted and mixed during the incubation stage. After 10 min of incubation, the SPME fiber was injected into the vial up to 43 mm for extraction of the volatiles from the headspace for 20 min and the fiber was then injected into the GC injector port. Each sample was analyzed in duplicate.

Sonicated samples: IE C18:0 20 and 30%: With the other conditions like the liquid samples, the incubation temperature was kept at the crystallization temperature which was 35 and 40 °C for the IE C18:0 20 and 30% samples, respectively. Each sample was analyzed in duplicate.

IE C16:0 30%: Since the incubator temperature range was 30 - 200 °C and was above the crystallization temperature of the IE C16:0 30% sample (25 °C), the IE C16:0 30% sample was not incubated in the incubator. The SPME fiber was injected into the headspace of the sample vial at room temperature. Each sample was analyzed in duplicates.

IE C16:0 20%: The crystallization temperature for IE C16:0 20% sample was 16 °C which was lower than the room temperature. Therefore, IE C16:0 20% sample melted during shipment and hence was not analyzed for the volatiles from sonicated samples.
A Supelco SPME 85 μm Carboxen/PDMS Stableflex Fiber Assembly (Sigma Aldrich, St. Louis, MO, U.S.A) was used as the SPME fiber for absorption of the volatiles from the headspace of the sample vials. After the extraction of the volatiles the SPME fiber was injected into the GC injector port and the volatiles were desorbed from the fiber at 250 °C for 5 min.

(iii) Gas chromatography - Mass spectroscopy (GC-MS)

The volatiles from the fat samples were separated using a Gas chromatograph (6890A, Agilent Technologies, Santa Clara, CA, U.S.A.) and identified using a mass spectrophotometer (5975B VLMSD, Agilent Technologies). A VF-5ms column with a length of 30 m and 0.25 mm I.D. and 1 μm film was used in the GC for the separation. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The GC oven was programmed for 70 °C for 2 min, then ramp of 20 °C/min to 230 °C. The MS was run at an electron mode of 70 and the mass range was 40-550.

The volatiles were identified using the NIST mass spectral search program. The three target ions were selected for each standard using the library and these ions were further used to identify and quantify the amount of standard in the samples.

Sensory Evaluation

A descriptive panel was used to evaluate the flavor intensity of 2-butanone and 2-nonanone. A Qualtrics survey was advertised along with the details of the research to recruit individuals from the local community who were 18 years or older, not pregnant, and did not have any food allergies. The panelists were chosen on first come basis. Seven panelists were chosen for the 2-butanone study while 8 panelists were chosen for the 2-nonanone study. The gender ratio in either studies was not symmetrical and since this
was a descriptive panel that was trained to identify the flavors, the non-symmetrical
gender ratio should not affect the results. Each participant was met with individually and
the research details were explained to them. This study was approved by the IRB at Utah
State University (IRB protocol number 5995). Consent forms were signed by the
panelists prior to the actual sensory training. Panelists selected for the 2-butanone test did
not have any experience in tasting lipids. Some of the panelists from the 2-butanone
sensory were selected for the 2-nonanone sensory and therefore had some experience in
descriptive sensory testing. Each sensory training session was between 45 min to 60 min
long. The training was performed in a round table format. Panelists were given 1 mL of
sample in micro-centrifuge tubes and the panelists were asked to pour the sample on the
spoon and taste them. The training concentrations for 2-butanone in soybean oil were low
(1.33 µL/mL), medium (1.75 µL/mL) and high (2.5 µL/mL). These concentrations were
rated as 4, 7, and 11 on the line scale respectively. The training concentrations for 2-
nonanone in soybean oil were low (0.14 µL/mL), medium (0.25 µL/mL) and high (0.40
µL/mL). These were rated as 2, 7, and 10 on the line scale in the increasing order
respectively. A line scale was used to quantify the intensity of the flavor of the samples.
The panelists were exposed to the flavor of soybean oil as being clean and beany and
gradually introduced to increasing concentrations of 2-butanone in soybean oil. They
identified the overall flavor of 2-butanone in soybean oil as being “walnut -like” and that
of 2-nonanone as “soapy”. The panelists were given known samples and asked to mark
their response for each sample. Eight panelists including 4 females and 4 males were
hired in this study. One female participant dropped out of the study who was immediately
replaced by another panelists. A second female participant who also dropped out from the
study was replaced and trained but the replacement participant dropped out too.
Eventually, there were 7 panelists with 3 females and 4 males. The group average of the length of the response on the scale was calculated. In the next session the panelists were told about the class averages and the known tasting was performed again. This was followed by blind and known tastings on alternate days until the individual responses were closer to the group average. The panelists were made aware of the class average before all the tasting sessions to calibrate them to the concentrations. The panelists were asked to rinse their palate after tasting a sample using warm water and unsalted crackers. The training was performed for up to 5 weeks. Panelists were considered trained on an attribute based on their ability to evaluate identical samples as the same over multiple repetitions, as well as rate the samples similar to the entire panel. The fat samples (IE and PB) were presented to the panelists at the medium training concentration (1.75 µL/mL) during the training.

The training and tasting for 2-butanol was performed first followed by those for 2-nonenone in separate experiments. During the training, the panelists marked their responses on the 15-cm line scale on paper while the tasting of the samples was performed in sensory booths on computers. After training for each individual flavor compound, samples were presented to the panelists in individual booths in a randomized fashion. For each flavor compound, samples were presented in groups of 4 in a randomized and balanced manner using 3-digit random codes. The panelists were given a 15-min break between the replicate runs. The panelists were given the IE and PB samples “non-spiked” and “spiked” and the panelists were aware of these two conditions. The panelists were reminded to mark the perceived intensity of the added flavor (2-butanol
or 2-nonanone) in the sample and not the overall flavor of the sample. After tasting of each sample, they were asked to clean their palate with warm water and a bite of cracker and then rinse again in that order. Each sample was evaluated in duplicate and results were collected using computer software (SIMS 2000).

Triangle test for recruitment of second 2-butanolone descriptive analysis

The performance of a few participants on the earlier 2-butanolone test was not satisfactory which affected the overall results of the study. Hence, the 2-butanolone sensory analysis was repeated with more participants. For this sensory study, participants were selected based on a selection criteria of their performance on a triangle test. Twenty-nine panelists were recruited from the local community using a Qualtrics questionnaire and participated in the screening test for the second sensory test with 2-butanolone. Five sets of samples were presented to the panelists. Each set contained 3 samples (2 same and 1 different). The panelists were asked to taste all three samples and identify the different sample and mark their responses on the SIMS software. Samples used for the triangle test were soybean oil and soybean oil spiked with the lowest concentration of 2-butanolone used in the previous sensory test which was 1.33 µL/mL in soybean oil. Participants were given 1 mL of sample in microcentrifuge tubes. Spoons were provided and the panelists were asked to pour the sample onto the spoon and taste it. The panelists were told to spit the sample in a spit cup and rinse with warm water after tasting it. An unsalted cracker was provided to clean the palate after tasting. Some of the panelists had participated in the previous sensory test and hence had some sensory experience. All the panelists signed the consent forms prior to the start of the research.
Sensory Evaluation of second 2-butanone descriptive analysis

The training was performed in similarly to the prior sensory test. The panelists were trained on 4 concentrations of 2-butanone in soybean oil which were very low (0.8 µL/mL), low (1.33 µL/mL), medium (1.77 µL/mL) and high concentration (2.50 µL/mL). The panelists marked their responses on a 16-point category scale with each category representing scale from 0 to 15. A rating of zero corresponded to no flavor in the sample while rating of 15 corresponded to the highest flavor intensity. This scale allowed to mark the intensity of the 2-butanone in increments of single numbers, which was easier to train the panelists compared to the line scale used in the previous training. The samples were rated as 1, 3, 7 and 10 in the increasing order of concentration, respectively. The panelists were trained for 3 weeks and the tasting of the samples were conducted in the fourth week. The panelists were considered trained for identification and quantification of 2-butanone based on their accuracy and precision in determining the intensity of blind samples over multiple tests and overall performance of the group together. Each IE and PB sample was presented to the panelists in duplicates in a randomized fashion. During sample tasting, the participants were given 4 samples and the panelists recorded their perceived intensity of 2-butanone in the samples on the SIMS2000 software. After a break of 15 min, the duplicates of the samples were presented in randomized fashion. The crystallized samples were given directly on a spoon for tasting.

Statistical Analysis

The SPME data for the crystallized samples and the sensory of second 2-butanone descriptive analysis data was analyzed by a two-way ANOVA followed by Tukeys’ multiple comparison test as a post hoc test (α = 0.05). All the other data presented here is
an average of duplicate analysis and was analyzed by a three-way ANOVA followed by Tukeys’ multiple comparison test as a post hoc test (α = 0.05). There were three main effects of sonication (without and with HIU), amount of saturated fatty acids at the sn-2 position (20% vs. 30%) and the type of fatty acid at the sn-2 position (palmitic acid vs. stearic acid). The presence or the absence of the spiking with the flavor compound was also treated as the main effect when appropriate. There were no random effects in the model. Statistical analyses were performed by Graphpad Prism software.

Results and Discussion

Solid phase micro-extraction

The octanol/water partition coefficient (k_{o/w}) for 2-butanone, 2-nonanone, ethyl butyrate is 1, 1380 and 80, respectively [11] and for butyric acid is 6.16 (14). The k_{o/w} value is the proportion of the concentration of any given compound in octanol and water at the same temperature, at equilibrium. The lower the k_{o/w} value the greater is the partition in water and hence the compound can be considered as less lipophilic. Among the compounds listed above the decreasing order of lipophilicity would be 2-nonanone, ethyl butyrate, butyric acid and 2-butanone. The compounds mentioned above were added to the PB and IE fats mentioned in the materials and the volatiles released in terms of peak area was measured by SPME GC MS. The comparison of the amount of volatiles of these four compounds released from all the fat samples (liquid) are presented in Fig. 7-1. Higher peak area values indicate higher amount of volatiles released. Due to the lowest k_{o/w} values, and hence least lipophilicity, peak area was highest for the 2-butanone and butyric acid and hence the amount of volatiles released were highest based on the numerical comparisons of the data presented in Fig. 7-1. Similar trend was also observed
by Frank et al. for 2-butanol volatiles (9). For the PB C16:0 20% sample, the peak area for 2-butanol and butyric acid was $2.65 \times 10^7 \pm 2.73 \times 10^6$ and $2.71 \times 10^7 \pm 1.78 \times 10^7$, respectively. The $k_{o/w}$ values for 2-nonenone and ethyl butyrate were comparatively higher than 2-butanol and butyric acid and hence lipophilicity of 2-nonenone and ethyl butyrate was higher, based on the data presented in Fig. 7-1. The peak area of PB C16:0 20% sample for 2-nonenone and ethyl butyrate was $2.59 \times 10^6 \pm 1.77 \times 10^5$ and $3.04 \times 10^6 \pm 4.58 \times 10^5$ respectively.

For the 2-butanol, the 3-way ANOVA comparisons showed a significant one way interaction ($p<0.05$) between the type of fat (PB vs. IE) and the amount of saturated fat at the sn-2 position (20% vs. 30%). There was also a significant interaction between the said two fixed effects ($p<0.0001$). However, the data showed that the three way interaction between the type of fat x type of saturated fatty acid x amount of saturated fatty acids at the sn-2 position was not significant for the 2-butanol volatiles data ($P>0.05$). Among the different fat samples, the peak area was significantly lower for the IE samples with 20% of the fatty acids at the sn-2 positions being palmitic or stearic ($p = 0.0001$). This means that the 2-butanol was retained in the IE samples with 20% of the fatty acids at the sn-2 position and fewer volatiles were released by these samples. This effect was more evident for the 2-butanol samples. The peak area for the IE C16:0 20% and IE C18:0 20% sample was $1.20 \times 10^7 \pm 3.66 \times 10^5$ and $1.39 \times 10^7 \pm 6.72 \times 10^5$. Also, in general, the IE samples released fewer volatiles than the PB samples for the 20% samples ($p<0.05$) and for the 30% the amount of volatiles measured by the peak area were similar to those of the corresponding PB samples ($p > 0.05$).
There were no differences in the peak area values of butyric acid among all the samples (p>0.05). Also, none of the interactions were significant for volatiles of butyric acid (p>0.05).

For the ethyl butyrate samples, the physical blends containing stearic acid had the highest peak area (<0.0001). This indicates that greater amount of volatiles were released by these samples while the volatiles were retained by the other fat samples. The peak area for the PB C18:0 20% and PB C18:0 30% sample was 1.90 x 10^7 ± 5.14 x10^5 and 1.68 x10^7 ± 2.70 x10^5. The one-way interaction between the type of fat and the type of fatty acid at the sn-2 position were significant along with the two way interaction between these two factors (p<0.05). However, there was no 3-way interaction between the type of fat x type of fatty acid x amount of saturated fatty acid at the sn-2 position.

For 2-nonanone, except for the one way interaction between the type of fat (PB vs. IE) (p>0.05), all the other one way, two and three way interactions were significant for the 2-nonanone volatiles data (p<0.05). The amount of volatiles as indicated by the peak area was lower for the IE C16:0 20% sample (1.27 x 10^6 ± 3.03 x 10^5) (p<0.05), like 2-butanone, but similar trend was not observed for the IE C18:0 20% sample. There were no differences among the other samples (p>0.05). The flavor compounds are well absorbed into the liquid fat. When these spiked samples crystallize, they trap the flavor compounds into the crystalline network and may thus change the release of the volatiles. However, since all the samples in this study were analyzed at 70 ºC, all the samples were in liquid state during measurement. Differences were observed among the peak area of different liquid samples but no specific trend was observed among the different
Figure 7-1: Peak area for the release of 2-butanone (A), butyric acid (B), ethyl butyrate (C) and 2-nonanone (D), from liquid samples of the PB C16:0 20% and IE C16:0 20%, PB C16:0 30% and IE C16:0 30%, PB C18:0 20% and IE C18:0 20% and PB C18:0 30% and IE C18:0 30% as quantified by SPME. The data presented is the mean value of standard error of two replicates. Columns with the different alphabets within the same graph are significantly different (α = 0.05).
compounds. The differences may be due to the specific effect of the flavor compound and the fat types used which however did not seem to translate across different flavors.

The peak area for the released volatiles from the crystallized IE fats is shown in Fig. 7-2. For the 2-butanone samples, the peak area for the sonicated IE C18:0 30% sample was lower than all the other samples (p < 0.05). In general, the peak area of the IE C16:0 30% crystallized samples was higher than that of the liquid samples (p < 0.05), while for the sonicated IE C18:0 30% sample, it was lower (p < 0.05). The higher peak area indicated that there was less retention of the 2-butanone by the crystallized fat. The 2-butanone was added to the fat after crystallization and gently stirred to avoid melting of the crystals. According to Relkin et al. (7), when the sample has more liquid fat, it holds the flavor compound better than the solid fat as the liquid fat acts as a solvent for the flavor compound. In case of crystallized samples, the only liquid fat that was present was that entrapped between the crystals. Since, the amount of liquid fat was lower, the solvent for the flavor was lower and hence it was released more than in case of liquid samples. Therefore, the amount of volatiles in the headspace was higher. Also, in general, the volatiles released from the IE C18:0 samples were higher than those of the IE C16:0 samples. This might indicate that there would be a lower retention of flavors by the crystallized IE C18:0 samples.

For the butyric acid samples, contrary to the 2-butanone data, the peak area was highest for the sonicated IE C18:0 30%. The peak area for the liquid IE C16:0 30% sample was significantly lower than the crystallized samples (p < 0.05). In case of the IE C18:0 20% sample, the peak area of the liquid and the crystallized samples were similar.
Figure 7-2: The peak area for the release of 2-butanolone (A), butyric acid (B), ethyl butyrate (C) and 2-nonanone (D), no HIU and with HIU IE C16:0 30%, IE C18:0 20% and IE C18:0 30% as quantified by SPME. Data was unavailable for the no HIU and with HIU IE C16:0 20%. The data presented is the mean value and standard error of the mean of two replicates. Columns with the different alphabets within the same graph are different (α = 0.05)
For the IE C18:0 30% sample, the peak area of the liquid sample was higher than the non-sonicated crystallized sample but was lower than the sonicated crystallized sample.

In case of 2-nonanone volatiles the peak area was higher for the IE C18:0 samples compared to the IE C16:0 samples. Application of HIU did not affect the amount of volatiles released from IE C18:0 30%, IE C18:0 20% and IE C16:0 30%. This may be due to the crystalline network created by sonication where less of the flavor compound entered the bulk of the crystals and hence higher volatiles may have been released. When compared to the peak area of 2-nonanone from the liquid samples, the crystallized samples, in general had higher peak area (p < 0.05).

Roberts et al. (8) discussed the comparison of the release of the volatiles from liquid vs. solid lipids. They mention that presence of solid fats in the carrier fat decreases the penetration of the flavor compounds into the bulk of the crystals and this effect aggravates with the increase in the solid fat content. In case of the samples that were crystallized with and without sonication, sonication induced a change in the crystalline network and hence the microstructure of the samples. This change affected the penetration of the flavor in the bulk of the crystals and hence higher volatiles were released.

**2-butanol sensory analysis**

Due to the duration of the project and the availability of the sample size, it was decided to conduct the descriptive analysis with only two compounds- 2-butanone and 2-nonanone. As discussed earlier, these compounds have extreme lipophilicity measured by their ko/w value which was 1 for 2-butanone and 1380 for 2-nonanone and hence they were chosen for the study. Along with the SPME analysis, descriptive analysis was also
performed to understand if similar trends in flavor perception are compared to volatiles analysis by SPME.

The primary objective of this study was to understand if, at the same 2-butanone concentration the maximum perceived intensity of flavor of 2-butanone changed with the type of fat or with the physical state and crystal structure. The training was performed using soybean oil spiked with the flavor compounds because soybean oil has a clean flavor. This allows participants to focus on the flavor of the added compound and hence further calibration for different concentrations of the flavor compound in the soybean oil. The average line scale scores along with their standard errors for the samples are shown in Fig. 7-3. In general, from Fig. 7-3, the intensity of attribute for the “non-spiked” samples were lower than the scores of the “spiked” samples, but not different (p>0.05). This shows that the panelists in most samples, could identify the absence and presence of the added flavor. Multiple comparisons did not show any differences in the perceived intensity of the added 2-butanone in PB and/or IE samples (p>0.05). The only difference was found between the IE C16:0 20% sample without 2-butanone and IE C16:0 30% with 2-butanone (Fig. 7-3A). From this data, it can be inferred that with the change in the amount of palmitic acid at the sn-2 position, there was no change in the perceived intensity of 2-butanone in the fat samples (Fig. 7-3A). Also, there was no difference in the perceived intensity of 2-butanone in PB or IE samples (Fig. 7-3A). Significant one-way interaction was observed between the spiked and non-spiked samples and a two-way interaction between the spiking and the type of sample (PB vs. IE).
Figure 7-3: Flavor intensity of 2-butanone in liquid PB and IE C16:0 20%, PB and IE C16:0 30%, PB and IE C18:0 20% and PB and IE C18:0 30% (A-B) and with and without HIU IE C16:0 20%, IE C16:0 30%, IE C18:0 20% and IE C18:0 30% (C-D). For each sample, data is presented for “non-spiked” and “spiked” sample. Columns with the different alphabets within the same graph are different ($\alpha = 0.05$).
In Fig. 7-3B, in general, there were no statistical differences between the spiked and non-spiked stearic based PB and IE samples (p>0.05) except for the spiked C18:0 30% samples (p<0.05). The PB C18:0 30% sample, had a melting point of 60 °C [6], which caused the sample to crystallize immediately upon tasting. Since the hardening of the fat inside the palate was not a very pleasant experience, the panelists were more focused on removing the sample from their mouth than on the intensity. In the short duration that the sample was in their mouth, they explained it was hard to identify and quantify the flavor of the 2-butanone in the sample resulting in a very low flavor intensity. The IE C18:0 30% sample had a comparable clean flavor and was liquid at body temperature, it was easy for the panelists to quantify the added flavor. Hence, the intensity of attribute for the PB and IE C18:0 30% samples were significantly different. Although the intensity of attribute for the spiked PB with different levels of stearic acid were different, this may have been due to the previously mentioned crystallization of the fat inside the palate. Significant one-way interaction was found between all the main fixed effects. A two-way interaction was also significant between the amount of saturated fat at the sn-2 position and the type of fat. There was no three-way interaction among the samples.

A 3-way ANOVA comparisons (not shown) were also made between the intensity of attribute of IE and PB samples with 20% palmitic or stearic acid at the sn-2 position and 30% palmitic or stearic acid at the sn-2 position. There were no differences in the intensity of attribute of the “spiked” PB samples and/or the IE samples containing 20% saturated fats. Among the samples with 30% saturates, the intensity of attribute of the
spiked PB C18:0 30% sample was lower than the spiked IE C18:0 30% sample ($p = 0.02$).

In Fig. 7-3C, the effect of sonication and the amount of palmitic acid at the sn-2 position of the samples was studied on the flavor intensity of 2-butanone. Like the previous results, the “non-spiked” samples had a lower intensity of attribute than the “spiked” samples due to the presence of 2-butanone in the later samples. From the data in Figure 7-3C, it can be inferred that there were no differences in the perceived intensity of the 2-butanone in the sonicated or non-sonicated IE samples irrespective of the amount of palmitic acid at the sn-2 position ($p>0.05$). This supports the trend observed for the SPME data where there were no significant differences in the volatiles released. No differences were observed between the intensity of attribute between the IE C16:0 30% and the IE C16:0 20% samples. The ANOVA analysis shows a significant one-way interaction between presence of spiking and also between the amount of saturated fatty acids at the sn-2 position. There were no two-way or three way interactions between the samples.

The sensory data for the crystallized IE samples with stearic acid are presented in Fig. 7-3D. The intensity of attribute for the “non-spiked” and “spiked” IE C18:0 20% sample were statistically different. However, there were no differences in the sonicated and non-sonicated samples. The overall scores of the crystallized IE C18:0 30% were lower than the IE C18:0 20% samples. This correlates well with the SPME data for the sonicated IE C18:0 30% sample, but the non-sonicated sample data did not follow trend. No significant differences in the intensity of attribute were observed upon sonication and or spiking with 2-butanone. There was no three way interaction among the fixed effects.
Upon comparison of the intensity of attribute of samples with similar saturates but different type of fatty acids (data not shown), there were no significant differences in the intensity of attribute of the “spiked” sonicated and non-sonicated samples containing 20% palmitic or stearic acid. Similar results were obtained for the samples containing 30% palmitic or stearic acid. Comparisons were also made by 2-way ANOVA to compare the intensity of attribute of liquid, non-sonicated and sonicated samples and for all the four IE samples. There were no significant differences in the intensity of attribute of the any of the IE samples, with the change in physical or chemical structure.

The study is based on the hypothesis that the change in the fatty acid or TAG composition or the physical state of the fat will change the flavor release of the fat. However, based on the results from the panelists in this study, no significant trends were observed these changes in fat on the flavor perception of 2-butanone. The lack of significant differences among the intensity of attribute for liquid and crystallized samples suggest that the performance of the panelists in terms of accuracy and precision as a group leading to high standard deviations may have been responsible for the lack of differences thereof.

2-nonenone sensory analysis

The flavor intensity of 2-nonenone in the PB and IE containing different levels (20% or 30%) and types (palmitic or stearic acid) of fatty acids were studied in this research. The mean intensity of attribute along with their standard error for the 2-nonenone flavor in the IE and PB samples are presented in Fig. 7-4 A-D. The intensity of attribute of the “non-spiked” samples were lower than the “spiked” samples (p<0.05) and this shows that panelists were better trained at identifying the spiked sample compared to
Figure 7-4: Flavor intensity of 2-nonanone in liquid PB and IE C16:0 20%, PB and IE C16:0 30%, PB and IE C18:0 20% and PB and IE C18:0 30% (A-B) and without and with HIU IE C16:0 20%, IE C16:0 30%, IE C18:0 20% and IE C18:0 30% (C-D). For each sample, data is presented for “non-spiked” and “spiked” sample. Columns with the different alphabets within the same graph are significantly different ($\alpha = 0.05$).
the 2-butanol test. Fig. 7-4A shows that the intensity of attribute of the spiked PB C16:0 20% sample was significantly lower compared to the spiked IE C16:0 20% sample (p = 0.03). In contrast, the data also shows that there were no significant differences in the intensity of attribute between the spiked PB and IE C16:0 30% (p > 0.05). Upon comparison of the PB and IE C16:0 20% with the C16:0 30% samples, there were no significant differences in the intensity of attribute of all the samples. This data shows that the perceived intensity of 2-nonanone was not different upon interesterification of the PB C16:0 30% sample or upon increase of the palmitic acid content of the sample (from 20% to 30%).

The sensory data for the PB and IE samples with stearic acid are shown in Fig. 7-4B. For both the IE and PB C18:0 20% and 30% samples, the intensity of attribute and hence the perceived intensity of the 2-nonanone in the “non-spiked” samples was significantly lower than the “spiked” samples. This suggests that panelists could identify the presence and absence of 2-nonanone in the fat samples. However, there were no significant differences among the intensity of attribute of any of the “spiked” PB and IE samples with either levels of stearic acid (20% or 30%). This suggests that with the change in the level of stearic acid at the sn-2 position, or upon interesterification, there was no change in the perceived intensity of the 2-nonanone in any of the fat samples.

Comparisons were also made to compare the intensity of attribute of 2-nonanone intensity between the (i) PB and IE C16:0 20% & PB and IE C18:0 20% samples and (ii) PB and IE C16:0 30% & PB and IE C18:0 30% samples (data not shown). Each data set (i and ii) were compared by 3-way ANOVA and the intensity of attribute of each fat was compared with the others in the group. Similar to results of the Fig. 7-4A and 4B, the
intensity of attribute of the “non-spiked” samples were significantly lower than the “spiked” samples. The intensity of attribute of the IE C16:0 20% sample was significantly higher than the IE C18:0 20% sample. In contrast, the scores of the PB C16:0 20% and PB C18:0 20% samples were not statistically different. For the ii data set, no differences were found in the scores of the PB and IE of the C16:0 30% and C18:0 30% samples.

The data on the effect of sonication on the perceived intensity of the 2-nonanone flavor are presented in Fig. 7-4C and 4D. From Fig. 7-4C, there were no significant differences in the intensity of attribute of the sonicated and non-sonicated IE samples (p > 0.05). Also, there were no differences among the intensity of attribute of the IE C16:0 20% and IE C16:0 30% samples (p > 0.05).

From Fig. 7-4D, no statistical differences can be seen among the sonicated and non-sonicated IE C18:0 20% samples or the IE C18:0 30% samples (p > 0.05). However, non-sonicated IE C18:0 20% sample had a significantly lower score than the IE C18:0 30% sample (p < 0.01).

Two comparisons with three-way ANOVA were performed among the intensity of attribute of the sonicated and non-sonicated- (i) IE C16:0 20% and IE C18:0 20% and (ii) IE C16:0 30% and IE C18:0 30% samples. Among the sonicated and non-sonicated IE C16:0 20% and IE C18:0 20%, the score of the non-sonicated spiked IE C18:0 20% sample was significantly lower than the non-sonicated IE C16:0 20% samples (p = 0.03). On the other hand, the scores of the spiked sonicated and non-sonicated IE C16:0 20% and 30% samples were not different (p> 0.05). This suggests that crystallized IE samples
containing 30% saturates do not differ in flavor release of 2-nonanone at the same concentration irrespective of the type of fatty acid or sonication condition.

Finally, a three-way ANOVA was also performed to compare the perceived 2-nonanone intensities among the liquid, non-sonicated, and sonicated (i) IE C16:0 20% (ii) IE C16:0 30% (iii) IE C18:0 20% (iv) IE C18:0 30% samples. The analysis showed that the physical state of the fat or the crystalline network in the sample did not affect the perceived intensity of 2-nonanone at the same concentration.

In these two sensory studies with 2-butanone and 2-nonanone, there was poor performance in terms of accuracy or precision from some of the panelists. Due to the constraint on the sample availability we had optimized the number of panelists in the study to 7-8 people. In each of these studies, the number of such poor performing panelists were 3-4. If the data from these panelists were deleted, we would have data from 4 panelists which was not sufficient to draw conclusions on the objectives in this study. The data presented in Fig. 7-3 and Fig. 7-4 used all the data irrespective of the panelists performance. Also, the line scale was hard for the panelists to master.

Hence, it was decided to repeat the study for 2-butanone with 15 panelists which were chosen based on their performance on a triangle test to distinguish between samples. The previously used line scale was changed to category scale from 0 to 15 with each number as a single category for the scales.

2-butanone sensory-part II

Like the previous experiments, the objective of the study was to understand if the perceived intensity of 2-butanone varied with the change in the type of fat (different fatty acid, TAG composition and/or the crystalline network). Among the participants in the
triangle test, 15 participants who scored either 4 or 5 correct answers were selected to participate in the study. A panelists dropped out during the study and the data is the mean of 14 panelists. Along with the selection criteria, the triangle test showed that the panelists could identify the presence/absence of 2-butanone in the samples. Hence in the sample tastings, the panelists were only given “spiked” samples at the low concentration (1.33 µl/mL).

The data for this study is presented in Fig. 7-5A-D. In Fig. 7-5A intensity of attribute of the PB and IE C16:0 20% and 30% were compared. The data showed that there were no significant differences in the perceived intensity of the PB or IE samples C16:0 20% or 30% or between 20 and 30% samples. This suggests that there was no change in the flavor release of 2-butanone upon interesterification or with the change in the amount of palmitic acid at the sn-2 position. This results correlates well with the findings from the previous 2-butanone study.

Data for the C18:0 samples is presented in Fig. 7-5B. There was no difference in the intensity of 2-butanone in the PB and the IE sample with same amount of stearic acid. However, the intensity of attribute for PB C18:0 20% sample was significantly higher than that of PB C18:0 30% sample (p = 0.03). This may have been because of the previously stated reason that the melting point of the PB C18:0 30% sample was higher and it crystallized immediately upon consumption. In the very short duration that the sample was liquid, the intensity of 2-butanone was not felt very strongly and hence it was lower. In the previous 2-butanone sensory study, the flavor intensity of 2-butanone in PB C18:0 30% was significantly lower than IE C18:0 30%. However, this difference was not observed in the current study.
Figure 7-5: Flavor intensity of 2-butanone in liquid PB and IE C16:0 20%, PB and IE C16:0 30%, PB and IE C18:0 20% and PB and IE C18:0 30% (A-B) and sonicated and non-sonicated IE C16:0 20%, IE C16:0 30%, IE C18:0 20% and IE C18:0 30% (C-D). All the samples are “spiked” samples. Columns with the different alphabets within the same graph are significantly different ($\alpha = 0.05$).
Comparisons were also made using 2-way ANOVA and Tukeys’ multiple comparison test between samples containing the same level of saturated fat irrespective of the type of saturated fatty acids- (i) PB & IE C16:0 20% and PB & IE C18:0 20% (ii) PB & IE C16:0 30% and PB & IE C18:0 30%. No differences in the intensity of attribute of 2-butanone was found among samples with 20% palmitic or stearic acid (PB and/or IE). However, the intensity of attribute of 2-butanone in PB C18:0 30% sample was significantly lower than that in PB C16:0 30%.

The flavor release of IE samples crystallized with and without sonication is presented in Fig. 7-5C. No differences were observed for the intensity of attribute of same samples (IE C16:0 20% or IE C16:0 30%) and between samples (IE C16:0 20% and IE C16:0 30%) under sonicated and non-sonicated conditions. In Fig. 7-5D, the flavor intensity for the 2-butanone were same for the sonicated and non-sonicated sample. The intensity of attribute for the non-sonicated IE C18:0 30% sample was significantly higher than the sonicated IE C18:0 30% sample and the sonicated and non-sonicated IE C18:0 20% sample (p< 0.05). Upon comparison of the intensity of attribute of samples with the same amount of saturated fatty acid (IE C16:0 20% and IE C18:0 20%), the scores IE C18:0 20% sample (both sonicated and non-sonicated samples) were significantly lower than sonicated IE C16:0 20% sample (data not shown). Among the non-sonicated samples, the IE C18:0 20% intensity of attribute was significantly lower than IE C16:0 20%. The scores of the non-sonicated IE C16:0 20% and sonicated IE C18:0 20% sample were statistically not different.

Among the intensity of attribute of the crystallized IE C16:0 30% and IE C18:0 30% samples, the sonicated IE C18:0 30% samples had the statistically lowest score (p <
Among the crystallized samples, following conclusions can be drawn: (i) The intensity of attribute of 2-butanone in IE C16:0 was higher than the IE C18:0 samples (ii) Among the C18:0 samples, non-sonicated IE C18:0 30% had the highest score, which was not statistically different from the C16:0 samples.

Furthermore, the intensity of attribute of each IE sample was compared as liquid, non-sonicated, and sonicated by one way ANOVA (data not shown). There were no differences in the intensity of attribute among these states for the IE C16:0 20% and IE C18:0 20% sample. For the IE C16:0 30% sample, the liquid sample had the significantly lower score than the sonicated sample. The sonicated sample had the highest score. In contrast for the IE C18:0 30% sample, the non-sonicated sample had the highest intensity of attribute, while the sonicated sample had the lowest score which although was not significantly different from the liquid sample.

Although no trends in the change of perceived intensity of 2-butanone from the samples can be made from this study, it is clear that solid fat content of the samples, in general affect the flavor release. Since the liquid samples had not solid fat content, the flavor release among the different samples was similar. The exception to this was PB C18:0 30% samples since it crystallized at body temperature and hence the flavor release was hampered due to the increase in solid fat content of the sample. Similarly, no direct correlations can be made with the chemical composition of the crystallized fat and the flavor perception from this samples. The differences among these samples may be attributed to the amount of solid fat content in these samples.
Conclusion

This study shows that the physical state along with the crystal network of the fat affects the flavor release. The SPME analysis of samples spiked with 2-butanol showed that higher number of volatiles were released by PB samples compared to the IE and the amount also lower for IE with lower levels of SFA at the sn-2 position. Based on descriptive analysis, for liquid samples, there were no significant differences among the samples with different FA and TAG compositions. For samples with tristearin, even if the sample was served liquid, due to the higher differential between the body temperature and the melting point of the fat, the sample crystallized in the mouth immediately upon swallowing which trapped the added flavor in the crystalline network and thus decreased the flavor release from the samples. Sonication was shown to increase the release of 2-nonanone from IE C18:0 30% samples, however the flavor release was either lower or remained the same for the other IE samples. Similar findings were not observed for the 2-butanolone samples.

REFERENCES


CONCLUSION

The best trans fat substitutes are the ones with physical properties and oxidation stability similar to those observed in partially hydrogenated oils. With the current trend of healthy eating, low saturated healthier fats can be considered as trans fat alternatives. The lacking of appropriate physical properties of these low saturated fats can be induced by sonication of the fat with high intensity ultrasound (1-3).

The effect of HIU on the crystalline behavior of fats also depends on the other processing conditions used during crystallization which includes temperature, rate of cooling, and agitation, among others. Results showed that a slight decrease in HIU effectiveness is observed when a crystallizing fat is stirred or agitated during and after sonication. Sonication generates cavities or bubbles responsible for inducing primary and/or secondary nucleation in the crystallizing media. These bubbles might dissolve faster in the presence of agitation affecting the interaction of crystallizing species in the medium and leading to a slight aggregation of crystals and hence a decrease in the rheological parameters. At low temperatures, when agitation was stopped just prior to sonication, HIU generated a fat network with superior rheological properties which was concluded to be the most efficient processing condition for HIU application.

The studies on the effect of HIU on the crystalline behavior of IE and PB fats with either stearic or palmitic acid at the sn-2 position showed that effect of HIU depends on the composition of the fat. HIU was more effective in changing the crystallization behavior of the IE C16:0 30% than the IE C16:0 20% samples. Although HIU induced smaller crystals in both the samples, higher saturation in IE C16:0 30% samples developed a stronger crystalline network that contributed to an increased viscosity and elasticity among the
Figure 8-1 Proposed differences in the crystalline network of the A. lower saturated fat sample B. higher saturated fat sample

Samples. A proposed comparison of the differences in the crystalline network and the amount of crystals of the IE fats with different levels of saturated fatty acids upon sonication at the same supercooling is presented in figure 8-1. The crystalline network in figure 8-1 A shows fewer crystals than those in figure 8-2 B indicating a denser network in samples with higher amount of saturated fatty acids. Also, although HIU induced smaller crystals in the microstructure, not all crystals are the same size. When HIU is applied after some crystals are formed in the system, not all crystals are broken down due to high shear associated with the bubble implosion. Hence, a few larger crystals are also seen in the sample.

This study also showed that a higher percent of high melting TAG in the PB although gave the samples a higher SFC, it had in general, inferior rheological properties than the IE. This happened due to the poor crystalline network formed by TAG with extreme melting points which trapped the liquid components between the solid crystals. The IE samples had TAG with melting points not as extreme as PB and hence crystallized together and formed a better network than IE.
At the same supercooling level, the samples with stearic acid at the sn-2 position were more viscous than the samples with palmitic acid at the sn-2 position, due to the higher melting FA and TAG content. In contrast to the palmitic samples, HIU was effective in both the IE C18:0 20 and 30% samples at inducing smaller crystals and increasing the viscosity and the viscoelastic parameters. The tristearin content of IE C18:0 30% sample was higher than IE C18:0 20% sample. In both these samples, SSS nucleated first among the TAG in the fat samples. HIU, by mechanism of secondary crystallization, broke the SSS crystals which acted as nuclei and promoted further crystallization of lower melting TAG around them. A proposed mechanism for this phenomenon is shown in figure 8.2. The higher melting TAG are SSS while the crystallizing lower melting TAG are SOS and OOS along with their positional isomers. This induction of crystallization of the lower melting TAG which was higher in IE C18:0 30% than the IE C18:20% samples which affected its crystalline network contributed to the differences in the rheological parameters between these samples (figure 8-1). We also propose a possible co-crystallization of TAG molecules as shown in figure 8-3. This figure shows a possible co-crystallization of SSS and OSO.
Similar to the PB C16:0 samples, the PB C18:0 samples had faster crystallization kinetics and a higher SFC compared to the IE fats due to the much higher SSS content which nucleated first and propagated further crystallization. However, the strength of the crystalline network of the PB C18:0 samples was lower than the IE C18:0 samples based on their rheological comparisons.

Post dry-fractionation, the IE C18:0 30% had no SSS, and hence the major TAG in the f-IE were OSS and OOS, which had much lower melting point than SSS. Although the f-IE had lower rheological properties than IE C18:0 30% samples, HIU affected how the TAG in the f-IE interacted and hence the crystalline network was changed by sonication.

We propose that this effect may be due to possible secondary crystallization in the sample induced by sonication as shown in figure 8-2. This effect significantly increased the viscoelastic properties of the fat thus supporting the hypothesis and proving the effectiveness of HIU in low saturated fats with lower melting TAG.

The study on the release of the 2-butanone and 2-nonanone from the IE and PB fats with saturated fatty acids at the sn-2 position showed that in the liquid state, the flavor

Figure 8-3: The possible co-crystallization of TAG in the fat sample
release from all the samples was similar. However, between physical states of the samples, crystallized IE samples had higher flavor release compared to the liquid samples. Although only in some samples, HIU induced crystalline network release more flavor than the non-sonicated samples. However, a trend could not be established in this case. Results from this study did not support our hypothesis that the chemical composition and the sonication induced crystalline network affects the flavor release from the fat.

In summary, this dissertation has worked on explaining the effects of HIU on fats with different fatty acid, TAG, and level of saturation on their functionality using the same HIU operating conditions. Results show that HIU does affect the crystallization behavior of all the fats by changing either the SFC, microstructure, melting characteristics or rheology. However, increase in saturation and the presence of higher melting TAG does make HIU more effective in the system. Future research may be needed in terms of a systematic study of the effect of HIU on fats with increasing SFA levels (from 0% SFA) to determine a minimum threshold saturation level where HIU would be effective in changing the functionality of fats. This can be compared with different types of fatty acids or TAG to understand if the threshold remains the same for different fatty acids/TAG. Systematic research on understanding HIU conditions needed in terms of tip size, amplitude and duration of sonication can be done to induce required characteristics in the sample in these samples with different types and levels of SFA. These characteristics may include a specific type of crystalline network, polymorphic form generation, alteration of viscosity and the viscoelastic properties, etc. Future research with the IE fats with SFA at the sn-2 position may also involve their use in food products in comparison to traditional fats to compare their performance and consumer acceptability.
REFERENCES


Table 1. Triacylglycerol composition of IESBO based on ECN number ("Reprinted (adapted) with permission from (Ye Y, Wagh A, Martini S (2011) Using high intensity ultrasound as a tool to change the functional properties of interesterified soybean oil, J Agric Food Chem 59:10712-10722). Copyright (2011) American Chemical Society.")

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<th>ECN*</th>
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<tr>
<td>38</td>
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<td>40</td>
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<td>48</td>
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*ECN = CN – 2n, where CN is the total carbon number of the TAG and n is the total number of unsaturations.
Table 2. Fatty acid of IESBO ("Reprinted (adapted) with permission from (Ye Y, Wagh A, Martini S (2011) Using high intensity ultrasound as a tool to change the functional properties of interesterified soybean oil, J Agric Food Chem 59:10712-10722). Copyright (2011) American Chemical Society.")

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</table>

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EFFECT OF HIGH INTENSITY ULTRASOUND ON THE CRYSTALLIZATION BEHAVIOR OF INTERESTERIFIED FATS

Dr. SILVANA MARTINI
Utah State University
Dec 2017
Invoice

Jetta V Kadamne
564 E 400 N
Apt 12
Logan, UT 84321
United States
Attn: Jetta Kadamne

0.00 USD

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v 1.1

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Format: Print, Electronic
Portion: chart/graph/table/figure
Number of charts/graphics/tables/figures: 1
The requesting person/organization is: Jeta Vijay Kadamne
Title or numeric reference of the portion(s): Chapter 7, Figure 1. Schematic showing the structural hierarchy defined during the formation of a fat crystal network
Title of the article or chapter the portion is from: Microstructure
Editor of portion(s): N/A
Author of portion(s): Suresh S. Narine, ALEJANDRO G. MARANGONI
Volume of serial or monograph: N/A
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Strong background in food science, fats and oils and crystallization in foods. Highly self-motivated and result oriented, hardworking with a passionate attitude towards learning. Skilled at effective communication (Verbal and written) data presentation and reporting. Skilled at experimental design and statistical analysis.

INDUSTRIAL EXPERIENCE
1. **Mead Johnson Nutrition** (Staffed through Aerotek Staffing Agency)  
   Research Associate  
   Feb’13-July’13  
   • Led the effort to establish the use of a new LUMiSiZer instrument in daily R&D product assessment  
   • Developed a method to assess emulsion stability using LUMiSizer and demonstrated the use of the equipment to the department  
   • Analyzed several existing and new samples and presented the summary of LUMiSizer data

2. **Adecco Staffing agency**  
   Research Technician  
   Apr’12 –Dec’12  
   • Evaluated new hydrocolloids and their levels on body, texture and sensory characteristics of the dairy beverage  
   • Developed dairy beverage prototypes at bench scale and mid-scale pilot plant units  
   • Worked on vegetable oil fractionation and analyses of the fractions by DSC and NMR  
   • Product stability by particle size, LUMiSizer and characterization by viscometer, UV vis and texture analyzer

3. **Nestle PTC, Marysville, Ohio, USA**  
   Research Intern  
   Feb’11 – Feb’12  
   • Evaluated feasibility of several fat types in a non-dairy creamer based on their solid fat content and summarized the findings  
   • Developed prototypes to study stability of the emulsion by particle size, LUMiSizer and Rheometer  
   • Performed literature review and setting up latest methods for oil analyses on DSC and for phospholipids and lyso-phospholipids on HPLC-ELSD  
   • Led the effort on upgrading Rancimat unit including validation of results against the older version

4. **Nestle PTC, Marysville, Ohio, USA**  
   Research Intern  
   Jan’10 - Aug’10  
   • Worked with the project team to develop purchasing specification for palm oil  
   • Sourced fat samples from different suppliers  
   • Through literature review, identified analytical methods to measure primary & secondary oxidation in fats/oils and further characterization by Rancimat, DSC and NMR  
   • Supported the Product Development team in adopting these methods for fat testing prior to pilot plant trials
• Participated in shelf life sensory testing of beverages and worked on correlating the sensory and analytical data
• Presented recommendations for palm oil quality based on the experimental data to the leaders

5. Godrej Foods, Ltd., Mumbai, India
   Summer Plant training  May ‘07 - Jun ‘07
   • Studied the manufacturing scale neutralization, bleaching, filtration and deodorization of edible oils
   • Studied the manufacturing of hydrogenated soybean oil and chemical assessment of initial oil quality

EDUCATION
Ph.D. in Nutrition, Dietetics and Food Science 2014 – 2017
“Effect of High Intensity Ultrasound (HIU) on the crystallization behavior of Interesterified fats”
   • Characterized the crystallization behavior of Interesterified fats (IE) in the presence of High Intensity Ultrasound
   • Identified differences in crystallization behavior of IE with Stearic or Palmitic acid at the sn-2 position
   • Lead a descriptive sensory panel to study the effect of fat composition on flavor release from fats
   • Instructed laboratory sessions in ‘Sensory evaluation of foods’ to graduate and undergraduate students
   • Good understanding of Dairy chemistry and cheese making and heat processing techniques in the dairy industry
Utah State University, Logan, UT  Thesis
Advisor: Dr. Silvana Martini

M.S. in Food Science 2008 - 2010
“Direct measurement of Conjugated Linoleic Acid (CLA) in CLA-rich Soybean Oil by FTIR spectroscopy”
   • Developed and validated rapid analyses methods for CLA fatty acid in oil and in potato chips by ATR-FTIR as a replacement for GC-FID
   • Designed and developed a rapid method for oil extraction from potato chips
University of Arkansas, Fayetteville, AR  Thesis Advisor: Dr. Andrew Proctor

B. Tech. in Oils, Oleochemicals and Surfactants Technology 2004 - 2008
University Institute of Chemical Technology, Mumbai, India

PEER REVIEWED PAPERS
2. Kadamne, J.V. and Martini, S., “Sonocrystallization of interesterified soybean oil with and without agitation”, In review, JAOCs, October 2017


**CONFERENCE PRESENTATIONS**

1. “Effect of High Intensity Ultrasound (HIU) on the Crystallization Behavior of Interesterified and Physical Blends of High Oleic Sunflower Oil (HOSO) and Tristearin” Oral Presentation at AOCS Annual Meeting at Orlando, FL
   **May 2017**

2. “Effect of High Intensity Ultrasound, Agitation, and Crystallization Temperature on the Crystallization Behavior of Interesterified Soybean Oil” Oral Presentation at AOCS Annual Meeting and Expo at Salt Lake City, UT
   **May 2016**

3. “Effect of High Intensity Ultrasound (HIU) on the Crystallization Behavior of Interesterified and Physical Blends of High Oleic Sunflower Oil (HOSO) and Tripalmitin” poster at AOCS Annual Meeting and Expo at Orlando, FL.
   **May 2014**

4. “ATR-FTIR measurement of Conjugated Linoleic Acid (CLA) in CLA-rich Soybean Oil” oral presentation at AOCS Annual Meeting and Expo at Phoenix, AZ.
   **May, 2010**

5. “ATR-FTIR measurement of Conjugated Linoleic Acid (CLA) in CLA-rich Soybean Oil” poster at the 100th AOCS Annual Meeting and Expo at Orlando, FL.
   **May, 2009**

**MEMBERSHIPS AND AFFILIATIONS**

- American Oil Chemists’ Society (2009- Present)
- Institute of Food technologists (2015- Present)
- Food Science Club President, USU (Fall 2017)
- Peer reviewer for manuscripts for ACS publications
- American Institute of Chemical Engineers (2014-2015)

**AWARDS**

- Dr. Niranjan R. Gandhi and Mrs. Josephine N. Gandhi Assistantship at Utah State University 2015-2017
- Noelle and John Cockett Graduate Fellowship at Utah State University 2015
- Awardee, Pennsylvania Manufacturing Confectioners’ Association Student outreach program 2015
• Ph.D. Research Assistantship Year at Utah State University
  2014-2015
• M.S. Research Assistantship Year at University of Arkansas
  2008-2010
• Travel awards from
  o USU Graduate School
  o College of Agriculture and from
  o Center for Women’s and Gender studies
  o Department of Nutrition, Dietetics and Food science
    2015, ’16 & ‘17